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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.

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The authors declare no conflicts of interest.

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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.

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1 Introduction

2 In the UK, ~26% of adults are overweight (body mass index [BMI] = 27–29.9 kg/m²) and
3 ~35% obese (BMI = 30–40 kg/m²) (Baker, 2018). In addition to exacerbated weight-bearing
4 (Wearing et al., 2006), obesity is associated with negative health conditions such as
5 hypertension (Hall et al., 2015) and type II Diabetes (Toplak et al., 2016). To reduce body
6 weight and improve health in obese populations, lifestyle interventions (i.e., dietary
7 manipulation, physical activity) have been implemented, yet with low adherence and success
8 (Forman & Butryn, 2015). Therefore, there is an urgent need for new strategies to reduce
9 obesity prevalence and improve the health and wellbeing of this population. One innovative
10 strategy evidencing therapeutic benefits in this demographic is passive hypoxic exposure
11 (Urdampilleta et al., 2012; Verges et al., 2015; Hobbins et al., 2017).

12
13 Normobaric hypoxia is defined as a reduced/insufficient oxygen supply (lower inspired oxygen
14 fraction or FiO₂) to tissues leading to decreased arterial oxygen saturation (SpO₂) (Heinonen
15 et al., 2006). Workman and Basset (2012) found increased energy expenditure in overweight
16 males immediately following one continuous resting 3-h session at a target SpO₂ of 85% *versus*
17 normoxia. **However, continuous hypoxic exposure (several hours) versus normoxia may**
18 **exacerbate sympathetic nervous system activity, raising the risk of elevated blood**
19 **pressure, tachycardia and rate pressure product in ‘at-risk’ individuals** (White et al.,
20 1985; Wulsin et al., 2017).

21
22 Intermittent hypoxic exposure (IHE) includes cycles of hypoxia and normoxia lasting from a
23 few minutes to hours (Urdampilleta et al., 2012). Decreased cerebral oxygenation and blood

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1 pressure, during and immediately after one ~1 h IHE session, were found in healthy and
2 overweight individuals *versus* a normoxic baseline (Chacaroun et al., 2017; Costalat et al.,
3 2017). Although positive physiological responses have been reported, the aforementioned
4 studies utilised individually-tailored hypoxic intervals (~5-min) to reach a target SpO₂ (~75%),
5 rather than replicable standardized hypoxic/normoxic cycles. Currently no consensus regarding
6 best practice IHE (i.e., optimal cycle length and frequency) for maximising **physiological**
7 **responses** primarily exists for implementation in adults with obesity.

8
9 Between 3–15 cycles per session of moderate hypoxia (FiO₂ = 9.0–16.0%) is suggested **to**
10 reduce blood pressure and increase blood glucose tolerance (daily sessions over four weeks)
11 (Navarrete-Opazo & Mitchell, 2014). Evidence coming from animal studies indicates that
12 longer, less frequent cycles will induce higher physiological (i.e., tissue de-oxygenation) stress
13 *versus* shorter, more frequent cycles (Almendros et al., 2014). **However, this is currently**
14 **unknown in regards to obese individuals completing IHE for beneficial psycho-**
15 **physiological responses.**

16
17 Perception is a necessary component for investigating behaviour changes towards health
18 (Schutzer & Graves, 2004). Stavrou et al. (2018) reported impaired mood state (i.e., greater
19 depression and tension) during a 21-day hypoxic bed rest (FiO₂ = 15.0%) *versus* normoxia. To
20 date, there is a lack of data including perceptual measures during and following (~60-mins)
21 IHE. A low level of enjoyment is commonly cited as a reason for low/non-adherence to lifestyle
22 interventions (King et al., 1988). Therefore, **it is** relevant to assess perception during IHE and
23 potential influences of different cycles. Although exercise-based, studies have found lower
24 perceived exertion when SpO₂ is >94% during hypoxic exercise *versus* <94% (Romer et al.,

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1 2006; Khaosanit et al., 2018). For IHE, perhaps more frequent re-oxygenation phases during
2 shorter *versus* longer hypoxic cycles will permit more positive perception as SpO₂ will likely
3 increase during normoxic cycles (Urdampilleta et al., 2012).

4
5 The aim of this study was to therefore compare the acute effects of 60-min IHE sessions
6 including short, medium and long hypoxic/normoxic exposure cycles (matched total hypoxic
7 and normoxic exposure time) *versus* a control on the magnitude and time course of psycho-
8 physiological responses. We hypothesised that the physiological stimulus (i.e., tissue de-
9 oxygenation) would be greater, **yet with less favourable perceptual responses** (i.e., perceived
10 breathlessness), during longer *versus* shorter cycles, with medium being the ‘optimal’ trade-
11 off.

12 13 **Materials and methods**

14 *Participants*

15 Eight adults with obesity (5 females, 3 males; age: 37.0 ± 11.1 yrs; height: 169.8 ± 0.1 cm;
16 weight: 93.9 ± 8.7 kg; BMI: 33.0 ± 2.2 kg/m²; systolic and diastolic blood pressure: 117.4 ±
17 18.3 and 70.6 ± 11.4 mmHg, respectively) were recruited. Participants enrolled onto the study
18 following eligibility assessment that consisted of having a BMI of 30–40 kg/m², being
19 normotensive (90–120 and 60–80 mmHg systolic and diastolic blood pressure, respectively),
20 no known cardiovascular, metabolic or physiological illness/disease, sedentary (<1 h of
21 moderate-intensity exercise/week), and no exposure to altitude (≥2500 m) for >48 h within six
22 months. Written informed consent was obtained from all participants. This study was carried
23 out in accordance with the Declaration of Helsinki. Ethical approval was received from the
24 School of Applied Sciences Ethics Committee, London South Bank University (SAS1705).

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1 *Experimental design*

2 Participants attended the lab on five separate visits. First, they were familiarised with the study,
3 including procedures/measures involved, and eligibility was determined. Eligible participants
4 returned on four occasions at a similar time of day for the main experimental trials, each
5 separated by seven days to enable a washout period (Kelly & Basset, 2018). Each experimental
6 trial included a 60-min intervention, three of which included 30-min hypoxia ($FiO_2 = 12.0\%$)
7 and 30-min normoxia, and one in continuous normoxia (control) completed in a randomised
8 order. The cyclical interventions were a) $15 \times 2/2$ mins (SHORT), b) $10 \times 3/3$ mins (MEDIUM)
9 and c) $5 \times 6/6$ mins (LONG) in hypoxia/normoxia. All trials included a 60-min post-
10 intervention period (normoxia). Participants arrived at the lab following a 8-h fast but were
11 permitted to consume water. Participants were asked to replicate their dietary intake for
12 remaining trials after the first (24-h prior), and continue their habitual routine during their time
13 spent enrolled on the study.

14 *Intervention*

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

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1 **Fig. 1 near here**

2 *Hypoxic simulation*

3 Participants wore a facemask (Altitude Training Mask, Hypoxico Altitude Training Systems,
4 USA) connected *via* corrugated plastic tubing to a hypoxic generator (Everest Training Summit
5 II, Hypoxico Altitude Training Systems, USA) to simulate hypoxia. The FiO_2 provided was
6 12.0% (simulated altitude of ~4500 m), deemed safe in the population studied (Navarrete-
7 Opazo & Mitchell, 2014). An additional hypoxic generator was set at a 20.9% FiO_2 for
8 normoxic cycles, both outside of participant viewing, for blinding. A Hans Rudolph two-way
9 valve positioned along the corrugated tubing permitted switching between gases. Participants'
10 remained seated at all times during the intervention and instructed to maintain a normal
11 breathing pattern by the investigator ("*breath as normally as you would without wearing a*
12 *facemask*") prior to each trial.

13 *Physiological measures*

14 A pulse oximeter (Nellcor N-200E Pulse Oximeter, Medtronic, USA) attached to the
15 participants' index finger continuously estimated SpO_2 (%) during the 60-min intervention.

16 Near-infrared spectroscopy bi-polar optode sensors (NIRO-2000NX, Hamamatsu, Japan)
17 attached via double-sided adhesive tape and housed (3 cm apart, 775 Nm wavelength) within
18 rubber-cased hoods assessed oxygenation via tissue saturation index (TSI; %) of the left pre-
19 frontal cortex. **Probes were placed over the left prefrontal cortex to illuminate cortical**
20 **area between standard Fp1 and F3 locations according to the international EEG 10-20**
21 **system (Chacaroun et al., 2017).** TSI was calculated *via* emission and absorption of near-
22 infrared light to tissue, in accordance with the Beer-Lambert law. The data signal was
23 arbitrarily set to zero *via* internal factory settings. SpO_2 and TSI data were continuously

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1 sampled during the 60-min intervention at 10 Hz (Chacaroun et al., 2017) and recorded into
2 Spike2 (v8, CED, England).

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

9 *Perceptual measures*

10 Perceived mood state, breathlessness and motivation to exercise were assessed at baseline, T0–
11 T5, and 15, 30 and 60 min after the intervention. Participants were asked ‘*how are you feeling*
12 *right now?*’, ‘*how breathless do you feel right now?*’ and ‘*how motivated do you feel to*
13 *complete exercise right now?*’ by the investigator. Participants were instructed to verbally
14 specify a number on an 11-point scale anchored ‘*very bad*’ (-5) up to ‘*very good*’ (+5) for mood
15 state (Hardy & Rejeski, 1989), a 12-point scale anchored ‘*nothing at all*’ (0) up to ‘*very, very*
16 *severe*’ (10) for breathlessness (Ward & Whipp, 1989), and to adjust a 20 cm visual analog
17 scale anchored ‘*extremely low*’ (1) up to ‘*extremely high*’ (20) for motivation (Crewther et al.,
18 2016). Positive and negative affects were assessed via a 20-item 5-point Likert scale at baseline
19 and 15, 30 and 60 min after the intervention. Participants were instructed to answer how they
20 feel towards 20 emotions including ‘*interested*’, ‘*distressed*’ and ‘*excited*’, ranging from ‘*very*
21 *slightly or not at all*’ (1) to ‘*extremely*’ (5). Items were totaled for positive and negative
22 responses (Watson & Clark, 1988), and the ratio between the two (Diehl et al., 2011). Scale
23 order presentation was held constant across time points and participants.

24 *Data analysis*

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1 Data were processed offline into Excel (Microsoft Office, 2016). SpO₂ data were averaged for
2 time in hypoxia (30-min) and normoxia (30-min) for each IHE condition, and 60-min of
3 normoxia for control. TSI data samples (2-min) were exported in hypoxia (before T1–T5) to
4 compare an equal hypoxic duration between conditions at matched timepoints. **Perceptual data**
5 were obtained in normoxic conditions to **allow meaningful comparisons between IHE cycle**
6 **variations and the control trial, all under normoxic conditions.** TSI data were smoothed
7 using a 5-point moving average, and truncated *via* removal of the first and last 30-s of each 2-
8 min (T0–T5) data collection period (1 min). TSI data were normalized by calculating
9 percentage change from T0 (in normoxia) in each respective condition for statistical analysis,
10 due to possible sensor placement differences. Perceptual data collected during the 60-min
11 intervention and post-intervention periods were expressed as percentage change from T0 and
12 baseline, respectively.

13 Statistical analysis

14 Data distribution was assessed via a Shapiro-Wilk normality test. Normally distributed data
15 was analysed with a parametric two-way analysis of variance, aligned with a Sidak confidence
16 interval adjustment, to investigate the main effects of condition (SHORT, MEDIUM, LONG
17 *vs.* control), time (during intervention: T0, T1, T2, T3, T4 *vs.* T5; after intervention: baseline,
18 15, 30 *vs.* 60 mins post-intervention) and the condition × time interaction for TSI, blood
19 pressure and perceptual responses. SpO₂ data were compared for main effect of condition
20 (SHORT, MEDIUM, LONG *vs.* control) and environment (hypoxia *vs.* normoxia). Sphericity
21 was assessed via a Mauchly test, if violated, a Greenhouse Geisser correction was applied.
22 Partial eta-squared was calculated as an estimation of effect size (ES). Values of 0.01, 0.06 and
23 above 0.14 were considered as small, medium and large, respectively (Cohen, 2013). If data
24 were non-normally distributed, a related samples non-parametric Friedmans test was used. If
25 any significant effects were found, further post-hoc analysis was carried out *via* pairwise

1 comparisons to assess where the significance lay. Statistical testing was carried out in SPSS
2 (v21, IBM, Cambridge). Data are presented as mean \pm SD, and considered statistically
3 significant if $p \leq 0.05$ and a trend for significance if $p \leq 0.07$. Perceptual data are presented as
4 raw values if statistical significance was not reached for context.

6 **Results**

7 ***Responses during IHE***

8 *Physiological measures*

9 SpO₂ was lower during SHORT, MEDIUM and LONG hypoxic vs. normoxic cycles (-6%; p
10 < 0.001 , $F = 32.351$, ES = 0.822; Fig. 2). Pairwise comparisons revealed SpO₂ during hypoxic
11 LONG cycles were lower vs. MEDIUM (-3%; $p = 0.023$) and SHORT (-4%; $p = 0.054$; Fig.
12 2).

13 *****Fig. 2 near here*****

14 TSI decreased during SHORT ($68.9 \pm 3.6\%$), MEDIUM ($69.1 \pm 4.4\%$) and LONG ($68.7 \pm$
15 5.3%) vs. control ($72.6 \pm 4.9\%$; $p = 0.009$, $F = 8.237$, ES = 0.543; Fig. 3). Compared to T0,
16 TSI from T1–T5 were lower (-3%; $p = 0.011$; $F = 6.107$; ES = 0.543; Fig. 3). There was no
17 interaction effect on TSI ($p = 0.080$; $F = 2.997$; ES = 0.300).

18 *****Fig. 3 near here*****

19 *Perceptual measures*

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

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1 **Table 1 near here**

2 **Responses after IHE**

3 *Physiological measures*

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

5 **Table 2 near here**

6 *Perceptual measures*

7 There was a significant effect of condition ($p = 0.003$, $F = 6.617$, $ES = 0.486$) and time ($p =$
8 0.001 , $F = 17.779$, $ES = 0.717$) but no interaction ($p = 0.146$, $F = 1.946$, $ES = 0.218$; Fig. 5a)
9 on perceived breathlessness. Pairwise comparisons revealed perceived breathlessness greater
10 15 min after the intervention vs. baseline (+34%; $p = 0.040$; Fig. 6a). Breathlessness during
11 SHORT was lower vs. control 15–60 mins after the intervention ($p = 0.001$; Fig. 6a). Further,
12 SHORT tended to be lower than LONG 15–60 mins after the intervention ($p = 0.06$). Positive
13 affect decreased from 15–60 min post-intervention vs. baseline ($p < 0.05$), but was unaffected
14 by condition ($p > 0.05$; Fig. 6b). Perceived mood state, motivation to exercise, negative affect
15 and the ratio between positive and negative affect were unaffected by condition and did not
16 change over time ($p > 0.05$; Table 1).

17 **Fig. 4 near here**

18
19 **Discussion**

20 To our knowledge, this is the first study to compare the acute psycho-physiological responses
21 to SHORT (15 × 2/2 mins), MEDIUM (10 × 3/3 mins) and LONG (5 × 6/6 mins) cyclical
22 variations of IHE. During one 60-min (30 min hypoxia/30 min normoxia) IHE session, arterial
23 and brain oxygenation decreases *versus* control, independently of cycle length. **Compared to**

1 baseline, perceived breathlessness increased 15 min after IHE completion. This increase
2 tended to be smaller following SHORT than LONG. When implementing IHE, greater
3 desaturation is observed during longer compared to shorter hypoxic/normoxic cycles in
4 adults with obesity. However, IHE tends to be better tolerated perceptually with shorter
5 rather than longer cycles.

6 *Responses during IHE*

7 *Physiological measures*

8 We found that IHE decreased SpO₂ (during hypoxic cycles) *versus* control. Further, LONG (-
9 7%) led to larger decreases than MEDIUM (-3%) and SHORT (-1%) *versus* normoxic cycles
10 of each respective condition. Although the hypoxic duration was matched across IHE (30 min),
11 the extent of SpO₂ decrease is aligned with hypoxic/normoxic cycle length. This may be due
12 to acute hypoxic exposure inducing a progressive decline in SpO₂ (Botek et al., 2015) that is
13 more evident in longer *versus* shorter cycles. SpO₂ has been found to decrease continuously
14 during exposure to hypoxia at rest (FiO₂ = 9.6%) for up to 10 min *versus* a normoxic baseline
15 in healthy individuals (-26% 0–10-min) (Krejčí et al., 2018). However, SpO₂ values during
16 hypoxic cycles of IHE presented in the current study (SHORT = 94.2%; MEDIUM = 93.1%;
17 LONG = 90.1%) may be considered clinically insignificant. Hence, SpO₂ below 90% has been
18 defined as a state of hypoxemia (Basnet et al., 2006). To reach greater levels of desaturation
19 (hypoxemic state) alone, it is likely that IHE protocols consisting of longer rather than
20 shorter hypoxic/normoxic cycles would be recommended. Overall, cyclical variations of
21 IHE impacts on the subsequent decreases in SpO₂, with longer cycles inducing lower values.
22 TSI of the pre-frontal cortex decreased during all IHE cycles in reference to control. Here, we
23 speculated that longer cycles of IHE would lead to larger decreases in pre-frontal cortex
24 oxygenation *versus* shorter cycles (Verges et al., 2012), but this was not the case. Rupp et al.

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1 (2016) reported decreases in pre-frontal cortex oxygenation (-3%) and SpO₂ (-9%) during 2-
2 min IHE cycles (FiO₂ = 11.0%) for 45 min *versus* a normoxic baseline in healthy individuals,
3 similar to the current study (TSI: -3%; SpO₂: -5%). Chacaroun et al. (2017) also reported
4 decreases in cerebral oxygenation (-6%) during IHE (7 × 5-min hypoxia/3-min normoxia) at a
5 target SpO₂ of 70–80%. Overall, it seems that larger SpO₂ decreases lead to measureable
6 differences in pre-frontal cortex de-oxygenation. A greater hypoxic dose than that used in the
7 current study (FiO₂ = 12.0%) may have led to larger SpO₂ decreases, and as such, pre-frontal
8 cortex oxygenation, during both SHORT and LONG. Notably, TSI decreases occurred and
9 were maintained from T1–T5 *versus* T0. It was previously stated that more than 30-min
10 continuous hypoxic exposure is required to obtain quantifiable decreases in TSI of the pre-
11 frontal cortex (Chacaroun et al., 2017). Under the present circumstances, IHE comprised of
12 varying hypoxic cycles totaling 30-min induced similar de-oxygenation levels in the pre-frontal
13 cortex of adults with obesity, independent of cycle length, which was maintained for 60-min.

14 *Perceptual measures*

15 During IHE, no changes in perceived mood state, breathlessness and motivation to exercise
16 between IHE cycles were reported or *versus* control. During a 21-day bed rest in hypoxia (FiO₂
17 = 15.0%), healthy individuals felt more depressed, tense and confused at days 14 and 21 *versus*
18 baseline (normoxia) (Stavrou et al., 2018). Although IHE and bed rest are passive modalities,
19 the negative affects during bed rest in combination with hypoxia are unlikely to occur during
20 IHE due to reduced exposure duration and inclusion of normoxic cycles. It was previously
21 reported that mood is negatively impacted during rest in continuous hypoxia (8 h; FiO₂ =
22 13.0%) *versus* baseline (normoxia) (de Aquino Lemos et al., 2012). Therefore, we anticipated
23 that SHORT would likely lead to better overall perception. However, no perceptual differences
24 were observed between conditions during IHE. This may be due to differences in exposure type
25 (intermittent *vs.* continuous), duration (30-min *vs.* 8-h), the hypoxic dose between studies (FiO₂

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1 = 12.0% vs. 13.0%), or little hypoxemia. We conclude here that perceptual responses during
2 IHE are maintained with all tested cycle variations.

3 ***Responses after IHE***

4 *Physiological measures*

5 Albeit with severe continuous hypoxic exposure ($\text{FiO}_2 < 8.0\%$), elevations in blood pressure
6 in humans and animals occur (White et al., 1985). Herein, we assessed blood pressure and
7 found no differences between baseline and post-intervention following IHE of a moderate
8 hypoxic level ($\text{FiO}_2 = 12.0\%$). Previous studies have found normalized blood pressure in
9 hypertensive patients following **regular IHE (~1–5-min hypoxic/normoxic cycles, $\text{FiO}_2 = 10–$**
10 **14%, daily for ~60-min, 10–14 days**) (Serebrovskaya et al., 2008). As there were no blood
11 pressure assessments during IHE in the current study, we cannot support this evidence. No
12 negative sympathetic nervous system activity effects are realized regardless of IHE cycle
13 length and is thus considered a safe therapy.

14 *Perceptual measures*

15 One unique finding includes greater perception of breathlessness 15-min post-intervention
16 *versus* baseline, which **tended to be** exacerbated following LONG *versus* SHORT. We believe
17 that this response occurred due to dyspnoeagenia, i.e., an evoked respiratory exertion without
18 increased physiological ventilation (Ward & Whipp, 1989). In adults with obesity,
19 breathlessness is a symptom often felt during rest (Gibson, 2001), which may explain the
20 increases in perceived breathlessness following control and IHE. Pulmonary ventilation
21 measured before and after IHE did not support the participants' increased perception of
22 breathing – supporting evidence of dyspnoeagenia. Importantly, increases in perceived
23 breathlessness **tended to be** smaller following SHORT, and greater following LONG. In

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1 summary, shorter IHE cycles may be preferential over longer because of a **marginal lowering**
2 **in the** the magnitude of post-intervention increases in perceived breathlessness **after IHE**.
3 Compared to baseline, positive affect was reduced 15–60-min post-intervention in all
4 conditions (including control). Stavrou et al. (2018) found reduced positive affect following a
5 21-day bed rest in hypoxic and normoxic conditions. Perceived mood state, motivation to
6 exercise and negative affect were maintained throughout IHE in the current study. As such, a
7 reduced positive affect may not be due to hypoxia *per se* but the lack of activity over time (>3-
8 h). Although positive affect was reduced following the 60-min intervention, it is unlikely
9 that this was due to the effect of IHE, or in particular, cyclical variation.

10 *Limitations and perspectives*

11 The current study has several limitations. Firstly, our sample size was small (n = 8) implying
12 that our findings should be interpreted with caution. Conclusions from this data are made only
13 from stage I obesity, which may differ to stage II and III, **and between genders** such as larger
14 psychophysiological stress (Stengel et al., 2013). Secondly, we used one hypoxic dose (FiO₂ =
15 12.0%) throughout IHE. Further studies should verify whether a **more severe** FiO₂ **(lower than**
16 **that used in the current study) during longer hypoxic/normoxic cycles, which will likely**
17 **maximise the desaturation achieved during IHE, does not lead to negative effects on**
18 **perceptual responses**. IHE combined with exercise may potentiate further positive responses,
19 at least short-term, than IHE alone due to added physical activity. As such, chronic studies
20 implementing 2-min IHE (and exercise) cycles on a regular basis (3–4 times per week, over
21 4–6 weeks) (**Hobbins et al., 2017**) which are likely to improve aspects of health, are needed
22 since the current study is acute-focused. The findings of this study sheds some light on
23 disregarded perceptual responses.

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1 **Conclusion**

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

Peer Review ONLY/Not for Distribution

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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 (SpO₂) during the intervention. Values are presented as mean \pm 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; Φ denotes a statistically significant difference ($p = 0.03$) vs. normoxia for a given condition; \S 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 \pm 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.

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2
3 **Fig. 4:** Perceived breathlessness (a) and positive affect (b) data measured during the post-
4 intervention period after 15, 30 and 60 mins. Values are calculated as a percentage difference
5 from baseline and are presented as mean \pm SD. Short = 2:2 mins; Medium = 3:3 mins; Long =
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10 6:6 mins condition. * denotes a statistically significant ($p < 0.01$) effect of condition; # denotes
11 a statistically significant ($p < 0.01$) effect of time; † denotes a statistically significant ($p < 0.01$)
12 difference vs. baseline; Ø denotes a statistically significant ($p < 0.02$) difference vs. 15 mins;
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‡ denotes a statistically significant ($p < 0.01$) difference vs. control; § denotes a statistical trend
($p < 0.06$) difference vs. Long.

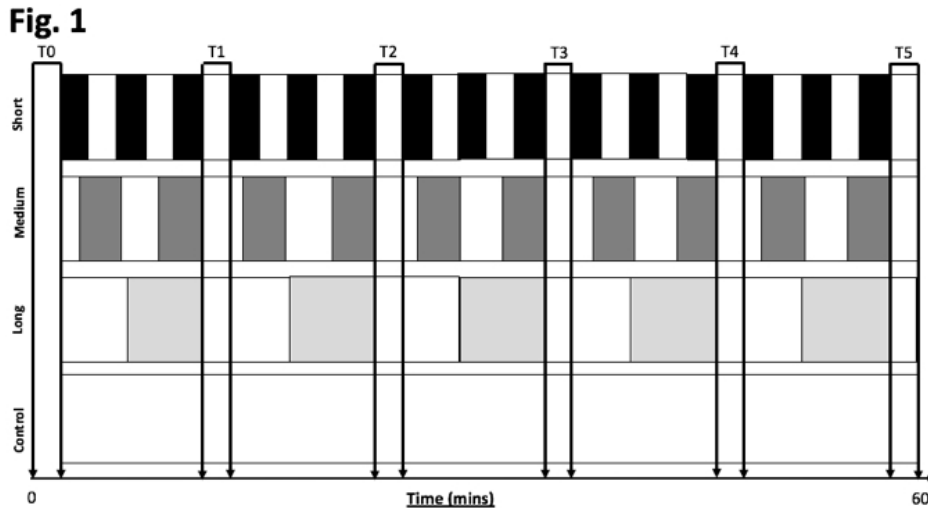


Fig. 1: Overview of the 60-min intervention completed during each experimental trial. Participants were passively exposed to hypoxia (coloured bars) interspersed with exposure to normoxia (white bars). The time spent in hypoxia and normoxia per intervention was 30 mins, achieved via different cyclical 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.

338x190mm (54 x 54 DPI)

Fig. 2

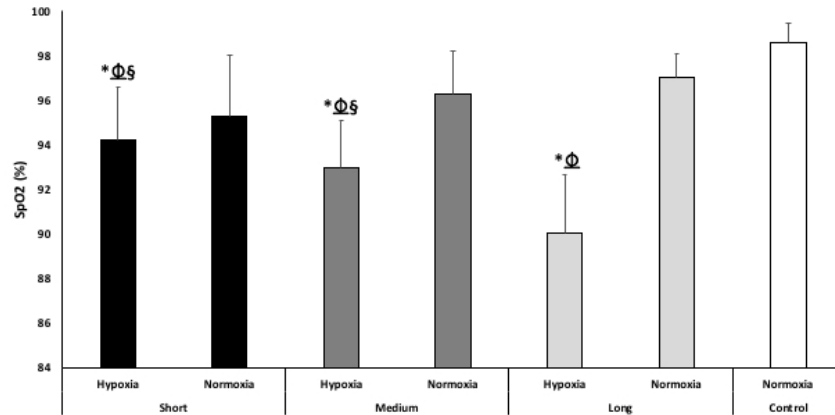


Fig. 2: Arterial oxygen saturation (SpO₂) during the intervention. Values are presented as mean ± SD during hypoxic and normoxic periods (average of 30 min) for Short, Medium and Long, and average of 60 mins for control. Short = 2:2 mins; Medium = 3:3 mins; Long = 6:6 mins. * denotes a statistically 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

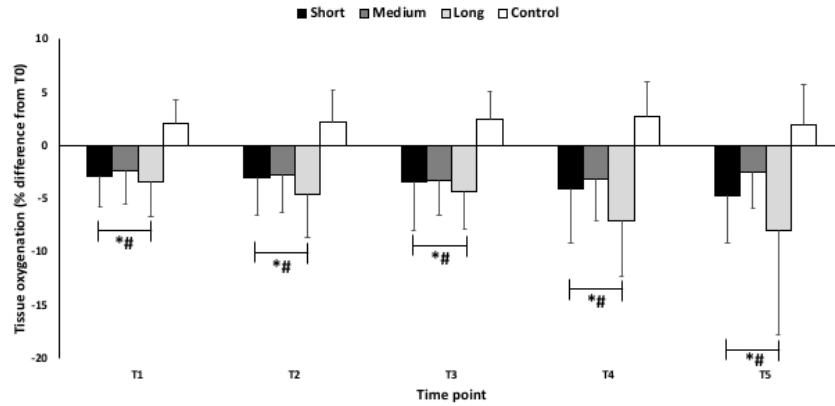


Fig. 3: Tissue saturation index (TSI) data of the pre-frontal cortex measured during the intervention at 8 (T1), 20 (T2), 32 (T3), 44 (T4) and 56 (T5) mins in hypoxia during Short, Medium and Long cycles and in normoxia during control. T1 – T5 values are calculated as a percentage difference from T0 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.

338x190mm (54 x 54 DPI)

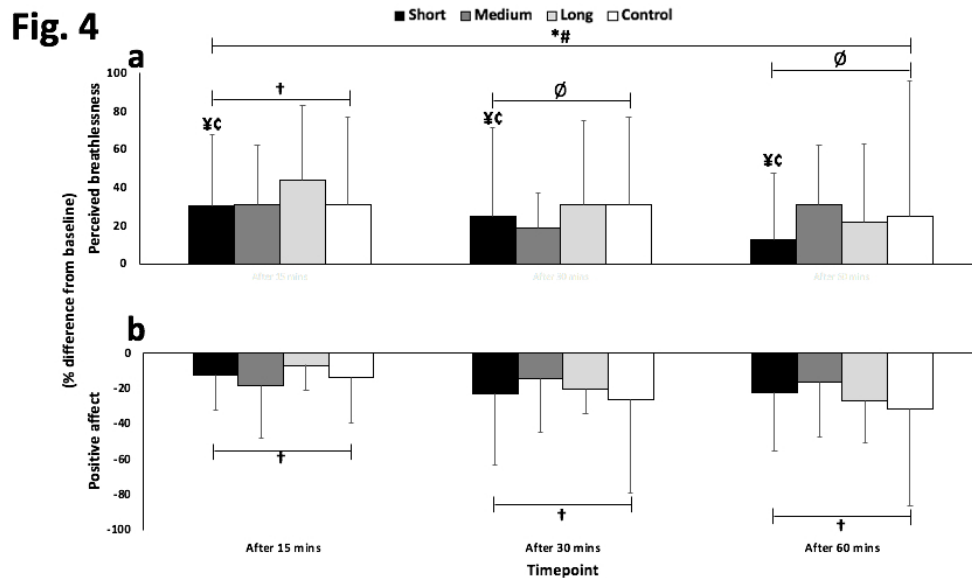


Fig. 4: Perceived breathlessness (a) and positive affect (b) data measured during the post-intervention period after 15, 30 and 60 mins. Values are calculated as a percentage difference from baseline and are presented as mean \pm SD. Short = 2:2 mins; Medium = 3:3 mins; Long = 6:6 mins condition. * denotes 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; ∅ 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.

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.

| Measure | Condition | Timepoint | | | | | | ANOVA <i>p</i> value (effect size) | | |
|------------------------|-----------|--------------|--------------|--------------|--------------|--------------|--------------|------------------------------------|--------------|--------------|
| | | T0 | T1 | T2 | T3 | T4 | T5 | Condition | Time | Interaction |
| Mood state | Short | 4.13 ± 0.83 | 3.88 ± 1.13 | 3.75 ± 1.67 | 3.75 ± 1.75 | 3.38 ± 2.00 | 2.50 ± 2.00 | 0.584 (0.08) | 0.657 (0.08) | 0.727 (0.04) |
| | Medium | 3.63 ± 1.30 | 3.38 ± 1.69 | 3.63 ± 1.60 | 3.25 ± 2.19 | 3.50 ± 2.00 | 3.25 ± 1.98 | | | |
| | Long | 4.00 ± 1.20 | 3.63 ± 1.51 | 3.38 ± 1.85 | 3.13 ± 1.96 | 3.13 ± 2.10 | 2.75 ± 2.38 | | | |
| | Control | 3.13 ± 1.36 | 3.75 ± 1.04 | 3.25 ± 1.17 | 3.13 ± 1.64 | 3.13 ± 1.89 | 2.88 ± 2.03 | | | |
| Breathlessness | Short | 0.63 ± 1.03 | 1.19 ± 1.49 | 1.56 ± 1.88 | 1.31 ± 1.33 | 1.38 ± 1.41 | 1.19 ± 1.07 | 0.220 (0.21) | 0.101 (0.35) | 0.359 (0.14) |
| | Medium | 0.75 ± 1.16 | 1.06 ± 1.15 | 1.06 ± 1.47 | 1.13 ± 1.09 | 1.13 ± 1.46 | 1.06 ± 1.15 | | | |
| | Long | 0.63 ± 0.64 | 1.88 ± 1.25 | 1.81 ± 1.19 | 2.00 ± 1.85 | 1.94 ± 1.66 | 1.75 ± 1.67 | | | |
| | Control | 0.69 ± 0.88 | 1.00 ± 1.04 | 1.19 ± 1.07 | 1.31 ± 1.10 | 1.25 ± 1.39 | 1.13 ± 1.13 | | | |
| Motivation to exercise | Short | 12.63 ± 3.20 | 13.00 ± 2.73 | 12.88 ± 2.47 | 12.25 ± 3.58 | 12.75 ± 3.24 | 12.13 ± 3.36 | 0.435 (0.10) | 0.287 (0.16) | 0.351 (0.13) |
| | Medium | 12.50 ± 2.98 | 13.00 ± 2.93 | 12.63 ± 3.25 | 13.50 ± 3.63 | 13.75 ± 3.20 | 13.38 ± 3.66 | | | |
| | Long | 11.75 ± 5.12 | 10.88 ± 5.08 | 11.00 ± 5.78 | 11.25 ± 5.82 | 11.88 ± 5.87 | 11.50 ± 5.42 | | | |
| | Control | 12.13 ± 3.91 | 12.38 ± 3.29 | 13.25 ± 2.87 | 13.50 ± 2.88 | 13.50 ± 3.16 | 10.88 ± 3.91 | | | |

Values are presented as group means ± SD. Short = 2:2 mins; Medium = 3:3 mins; Long = 6:6 mins.

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.

298x154mm (72 x 72 DPI)

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.

| Measure | Condition | Timepoint | | | | ANOVA <i>p</i> value (effect size) | | |
|------------------------------------|-----------|----------------|----------------|----------------|----------------|------------------------------------|--------------|--------------|
| | | Baseline | +15 mins | +30 mins | +60 mins | Condition | Time | Interaction |
| Systolic blood pressure (mmHg) | Short | 113.38 ± 10.97 | 108.63 ± 13.35 | 117.75 ± 12.21 | 113.75 ± 17.09 | 0.075 (0.27) | 0.661 (0.07) | 0.392 (0.13) |
| | Medium | 112.00 ± 15.36 | 116.57 ± 21.93 | 112.13 ± 13.58 | 110.50 ± 14.99 | | | |
| | Long | 116.14 ± 12.73 | 116.38 ± 16.05 | 122.25 ± 15.71 | 121.43 ± 12.51 | | | |
| | Control | 112.43 ± 17.55 | 115.86 ± 15.60 | 114.14 ± 13.03 | 114.00 ± 14.47 | | | |
| Diastolic blood pressure (mmHg) | Short | 76.63 ± 5.88 | 74.00 ± 7.19 | 73.88 ± 8.13 | 76.75 ± 8.70 | 0.288 (0.16) | 0.331 (0.14) | 0.584 (0.09) |
| | Medium | 72.38 ± 10.27 | 73.43 ± 12.39 | 73.75 ± 7.78 | 77.50 ± 9.44 | | | |
| | Long | 76.71 ± 6.37 | 76.25 ± 8.26 | 79.25 ± 8.22 | 79.00 ± 7.96 | | | |
| | Control | 72.71 ± 9.30 | 75.43 ± 9.50 | 75.86 ± 10.24 | 76.43 ± 7.41 | | | |
| Mood state | Short | 4.63 ± 0.74 | 3.63 ± 1.69 | 3.75 ± 1.67 | 4.25 ± 1.16 | 0.536 (0.06) | 0.710 (0.03) | 0.672 (0.05) |
| | Medium | 3.63 ± 1.51 | 3.75 ± 1.75 | 3.88 ± 1.46 | 3.75 ± 1.75 | | | |
| | Long | 4.25 ± 1.16 | 3.63 ± 1.85 | 3.50 ± 1.31 | 3.88 ± 1.73 | | | |
| | Control | 3.25 ± 1.28 | 3.88 ± 1.81 | 3.75 ± 1.49 | 3.63 ± 1.41 | | | |
| Motivation to exercise | Short | 13.38 ± 3.29 | 12.88 ± 3.56 | 12.50 ± 2.98 | 13.25 ± 2.82 | 0.473 (0.11) | 0.746 (0.05) | 0.375 (0.13) |
| | Medium | 12.63 ± 3.38 | 13.38 ± 3.50 | 13.25 ± 3.28 | 12.88 ± 3.18 | | | |
| | Long | 11.88 ± 5.59 | 10.38 ± 4.66 | 10.25 ± 5.20 | 11.75 ± 4.89 | | | |
| | Control | 12.38 ± 4.14 | 13.38 ± 2.97 | 13.38 ± 2.83 | 13.13 ± 1.96 | | | |
| Negative affect | Short | 10.13 ± 0.35 | 10.75 ± 1.39 | 10.75 ± 1.16 | 10.50 ± 0.76 | 0.411 (0.12) | 0.277 (0.16) | 0.444 (0.11) |
| | Medium | 10.25 ± 0.71 | 11.13 ± 2.10 | 11.13 ± 1.89 | 10.75 ± 1.39 | | | |
| | Long | 10.88 ± 1.13 | 11.00 ± 1.31 | 10.75 ± 1.16 | 10.50 ± 0.76 | | | |
| | Control | 10.50 ± 0.76 | 11.38 ± 1.77 | 11.00 ± 1.41 | 10.63 ± 1.19 | | | |
| Positive and negative affect ratio | Short | 2.98 ± 0.93 | 2.64 ± 1.19 | 2.59 ± 1.36 | 2.59 ± 1.31 | 0.346 (0.14) | 0.134 (0.28) | 0.793 (0.04) |
| | Medium | 2.69 ± 1.15 | 2.36 ± 1.41 | 2.46 ± 1.42 | 2.44 ± 1.36 | | | |
| | Long | 2.74 ± 1.24 | 2.65 ± 1.34 | 2.38 ± 1.19 | 2.36 ± 1.32 | | | |
| | Control | 2.78 ± 0.96 | 2.43 ± 1.11 | 2.39 ± 1.14 | 2.43 ± 1.32 | | | |

Values are presented as group means ± 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.

261x283mm (150 x 150 DPI)