HIGH ALTITUDE MEDICINE & BIOLOGY

High Altitude Medicine & Biology: http://mc.manuscriptcentral.com/highaltitudemedicine

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

Journal:	*High Altitude Medicine & Biology*
Manuscript ID	HAM-2019-0002.R2
Manuscript Type:	Regular Scientific Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Hobbins, Liam; London South Bank University School of Applied Science, Sport and Exercise Science Research Centre Girard, Olivier; Murdoch University, School of Psychology and Exercise Science Gaoua, Nadia; London South Bank University School of Applied Science, Sport and Exercise Science Research Centre Hunter, Steve; London South Bank University School of Applied Science, Sport and Exercise Science Research Centre
Keyword:	Intermittent hypoxia
Manuscript Keywords (Search Terms):	obesity, acute hypoxia, tissue oxygenation, perception

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

Authors: Liam Hobbins[1], Dr. Olivier Girard[2], Dr. Nadia Gaoua[1], Steve Hunter[1]

Affiliations: [1] Sport and Exercise Science Research Centre, London South Bank University, London, United Kingdom, [2] School of Psychology and Exercise Science, Murdoch Applied Sports Science (MASS) Laboratory, Murdoch University, Perth, Australia

Corresponding author:
Mr. Liam Hobbins
hobbinsl@lsbu.ac.uk
+44 (0)207 815 9137
Sport and Exercise Science Research Centre
School of Applied Sciences
London South Bank University
London, United Kingdom
SE1 0AA

Co-author contact information:
Dr. Olivier Girard
Olivier.Girard@murdoch.edu.au
+61 9360 2566

Dr. Nadia Gaoua
gaouan@lsbu.ac.uk
+44 (0)20 7815 7547

Steve Hunter
steve.hunter@lsbu.ac.uk
+44 (0)207 815 7965

Running title: Psycho-physiological responses to hypoxia.

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

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/m^2) 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

Mary Ann Liebert, Inc., 140 Huguenot Street, New Rochelle, NY 10801

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.,

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 SpO_2 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 psycho-physiological responses. We hypothesised that the physiological stimulus (i.e., tissue de-oxygenation) 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/m^{-2}; 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/m^{-2}, 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).

Mary Ann Liebert, Inc., 140 Huguenot Street, New Rochelle, NY 10801

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 (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 × 2/2 mins (SHORT), b) 10 × 3/3 mins (MEDIUM) and c) 5 × 6/6 mins (LONG) in hypoxia/normoxia. All trials included a 60-min post-intervention period (normoxia). Participants arrived at the lab following a 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.

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 FiO_2 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% FiO_2 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 SpO_2 (%) 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 pre-frontal 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 absorbtion 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. SpO_2 and TSI data were continuously

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 (Beevers 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_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 analaysed 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_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 significiance 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).

Table 1 near here

Responses after IHE

Physiological measures

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

Table 2 near here

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).

Fig. 4 near here

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_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_2 decrease is aligned with hypoxic/normoxic cycle length. This may be due to acute hypoxic exposure inducing a progressive decline in SpO_2 (Botek et al., 2015) that is more evident in longer *versus* shorter cycles. SpO_2 has been found to decrease continuously during exposure to hypoxia at rest (FiO_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_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_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_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.

Mary Ann Liebert, Inc., 140 Huguenot Street, New Rochelle, NY 10801

(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 measureable 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

= 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 (FiO_2 < 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 (FiO_2 = 12.0%). Previous studies have found normalized blood pressure in hypertensive patients following **regular** IHE (**~1–5-min hypoxic/normoxic cycles, FiO_2 = 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 dyspnoeagenia, 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 dyspnoeagenia. Importantly, increases in perceived breathlessness **tended to be** smaller following SHORT, and greater following LONG. In

Mary Ann Liebert, Inc., 140 Huguenot Street, New Rochelle, NY 10801

summary, shorter IHE cycles may be preferential over longer because of a **marginal lowering in the** 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 intervervention, 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 (FiO_2 = 12.0%) throughout IHE. Further studies should verify whether a **more severe** FiO_2 **(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.

Mary Ann Liebert, Inc., 140 Huguenot Street, New Rochelle, NY 10801

Acknowledgements

The authors would like to thank the participants for the time and commitment to the study and Mr. Bill Anderson for technical advice and support.

References

Almendros I, Wang Y, and Becker L (2014). Intermittent hypoxia-induced changes in tumor-associated macrophages and tumor malignancy in a mouse model of sleep apnea. American Journal of Respiratory and Critical Care Medicine 189(5): 593-601.

Baker C (2018). Obesity statistics briefing paper. House of Commons Library 336:1-20.

Basnet S, Adhikari RK, and Gurung CK (2006). Hypoxemia in children with pneumonia and its clinical predictors. The Indian Journal of Paediatrics 73(9):777-781.

Beevers G, Lip GY, and O'brien E (2001). ABC of hypertension: Blood pressure measurement: Part II—Conventional sphygmomanometry: technique of auscultatory blood pressure measurement. BMJ: British Medical Journal 322(7293):1043.

Botek M, Krejčí J, De Smet S, Gába A, and McKune AJ (2015). Heart rate variability and arterial oxygen saturation response during extreme normobaric hypoxia. Autonomic Neuroscience 190:40-45.

Chacaroun S, Borowik A, Morrison S, A., Baillieul S, Flore P, Doutreleau S, and Verges S (2017). Physiological responses to two hypoxic conditioning strategies in healthy subjects. Frontiers in Physiology 7:675.

Cohen J (2013). Statistical Power Analysis for the Behavioral Sciences. London: Routledge.

Costalat G, Lemaitre F, Tobin B, and Renshaw G (2017). Intermittent hypoxia revisited: a promising non-pharmaceutical strategy to reduce cardiometabolic risk factors? Sleep Breath.

Crewther BT, Carruthers J, Kilduff LP, Sanctuary CE, and Cook CJ (2016). Temporal associations between individual changes in hormones, training motivation and physical performance in elite and non-elite trained men. Biology of Sport 33(3):215.

de Aquino Lemos V, Antunes HKM, dos Santos RVT, Lira FS, Tufik S, and de Mello MT (2012). High altitude exposure impairs sleep patterns, mood, and cognitive functions. Psychophysiology 49(9):1298-1306.

Diehl M, Hay EL, and Berg KM (2011). The ratio between positive and negative affect and flourishing mental health across adulthood. Aging & Mental Health 15(7):882-893.

Forman EM, & Butryn ML (2015). A new look at the science of weight control: how acceptance and commitment strategies can address the challenge of self-regulation. Appetite 84:171-180.

Gibson GJ (2001). Obesity, respiratory function and breathlessness. Thorax 55(Suppl 1):S41.

Hall JE, do Carmo JM, da Silva AA, Wang Z, and Hall ME (2015). Obesity-induced hypertension: interaction of neurohumoral and renal mechanisms. Circulation Research 116(6),991-1006.

Hardy CJ, & Rejeski WJ (1989). Not what, but how one feels: The measurement of affect during exercise. Journal of Sport and Exercise Psychology 11(3):304-317.

Heinonen IHA, Boushel R, and Kalliokoski KK (2016). The circulatory and metabolic responses to hypoxia in humans–with special reference to adipose tissue physiology and obesity. Front Endocrinol 7:116.

Hobbins L, Hunter S, Gaoua N, and Girard O (2017). Normobaric hypoxic conditioning to maximize weight loss and ameliorate cardio-metabolic health in obese populations: a systematic review. Am J Physiol Regul Integr Comp Physiol 313:251-264.

Kelly LP, & Basset FA (2018). Acute normobaric hypoxia increases post-exercise lipid oxidation in healthy males. Frontiers in Physiology 8;293.

Khaosanit P, Hamlin MJ, Graham KS, and Boonrod W (2018). Acute effect of different normobaric hypoxic conditions on shuttle repeated sprint performance in futsal players. Journal of Physical Education and Sport 18(1):210-216.

King DS, Dalsky GP, and Clutter WE (1988). Effects of exercise and lack of exercise on insulin sensitivity and responsiveness. J Appl Physiol 64:1942–1946.

Krejčí J, Botek M, and McKune AJ (2018). Dynamics of the heart rate variability and oxygen saturation response to acute normobaric hypoxia within the first 10 min of exposure. Clinical Physiology and Functional Imaging 38(1):56-62.

Naverrete-Opazo A, & Mitchell GS (2014). Therapeutic potential of intermittent hypoxia: a matter of dose. Am J Physiol Regul Integr Comp Physiol 307:1181–1197.

Romer LM, & Dempsey JA (2006). Effects of exercise induced arterial hypoxaemia on limb muscle fatigue and performance. Clinical and Experimental Pharmacology and Physiology 33:391–394.

Rupp T, Peyrard A, Tamisier R, Pepin JL, and Verges S (2016). Cerebral and muscle oxygenation during intermittent hypoxia exposure in healthy humans. Sleep 39(6):1197-1199.

Schutzer KA, & Graves BS (2004). Barriers and motivations to exercise in older adults. Preventive Medicine 39(5):1056-1061.

Serebrovskaya TV, Manukhina EB, Smith ML, Downey HF, and Mallet RT (2008). Intermittent hypoxia: cause of or therapy for systemic hypertension? Experimental Biology and Medicine 233(6):627-650.

Stavrou NA, Debevec T, Eiken O, and Mekjavic IB (2018). Hypoxia exacerbates negative emotional state during inactivity: the effect of 21 days hypoxic bed rest and confinement. Frontiers in Physiology 9,26.

Stengel A, Hofmann T, Goebel-Stengel M, Elbelt U, Kobelt P, and Klapp BF (2013). Circulating levels of irisin in patients with anorexia nervosa and different stages of obesity–correlation with body mass index. Peptides 39:125-30.

Toplak H, Hoppichler F, Wascher TC, Schindler K, and Ludvik B (2016). Obesity and type 2 diabetes. Wiener klinische Wochenschrift 128:196-200.

Thijssen DH, Black MA, and Pyke KE (2011). Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. American Journal of Physiology-Heart and Circulatory Physiology 300(1).

Urdampilleta A, González-Muniesa P, Portillo MP, and Martínez JA (2012). Usefulness of combining intermittent hypoxia and physical exercise in the treatment of obesity. J Physiol Biochem 68:289–304.

Verges S, Chacaroun S, Godin-Ribuot D, and Baillieul S (2015). Hypoxic conditioning as a new therapeutic modality. Front Pediatr 3:58.

Verges S, Rupp T, and Jubeau M (2012). Cerebral perturbations during exercise in hypoxia. Am J Physiol Regul Integr Comp Physiol 302:R903–16.

Ward SA, & Whipp BJ (1989). Effects of peripheral and central chemoreflex activation on the isopnoeic rating of breathing in exercising humans. The Journal of Physiology 411(1):27-43.

Watson D, Clark LA, and Tellegen A (1988). Development and validation of brief measures of positive and negative affect: the PANAS scales. Journal of Personality and Social Psychology 54(6):1063.

Wearing SC, Hennig EM, Byrne NM, Steele JR, and Hills AP (2006). The biomechanics of restricted movement in adult obesity. Obes Rev 7:13–24.

Weir, BJB (1949). New methods for calculating metabolic rate with special reference to protein metabolism. J Phys 109:1-9.

White SG, Fletcher EC, and Miller CC (1985). Acute systemic blood pressure elevation in obstructive and nonobstructive breath hold in primates. J Appl Physiol 79:324–330.

Workman C, & Basset FA (2012). Post-metabolic response to passive normobaric hypoxic exposure in sedendary overweight males: a pilot study. Nutr Metab 9:103.

Wulsin L, Herman J, and Thayer JF (2017). Stress, autonomic imbalance, and the prediction of metabolic risk: A model and a proposal for research. Neuroscience & Biobehavioral Reviews.

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; Φ 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.

Mary Ann Liebert, Inc., 140 Huguenot Street, New Rochelle, NY 10801

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.

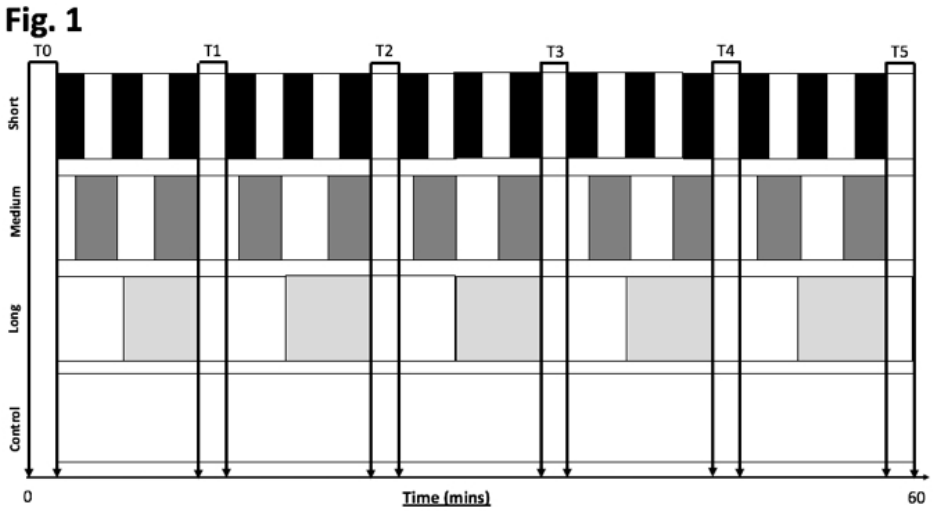

Fig. 1: Overview of the 60-min intervention completed during each experimental trial. Participants were passively exposed to hypoxia (coloured bars) interspersed with exposure to normoxia (white bars). The time spent in hypoxia and normoxia per intervention was 30 mins, achieved via different cyclical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

338x190mm (54 x 54 DPI)

Fig. 2

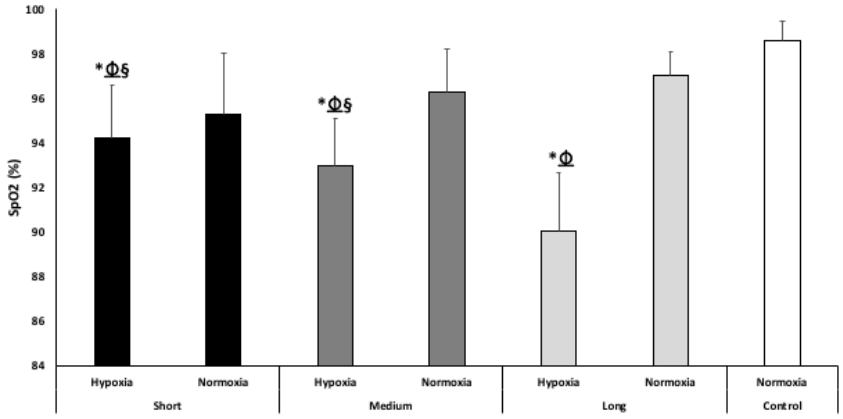

Fig. 2: Arterial oxygen saturation (SpO2) during the intervention. Values are presented as mean ± SD during hypoxic and normoxic periods (average of 30 min) for Short, Medium and Long, and average of 60 mins for control. Short = 2:2 mins; Medium = 3:3 mins; Long = 6:6 mins. * denotes a statistically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

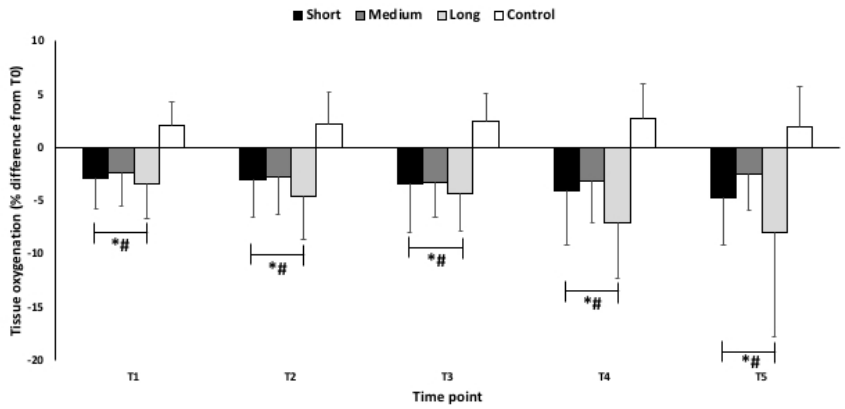

Fig. 3: Tissue saturation index (TSI) data of the pre-frontal cortex measured during the intervention at 8 (T1), 20 (T2), 32 (T3), 44 (T4) and 56 (T5) mins in hypoxia during Short, Medium and Long cycles and in normoxia during control. T1 – T5 values are calculated as a percentage difference from T0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

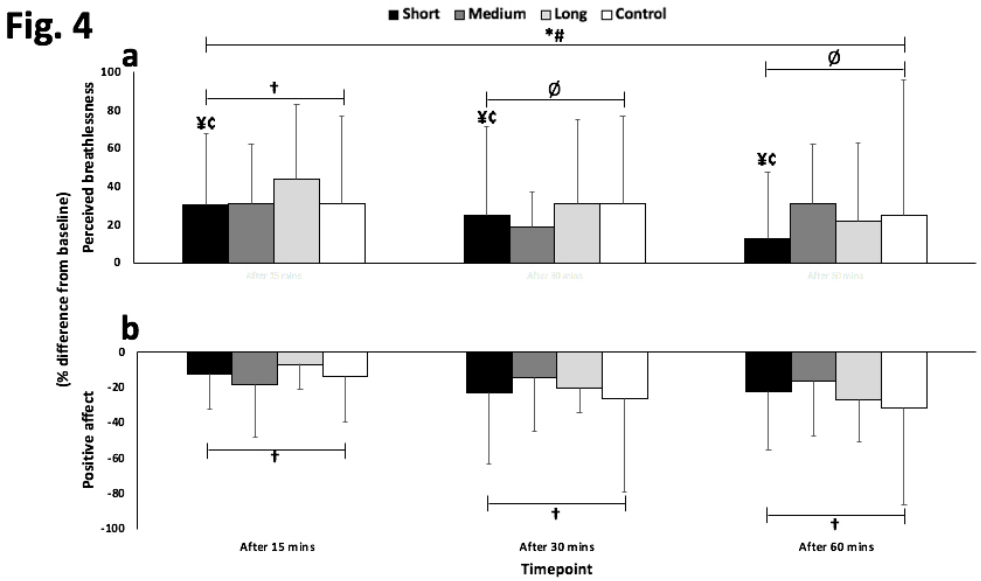

Fig. 4: Perceived breathlessness (a) and positive affect (b) data measured during the post-intervention period after 15, 30 and 60 mins. Values are calculated as a percentage difference from baseline and are presented as mean ± SD. Short = 2:2 mins; Medium = 3:3 mins; Long = 6:6 mins condition. * denotes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

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.

		Timepoint						ANOVA *p* value (effect size)		
Measure	**Condition**	**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.

		Timepoint				ANOVA *p* value (effect size)		
Measure	**Condition**	**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)