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Title

Normobaric hypoxic conditioning to maximise weight-loss and ameliorate cardio-metabolic health in obese populations: A systematic review

Authors

L Hobbins [1], S Hunter [1], N Gaoua [1], O Girard [2,3]

Institutions

[1] *Sport and Exercise Science Research Centre, London South Bank University, London, United Kingdom*

[2] *Athlete Health and Performance Research Centre, Aspetar Orthopaedic and Sports Medicine Hospital, Doha, Qatar*

[3] *ISSUL, Institute of Sport Sciences, University of Lausanne, Switzerland*

Address for correspondence

Mr. Liam Hobbins
hobbinsl@lsbu.ac.uk
Sport and Exercise Science Research Centre
School of Applied Sciences
London South Bank University
London, United Kingdom
SE1 0AA

Running head

Therapeutic use of hypoxia in obese individuals.

Key words

Obesity, hypoxia, altitude training, weight loss, cardio-metabolic health.

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Abstract

Background: Normobaric hypoxic conditioning (HC) is defined as exposure to systemic and/or local hypoxia at rest (passive) or combined with exercise training (active). HC has been previously used by healthy and athletic populations to enhance their physical capacity, and improve performance in the lead up to competition. Recently, HC has also been applied acutely (single exposure) and chronically (repeated exposure over several weeks) to overweight and obese populations with the intention of managing and potentially increasing cardio-metabolic health and weight loss. At present, it is unclear what the cardio-metabolic health and weight loss responses of obese populations are in response to passive and active HC. Exploration of potential benefits of exposure to both passive and active HC may provide pivotal findings for improving health and well being in these individuals.

Methodology: A systematic literature search for articles published between 2000 and 2017 was carried out. Studies investigating the effects of normobaric HC as a novel therapeutic approach to elicit improvements in the cardio-metabolic health and weight loss of obese populations were included.

Results: Studies investigated passive (n = 7; 5 animal, 2 human), active (n = 4; all human) and a combination of passive and active (n = 4; 3 animal, 1 human) HC to an inspired oxygen fraction (FiO_2) between 4.8–15.0%, ranging between a single session and daily sessions per week, lasting from 5 days up to 8 months. Passive HC led to reduced insulin concentrations (-37 – +22%) in obese animals and increased energy expenditure (+12 – +16%) in obese humans, while active HC lead to reductions in body weight (-4 – -2%) in obese animals and humans, and blood pressure (-8 – -3%) in obese humans, compared to a matched workload in normoxic conditions. Inconclusive findings, however, exist in determining the impact of acute and chronic HC on markers such as triglycerides, cholesterol levels and fitness capacity. Importantly, most of the studies that included animal models involved exposure to severe levels of hypoxia (FiO_2 = 5.0%; simulated altitude >10,000 m) that are not suitable for human populations.

Conclusions: Overall, normobaric HC demonstrated observable positive findings in relation to insulin and energy expenditure (passive), and body weight and blood pressure (active), which may improve the cardio-metabolic health and body weight management of obese populations. However, further evidence on responses of circulating biomarkers to both passive and active HC in humans is warranted.

Word count: 391

1. Introduction

Obesity has been labeled as the global epidemic of the 21st century (78). In the United Kingdom alone, 58% of women and 65% of men are considered to be overweight or obese, i.e., defined as having a body mass index (BMI) of 25–29.9 or ≥30 kg.m^2, respectively (49). Compared to the early 1990s, whereby obesity prevalence was estimated to be ~15%, those living in today's society have a 1 in 4 chance of becoming obese (49). Further, co-morbidities such as cardiovascular disease, type II diabetes and cancer are at greater risk of development in obese popuations resulting in the possibility of higher mortality rates (21).

Obesity is typically caused by a consistently positive energy balance, i.e., greater calories consumed *versus* those expended, which eventually leads to excess fat accumulation (28) – the negative impact of which is profound in terms of health consequences. Carrying additional weight can result in elevated blood pressure (7), metabolic deficiencies (28) and mechanical complications (11) amongst other factors – all of which create an increased functional demand on the body of obese individuals. Further, the increased mechanical demand during weight-bearing activities of obese populations may be deleterious on lower limb joints (i.e., knee and ankle) and limit the functional capabilities compared to healthy and normal weight populations (70). Aside from bariatric surgery, that is primarily available for the most severe cases (BMI ≥40 kg.m^2 [3]), various interventions including diet manipulation, caloric restriction, and increased physical activity and exercise (12), are proposed to counteract these problems.

For weight loss to be considered clinically significant, a change of ≥3% in body weight is required (12) – and then ≤3% change to be deemed as weight maintenance over the duration of several months (65). Typically, weight loss is achieved in the first six months of commencing a new diet and/or exercise programme, but a plateau is then reached and often the weight lost is subsequently regained (66). Given the inadequacy of current weight

management strategies, innovative approaches are warranted for clinically-relevant weight loss treatment and significant improvements in the health and general well-being of those who are overweight and obese beyond what is achieved to date.

Hypoxia is defined as a reduced (or insufficient) oxygen (O_2) supply to tissues caused by decreases in O_2 saturation of arterial blood (24). Hypoxic conditioning (HC) relates to passive (i.e., during rest) or active (i.e., during exercise) exposure to systemic (whole body) and/or local (tissue) hypoxia, resulting in a decrease in arterial O_2 availability (38). HC can be implemented acutely (single exposure) or chronically (multiple exposures over prolonged periods of time). Permenant residence in a hypobaric hypoxic (terrestrial altitude due to lower-than-sea level barometric pressure) environment has shown to reduce the likleyhood of becoming obese (68). Several studies have reported weight loss (1, 58, 81), reduced blood pressure (35, 61) and improved metabolic function (35, 61, 64, 72, 73) after a 1–3 week residential stay (e.g. hotel and food provided, light entertainment acitivites throughout the day, no structured exercise program) at terrestrial altitude (1500–8800 m). However, permanent living or travelling regularly to terrestrial altitude may not be feasible to all (i.e., re-location, elevated cost, lack of time). In obese populations, this practice could also lead to side effects such as physiological and metabolic deficiencies (44), including obstructive sleep apnea (30) or the development of acute mountain sickness (81).

Alternatively, exposure to normobaric hypoxia (or simulated altitude via a reduced inspired O_2 fraction [FiO_2]), is increasingly popular as the number of commercially-available devices permitting simulated hypoxic exposure is growing. Primarily, this intervention allows living at or near sea level and then exposing, periodically, individuals to hypoxic conditions at rest or whilst exercising. This is typically accomplished by breathing through a mask or staying in an environmentally controlled chamber/room/tent whereby the FiO_2 is typically reduced to 15–12% (equivalent to simulated altitudes of ~2600–4300 m). In sedentary

overweight males, for instance, passive acute (single 3-hour exposure session) normobaric HC increased energy expenditure and altered fuel utilisation (reduced glucose and increased lipid oxydation), while further passive HC (multiple 3-h exposure sessions on 7 consecutive days) magnified these metabolic adjustments (77). For a range of exercise intensities (55–65% of maximal O_2 uptake [$VO2_{max}$] / 60–70% of maximum heart rate [HR_{max}]) and similar levels of simulated altitude (~2600 m), other studies (18, 32, 46, 51, 76) have suggested that active HC induces specific molecular adaptations that do not occur when training in a normoxic environment (66). These positive adaptations, in particular, include increased basal noradrenaline levels (4), arteriole diameter and peripheral vasodilation (45), mitochondria number (66), glycolytic enzyme activity (16), insulin sensitivity (40), as well as reduced diastolic blood pressure (63) and leptin levels (29). Such physiological adaptations would in turn improve the metabolic phenotype of obese individuals.

Recent reviews have investigated the impact of O_2 availability as a therapeutic intervention for body weight management (56), intermittent hypoxia for fat loss and enhancement of cardiovascular health (66), the role of hypoxia in energy balance (28), hypoxic conditioning for several pathological diseases (67) and the effectiveness of hypoxic training on cardio-metabolic risk factors (71). Overall, these reviews tend to agree that both hypoxia and hyperoxia (i.e., environmental conditions posing a challenge to O_2 homeostasis) may play a significant role in the processes associated with obesity and weight loss paradigm. However, the aforementioned reviews are limited in terms of systematically examining the potential impact of passive and active HC on markers of cardio-metabolic health and well-being (71), while some focus solely on human research with no consideration of findings from animal models (66, 71). Further, combining the literature on HC of populations with a multitude of diseases (e.g., cardiovascular and pulmonary) does not provide conclusive evidence in relation to the specific treatment of obese populations (67). Due to the

146 mechanical restrictions and weight loading implications on lower limbs (i.e., on the knee and
147 ankle joints) when completing exercise in at-risk (obese, overweight and sedentary)
148 populations (70), the exploration of potential benefits of exposure to both passive and active
149 HC may provide pivotal findings for weight loss and maintenance strategies.
150 Therefore, the aim of this systematic review is to a) summarise the current literature
151 surrounding passive and active normobaric HC as a therapeutic method for improving cardio-
152 metabolic health and managing weight-loss in obese animals and humans, and b) offer
153 perspectives for future research within this area of literature.
154
155 **2. Materials and methods**
156 *2.1. Literature search*
157 A literature search was carried out in the Pubmed, ScienceDirect, Scopus, Web of
158 Science and SportsDiscus databases. The terms (intermittent hypoxia OR passive hypoxic
159 exposure OR hypoxic training OR altitude training OR live-low train-high) AND (obesity OR
160 overweight OR weight loss OR physiological response OR metabolic response OR
161 cardiovascular response) were combined to search the full text of experimental articles
162 published after 2000 and before January 2017. Each title, abstract and full text were assessed
163 for relevance to the topic and selected if they met the inclusion criteria as follows: an original
164 research article; randomised and controlled design; human or animal experimentation;
165 overweight (BMI: 25–30 $kg.m^2$), obese (BMI: 30–38 $kg.m^2$) and/or sedentary participants;
166 normobaric hypoxic intervention; assessment of at least one of the following parameters:
167 blood pressure, glucose concentrations, insulin levels or cholesterol; English language; and
168 published in a peer-reviewed journal. Exlcusion criteria were: athletic/sport
169 population/performance focus; involved obstructive sleep apnoea; clinical studies;
170 implemented hypobaric/no hypoxia; or included a physically active or adolescent population.

171 Only full text articles were reviewed. In addition to the literature search, references were
172 scanned for further relevant articles and were included if they met the inclusion criteria.
173
174 *2.2. Assessment of Methodological Quality*
175 A modified scale to assess the methodological quality of the studies retrieved in this
176 review was carried out following selection of full text articles. The modified version was
177 applied due to the greater representation for experiments employing a training intervention,
178 compared to the Delphi, PEDro and Cochrane scales (53). A 10 item quality rating guide
179 included the criteria listed below and guided the assessment scoring of each study as follows:
180 0 = clearly no; 1 = maybe; 2 = clearly yes; range = 0 (poor)–20 (excellent).
181 1. Inclusion criteria were clearly stated;
182 2. Subjects were randomly allocated to groups;
183 3. Intervention was clearly defined;
184 4. Groups were tested for similarity at baseline;
185 5. A control group was used;
186 6. Outcome variables were clearly defined;
187 7. Assessments were practically useful;
188 8. Duration of intervention was practically useful;
189 9. Between-group statistical analysis was appropriate;
190 10. Point measures of variability;
191
192 **3. Results**
193 *3.1. Search results*
194 Fig. 1 illustrates a flow chart of the search results. The search yielded a total of 212
195 publications. After removal of irrelevant titles, 23 items remained in relation to the focus of

196 the review, reduced to eight following abstract assessment, and subsequently four full texts
197 that met the inclusion criteria. Additionally, a further eleven full text items were added via
198 reference list searching.
199 **Fig. 1 near here***
200
201 *3.2. Methodological quality assessment*
202 The average quality of the 15 studies included in this review was 16/20 according to
203 Paul et al. (53). One study scored 20/20, and the lowest score was 12/20.
204
205 *3.3. Study characteristics*
206 Table 1 illustrates the details of the studies included in this review. Eight studies used
207 animal models (2, 6, 34, 37, 52, 55, 59, 79). Five of these implemented a protocol of passive
208 HC only (2, 34, 52, 55, 59), two active normoxic periods followed by passive HC (6, 79), and
209 one used passive and active HC combined (37). All animal studies included obese rodents
210 (mice or rats) aged between 3 and 24 weeks, seven used male (2, 6, 37, 52, 55, 59, 79) and
211 one involved female (34) models. Five of the animal model groups were genetically obese (2,
212 6, 55, 59, 79), while three were fed a high-fat diet (34, 37, 52). Other than one study stating
213 leptin deficiency in their animal models (34), no other difference in the health of animals
214 across studies was mentioned.
215 Seven of the eligible studies investigated human participants (18, 32, 46, 51, 69, 76,
216 77). Two of these employed passive HC only (69, 77), four active HC only (32, 46, 51, 76),
217 and one investigated both passive and active HC (18). Four of the human investigations were
218 composed of both males and females (18, 32, 51, 76), with the remaining three including
219 males only (46, 69, 77). Further, four studies used obese (BMI = 30–37.1 kg.m2 [18, 32, 51,
220 76]), one overweight (BMI = 27 kg.m2 [77]) and one sedentary (normal weight with a BMI

221 = 22.2 kg.m2 [69]) participants. The body composition of one participant cohort was not
222 reported (46). Participants were aged between 21–51 years. Where mentioned, participants
223 were free from hypertension (18, 77), diabetes (76), stroke (18), acute and chronic cardio-
224 vascular, pulmonary and respiratory diseases/infections (18, 69, 76, 77), barriers to physical
225 activity (32), altitude/hypoxic exposure (32, 76), medication to control weight or metabolism
226 (32, 69, 77), alcohol/drug abuse and smoking (33, 69, 76, 77), and exercise (32, 46, 69, 77)
227 within ≥3 months of enrolling.
228 **Table 1 near here***
229
230 *3.4. Animal studies*
231 *3.4.1. Passive hypoxic exposure*
232 The five investigations reviewed implemented two modes of passive HC, namely
233 intermittent and sustained hypoxia. Intermittent protocols adopted a pattern of 30 s of
234 exposure to hypoxia followed by 30 s of exposure to normoxia, lasting for 8 h (2) and 12–16
235 h per day (55). There were modifications to this approach in two of the investigations as
236 follows: 40 and 80 s of exposure to hypoxia and normoxia, respectively (52), and 2 x 15-min
237 periods of exposure to hypoxia interspersed with 5 and 10 min of exposure to normoxia (34).
238 Only Rodriguez et al. (59) implemented a sustained exposure period of 24 h per day. The
239 hypoxic level ranged between FiO_2 = 4.8% (2, 52, 55, 59) and 14.3% (34), while most studies
240 used a FiO_2 of ~5.0% (2, 52, 55, 59) All interventions involved daily exposure. Most studies
241 examined responses over a prolonged period of time (2–6 weeks [2, 34, 52, 59]), with only
242 Polotsky et al. (55) investigating both short-term (5 days) and long-term (12 weeks)
243 responses.
244
245 *3.4.2. Combined passive and active hypoxic exposure*

246 Chen et al. (6) and Wu et al. (79) implemented a live high-train low (LHTL)
247 intervention, with 90-min exercise sessions (moderate-intensity swimming) carried out in
248 normoxia, followed by sustained passive HC periods (8 h per day, FiO_2 = 14.0%). Lu et al.
249 (37) employed a live high-train high (LHTH) intervention, with implementation of 60-min
250 active HC (moderate-intensity running), and the remaining hours of the day living in the
251 same hypoxic environment (FiO_2 = 13.6%). These interventions ranged between 4–6 weeks.
252
253 *3.5. Human studies*
254 *3.5.1. Passive hypoxic exposure*
255 Wang et al. (69) and Workman & Basset (77) both implemented sustained passive HC
256 periods corresponding to a period of 60 min and 3 h, respectively. The hypoxic level during
257 these sessions was controlled via two methods: FiO_2 clamped at 12–15% (69), and
258 manipulation of FiO_2 to clamp the arterial O_2 saturation (SpO_2) at ~80% (77).
259 Whereas Wang et al. (69) implemented a 4-week intervention (5 days of exposure per
260 week, 60-min sessions), Workman & Basset (77) investigated responses to both a single 3-h
261 session as well as the same period of exposure and hypoxic level on an additional 6
262 consecutive days.
263
264 *3.5.2. Active hypoxic exposure*
265 Active investigations have used a live low-train high (LLTH) approach and
266 implemented exercise of a moderate intensity (55–65% $VO2_{max}$ / 60–70% HR_{max}). Exercise
267 programmes were typically cardiovascular-based (running, cycling, stepping [32, 46, 51,
268 76]), with one study adding strength training (40–50% of 1 repetition maximum, 3 sets of 15
269 repetitions, interspersed with 2–3-min rest periods [32]).

The FiO_2 in all studies was 15.0%. Typical exercise prescription included sessions of 60–90 min in duration, performed three times per week, over a 4-week period (32, 46, 76), with one study implementing a longer training period of eight weeks (51). Kong et al. (32) took their participants to a sea-level residential camp for 4 weeks, which permitted a greater amount of time for exercise per week (22 h) and dietary control. Although, the hypoxic group spent only 6 h in hypoxia per week (exercise modality unknown) with the remainder of the sessions (16 h) carried out in normoxic conditions.

3.5.3. Combined passive and active exposure

Gatterer et al. (18) utilised a combination of passive and active HC via a LLTH approach over a period of 8 months. Participants completed 90-min moderate intensity (65–70% of HR_{max}) exercise sessions on an exercise ergometer of their choice (cycle, treadmill, cross-trainer), immediately followed by 90 mins rest, all in hypoxic (FiO_2 = 12–14%) conditions, twice weekly.

4. Discussion

4.1. Animal studies

4.1.1. Passive hypoxic exposure

Table 2 presents the overall findings of the animal studies included in this review. Glucose concentrations are commonly measured in obese animals following passive HC as an indirect marker of insulin sensitivity, however, the findings of this measure are inconsistent. Polotsky et al. (55) and Ling et al. (34) both found reductions in fasting glucose concentrations following intermittent HC, despite exposure time/cycle (30 s :30 s *versus* 15 min :5–10 min, respectively) and severity of hypoxic exposure (FiO_2 = ~5.0% *versus* 14.3%, respectively) being largely different between protocols. In contrast, Briancon-Marjollet et al.

295 (2) reported significant glucose concentration increases in obese rats after 8 h of intermittent
296 (30 s :30 s) HC to an extreme hypoxic level (FiO_2 = 5.0%) per day over 2 weeks. Other
297 investigations have shown unchanged values when animals were exposed intermittently to
298 similar hypoxic levels using protocols of 40 s :80 s for 8 h (52) and 30 s :30 s for 12 h (55)
299 per day. It seems that the common response of glucose concentrations in obese animals,
300 passively exposed to varying levels of hypoxia, is yet to be verified. This variation in the
301 present findings may be partly explained through the differences in pre-analytical conditions
302 of sampled tissue, which was subsequently utilised for glucose concentration assessment
303 (84).

304 Insulin is receiving a great deal of attention due to its dominance in Type II Diabetes
305 control and development (26). In obese rats, insulin concentrations were unchanged
306 following intermittent HC for 8 h per day (40 s :80 s [52]; and 30 s :30 s [2]). This is perhaps
307 due to the severity of the hypoxic stimulus (FiO_2 = ~5.0%) blunting improvements in this
308 health marker (50). Only one study has reported significant increases in insulin levels, which
309 occurred following both a 5 day (+356%) and 12 week (+185%) hypoxic intervention in
310 obese mice (55). The highly significant increase in insulin concentrations shown here may
311 not actually be of benefit. Perhaps, exacerbation of insulin resistance occurred, leading to
312 hyperinsulinemia (79). It is interesting to note that the hypoxic level employed in these
313 studies was similar (FiO_2 = ~5.0%), and animals were intermittently exposed to hypoxia over
314 1 :1 (30 s :30 s) and 1 :2 (40 s :80 s) sequences. Reducing the severity of hypoxia during
315 exposure periods may prevent dramatic increases, as reported here by Polotsky et al. (55),
316 and protect against subsequent exacerbation and development of hyperinsulinemia. This
317 assumption, however, needs to be verified in an obese human population.

318 Varying findings of cholesterol following HC have been reported. Reductions in total
319 cholesterol were found following hypoxic exposure (15 min :5–10 min, 8 times per day for

40 days, FiO_2 = 14.3%) in both lean and obese mice (34). Contrastingly, an increase in total cholesterol values occured following HC (40 s :80 s for 8 h per day over 14 days, FiO_2 = 5.0%) in obese rats, and in control (no hypoxic exposure) lean and obese rats (52). In another study, no difference was reported in both lean and obese animals exposed to hypoxia (30 s :30 s for 8 h per day over 14 days, FiO_2 = 5.0%) or those who received no hypoxic exposure (2). Although the variance is apparent, it is difficult to interpret findings due to there being a lack of individual evaluation of levels of high-density (HDL) and low-density (LPL) lipoprotein. Increases in total cholesterol in response to HC may in fact be a result of an increase in the HDL/LDL ratio, which would actually be beneficial but is not yet clear in the current literature.

Leptin, a satiety hormone, is suggested to be associated with weight loss due to its action on hypothalamic metabolism and appetite suppression, potentiating a reduced energy intake (47). It is also considered a growing marker of weight loss during and following HC (50). Studies have reported increases in leptin in both hypoxic and normoxic groups (2, 52), but there was no assessment of body weight changes. Increases in serum leptin have been found following intermittent, moderate hypoxic and normoxic exposure of 15 min :5–10 min, respectively, compared to those who received no exposure to hypoxia (34), which was also aligned with slower rates of weight gain (+79% *versus* +100%, respectively). Notably, the animal models were fed a high-fat diet during the course of the intervention, which therefore may explain the reports of weight gain in this study. It could be that the weight gain was a result of increases in muscle mass of the animal models, but this measure was not assessed. In summary, a small amount of evidence suggests that leptin may be a marker associated with weight loss, due to the findings of slower weight gain following passive HC.

Triglycerides, an important part of fat storage (13), have been found to increase following intermittent (40 s :80 s) HC for 8 h per day over 2 weeks in obese rats (+30% [52]).

345 Notwithstanding, equal changes occurred in the control group (+30%), whom didn't received
346 any hypoxic treatment. In addition to this, mean arterial blood pressure increased similarly in
347 both groups. This may have been a result of the investigators feeding animals a high-fat diet
348 alongside the hypoxic intervention, and subsequently blunting the potentially beneficial
349 effects. These findings further highlight the co-morbidity relationship between obesity and
350 hypertension whilst consuming a high-fat diet, which will not be reduced with severe hypoxic
351 levels as shown in other studies (2, 52).

352 Finally, only three studies have measured body weight before and after a chronic
353 passive hypoxic intervention in obese animals. Ling et al. (34) found weight to increase
354 equally in the hypoxic group (+79%) and the control group (+78%). Rodriguez et al. (59)
355 reported weight to increase in the hypoxia group (+9%) but with slightly greater increases in
356 the control group (+13%). Previously, weight loss has generally been observed in the first
357 days of exposure (41, 42). However, this has not been the case in the present review. Finally,
358 Polotsky et al. (55) reported no change in any group. The discrepancy in findings of body
359 weight following passive HC presented here may be due to a lack of dietary control. From an
360 experimental perspective, controlling caloric intake may be difficult in animal models and
361 subsequently leads to disparate changes in weight (i.e., weight gain). Only Polotsky et al. (55)
362 stated what the animal models were fed throughout the intervention and reported no change
363 in body weight. The majority of available studies presented here actually report weight gain
364 after repeated passive HC over 4–12 weeks. To summarise, passive HC in obese animals, fed
365 a high-fat diet, does not lead to conclusive weight loss.

366 **Table 2 near here**

367

368 *4.1.2. Combined passive and active hypoxic exposure*

Unlike passive HC alone, reductions in fasting glucose and insulin responses have typically been found following a combination of passive HC and normoxic active periods (6, 79). Interestingly, the hypoxic level (FiO_2 = ~14.0%) as well as the duration and mode of exercise employed (1.5 h of swimming) was similar across studies. Notably, Wu et al. (79) also found reductions in fasting glucose concentrations within the group whom carried out normoxic exercise without passive HC. This raises questions as to whether exercise alone is more effective than a combination of exposure modes. Passive HC and normoxic active periods, when combined, could potentially improve metabolic and hormonal responses of obese animals. Pending confirmatory research, this could at least in part be ascribed to improved insulin sensitivity and cellular glucose uptake.

The primary question regarding the use of passive and active HC is whether it leads to more benefical health outcomes than a similar workload completed in normoxic conditions. Lu et al. (37) concluded that, compared to a control group, who received no exposure to hypoxia or exercise completion, obese rats lost significant amounts of weight, fat mass, LDL and total cholesterol after a combination of 60-min running sessions in hypoxic conditions (FiO_2 = 13.6%) and permenant residence in the same hypoxic environment, conducted over 4 weeks. Therefore, perhaps the increased physical workload, regardless of the conditions the animal models were in, led to improvements in cardio-metabolic health and reductions in weight. Notably, HDL cholesterol was reduced in the hypoxic group (37), presenting a negative effect of active hypoxic exposure, as HDL cholesterol is deemed as 'good' cholesterol (17). The change in HDL levels may be a reflection in the overall reduction in total cholesterol. Therefore, this may have led to a reduction in HDL and LDL, but with the maintenance of relative concentrations and HDL:LDL ratio.

The remaining two combined normoxic active periods and passive HC studies included in this review, which measured weight pre- and post-intervention, reported similar

findings. Both Chen et al. (6) and Wu et al. (79) implemented identical protocols consisting of daily 90-min swimming sessions in normoxic conditions followed by passive HC (FiO_2 = 14.0%, sustained for 8-h per day). Both studies found greater body weight attenuation of the obese animal models, in comparison to the increase in the control group (no passive and active exposure). Further, weight did not change in the group who completed exercise in normoxic conditions without passive HC (6, 79). These findings suggest that a combination of passive and active HC is possibly more beneficial for weight control than a matched workload in normoxia. To date, however, the mechanisms that induce this response remain unclear (28). Possible increases in daily metabolic rate of only those in the hypoxic groups, causing a negative energy balance, may have occurred. Or perhaps appetite was surpressed through increased leptin concentrations, resulting in a reduced calorie intake. However, neither of these reponses were assessed in these investigations.

4.2. Human studies

4.2.1. Passive hypoxic exposure

Table 3 presents the overall findings of the human studies included in this review. Only two studies included in this review have implemented passive HC in humans. Blood pressure remained unchanged following acute (single 3-h session) and short-term (3-h session per day for 7 days) exposure to a SpO_2 of ~80% (77). Additionally, unchanged body weights occurred following daily HC (1 h) for 4 weeks to severe (FiO_2 = 12.0%) and moderate (FiO_2 = 15.0%) hypoxia (69). However, the participants included in these studies had a healthy BMI (22–27 $kg.m^2$), yet deemed as sedentary, which may explain the ineffective treatment on blood pressure and body weight. Moreover, it could be suggested that the participant cohort in these studies (69, 77) required a more severe level of hypoxia to elicit positive responses. In support of this, recent reviews (50, 67) have indicated a linear continuum between no

419 additive effect and a deleterious effect with HC that is dependent on the severity of the
420 hypoxic stimulus. Therefore, previously employed passive HC protocols in humans may not
421 be beneficial to improve cardio-metabolic health (reduce blood pressure) or lose weight.
422 In their study, Workman & Basset (77) assessed metabolic responses, via a 30-min
423 metabolic rate determination test pre- and post-intervention. They found increases in energy
424 expenditure following acute (+16%) and short-term (+12%) HC, as did lipid metabolism
425 (+44% and +29%, respectively); whereas, glycogen metabolism decreased (-31% and -49%,
426 respectively). Collectively, these findings suggest that passive HC may be an effective
427 modality to induce a shift in fuel utilisation and expend a greater quantity of lipid-based
428 energy stores. Over a longer duration, this may lead to a substantially consistent negative
429 energy balance which may promote measurable weight loss. To date, such a protocol has not
430 been employed in an obese human population.
431 **Table 3 near here**
432
433 *4.2.2. Active hypoxic exposure*
434 Metabolic responses have been assessed following active HC (60–90-min moderate-
435 intensity cardiovascular activity, 3 sessions per week, 4–6 weeks, FiO_2 = 15.0%) in obese
436 humans. Netzer et al. (51) reported greater enhancements in triglycerides, total cholesterol
437 and HDL in those whom completed 8 weeks training for 90-min at 60% of HR_{max} in hypoxic
438 *versus* normoxic conditions. In other studies, no change has been found in both the hypoxic
439 and normoxic groups for triglycerides, total cholesterol and HDL following a similar exercise
440 intensity range and duration over 4 weeks (46, 76). Morishima et al. (46) also reported that
441 glucose concentrations decreased in both the hypoxic (-8%) and normoxic (-7%) group over
442 the course of the intervention. These findings are interesting as all intervention groups
443 exercised under the same hypoxic level and completed the same type of exercise at an

444 'absolute' intensity, i.e. an intensity regardless of the environmental condition. Consequently,
445 differences in findings may have been related primarily to the total duration of the studies (8
446 [51] *versus* 4 weeks [46, 76]). Therefore, it appears that further improvements in metabolic
447 markers such as triglycerides, total cholesterol and HDL with HC would require an
448 intervention of more than 4 weeks in duration for positive effects.

449 In two studies, fasting insulin reductions have been found in both hypoxic (FiO_2 =
450 15.0%) and normoxic exercise (60 mins, moderate intensity, 3 times per week, for 4 weeks)
451 groups over the course of an intervention (hypoxia: -37%, normoxia: -33% [76]; hypoxia: -
452 22%, normoxia: -36% [46]). Although not significant, baseline assessment in both studies of
453 insulin concentrations were ~2 arbitary units larger in the hypoxic compared to normoxic
454 group. Therefore, this may explain the insignificant effect of the hypoxic stimulus as those in
455 the control group started the intervention at a lower concentration. Additional consideration
456 of other hormonal markers, such as growth horomone and insulin-like growth factor, that
457 may further lead to enhancements of potential weight loss through promotion of mechanistic
458 responses (60) warrant further investigation.

459 Hypertension is extremely prevelant in obese populations, causing an increased strain
460 on an already laboured cardiovascular system (33). Kong et al. (32) implemented
461 cardiovascular- and strength-based exercise in an obese population and found significant
462 improvements of systolic (-8%) and diastolic (-7%) blood pressure after 4 weeks of 22 h of
463 exercise per week in the hypoxic group. Notably, their hypoxic group participants completed
464 6 h of the weekly training schedule (type of exercise session unknown) in a hypoxic
465 environment, with the remainder carried out in normoxic conditions. Whereas, those who
466 carried out all of the 22 h training load in normoxic conditions had less improvement in
467 systolic (-3%) and diastolic (-1%) blood pressures. Compared to the normoxic group,
468 Wiesner et al. (76) also reported a similar reduction in systolic (-2% *versus* -2%) but greater

reduction in diastolic (-4% *versus* -1%) blood pressures in the hypoxic group over a similar duration of 4 weeks, yet with a reduced volume of exercise (180 min per week). All in all, active HC demonstrates more supportive evidence for improved blood pressure responses compared to active normoxic periods. That said, a previous review (62) concluded significant benefits to blood pressure values following active HC compared to normoxic conditions in those with various cardiovascular diseases, including normalisation and 3 month maintenance of stage 1 hypertensive patients (39). It could also be suggested that multiple combinations of exercise (cardiovascular and strength) carried out in hypoxic conditions are more beneficial than cardiovascular exercise alone to reduce blood pressure in obese populations. This is supported by the findings of Kong et al. (32), perhaps through enhanced vascular endothelial growth factor transcription leading to improved human vasculature control and capillary action (82).

Reductions in heart rate, for a given exercise workload, have been observed for both hypoxic (-18%) and normoxic (-20%) groups post-intervention (32), yet only statistically significant in the normoxic group. In other studies, no change in heart rate during an exercise test before and after the intervention period was found in the hypoxic or normoxic group (46, 76) – although lactate accumulation was reduced in both intervention groups (hypoxic: -11%, normoxic: -13% [76]). It could be suggested that due to obese humans having a lower baseline fitness level compared to athletic and healthy populations, it is likely that any form of training will lead to an improved recovery response, via assessment of heart rate. Arguably, adding in an additional stimulus such as hypoxia likely reduces the potential of an increased recovery, and therefore, be less beneficial than the same workload in normoxic conditions.

Kong et al. (32) showed non-significant reductions in BMI (-6%) and weight (-7%) of the hypoxic group, however, obese humans in the normoxic group also showed non-

494 significant weight loss post-intervention (-4%). Netzer et al. (51) reported non-significant
495 reductions in weight and BMI in the hypoxic group, however, this did not occur in the
496 normoxic group. In another study, no change was found in BMI and fat mass following both
497 the hypoxic (FiO_2 = 15.0%) and normoxic intervention (moderate-intensity cycling, 3 times
498 per week, 4 weeks), but the normoxic group did lose slightly more weight after the
499 intervention compared to those in the hypoxic group (-1% *versus* -0.5%, respectively [46]).
500 Overall, reductions in weight, BMI and individual tissue mass are found following active HC
501 (moderate-intensity cardio-based exercise, 3 sessions per week, 4–8 week duration). This also
502 occurs without hypoxia but to a lesser extent. Non-significant improvements in these studies
503 may be strengthed if the small participant cohorts (~10 individuals per group) were increased
504 to permit a greater effect size. Alternatively, it could be considered that participants became
505 acclimatised to the hypoxic level (FiO_2 = 15.0%), which was consistently maintained
506 throughout the whole intervention period (4–8 weeks). This could have lead to a rapid plateau
507 of adaptations in body composition as the absence of periodisation may not perpetuate
508 beneficial gains.
509
510 *4.2.3. Combined passive and active hypoxic exposure*
511 Gatterer et al. (18) employed a 90-min moderate intensity (65–70% HR_{max})
512 cardiovascular-based active HC (FiO_2 = 14.0%) and a 90-min period of passive HC (FiO_2 =
513 12.0%) twice per week, for 8 months in obese males and females. After 5 weeks, similar
514 changes in both hypoxic and normoxic groups were reported for body weight (-2% and -1%)
515 and fat mass (+1% and -1%). After 3 months, these responses were futher improved in
516 comparison to the baseline assessment in the hypoxic (body weight: -4%, fat mass: -1%) as
517 well as normoxic (body weight: -3%, fat mass: -2%) group. Additionally, similar reductions
518 were found in both hypoxic and normoxic groups for values of systolic (-3% and -2%) and

519 diastolic blood pressure (-3% and -3%). Following completion of the 8 month intervention
520 period, those in the hypoxic group displayed reductions in fat mass (-1%) and blood pressure
521 (systolic: -4%, diastolic: -2%). However, similar responses were found in the normoxic group
522 (fat mass: -2%; systolic blood pressure: -6%; diastolic blood pressure: -5%). Interestingly,
523 body weight was equally reduced in both groups (-3%) post-intervention. In the only
524 available study, it seems that a combination of both passive and active HC has no added
525 benefit compared to a matched workload in normoxic conditions on weight loss and cardio-
526 metabolic responses assessed here. The main explantion would be that unaltered stimuli (i.e.,
527 hypoxic level, exercise intensity/duration) throughout the intervention lead to a near plateau
528 in most measures assessed over this 8 month period.
529
530 **5. Additional considerations**
531 At present, it is difficult to affirm that overall fitness is improved following active HC
532 *versus* similar exercise training in normoxia of obese populations. Exercise performance in an
533 obese population, assessed via total running distance over the course of a 4-week
534 intervention, showed a tendency of being higher in the hypoxic compared to the normoxic
535 group (+18% [32]). In contrast, workload during hypoxic in reference to normoxic sessions in
536 other studies was typically lower (-17.5% [76], -20% [46]). When exercising in hypoxia,
537 exercise may be perceived as 'harder' (majored internal load as evidenced by higher heart
538 rate, rating of perceived exertion or blood lactate values) *versus* a matched workload in
539 normoxia, leading to a reduced total workload. Therefore, it may be that obese humans
540 require multiple exercise modalities to continue exercising at a clamped intensity and
541 complete a greater total workload.
542 Cardiorespiratory fitness (VO_{2max}) is a key determinant of morbidity and mortality
543 (74). Following active HC (60-mins cardiovascular-based exercise, 55–65% VO_{2max}, 3 times

per week, for 4 weeks) non-significant increases in this determinant have been reported (46, 76). However, these enhancements were visible in both the hypoxic and normoxic exercise groups (+5.6% *versus* +3.1% [76], +12.6% *versus* +8.7% [46]; hypoxia *versus* normoxia, respectively). Taken as a whole, this could indicate that the mode of exercise is primarily responsible for gains (i.e., not the addition of the hypoxic stimulus). Undoubtedly, detection of adaptations to the intervention is paramount to select training intensity, modality and duration for successful interventions in obese populations. One may argue that the studies included in the present review have primarily implemented exercise performance tests that are overly challenging for obese populations, due to the requirement of exercising to volitional exhaustion (46, 76). Other sea-level training studies of obese populations have incorporated a 10-m walk test (23), a 6-minute step test (5) and a 6-minute walk test (27) to assess post-intervention changes in aerobic exercise performance. To date, the inclusion of such performance tests is lacking in the field of HC.

Other than one study which utilised a fixed SpO_2 (77), all studies presented in this review have implemented a fixed FiO_2 during exposure to hypoxia. One potential issue, however, is that the variance in individual response to a given simulated altitude is significant. In support of this, Hamlin et al. (22) concluded that for exposure to the same hypoxic level (FiO_2 = 10.0%), there is a greater inter-individual variability in the extent of arterial desaturation compared to a clamped SpO_2 of 75%. Additionally, obese humans are considered as having a higher 'resistance to hypoxia' in comparison to healthy humans, and thereby a delayed/minimal desaturation (or SpO_2 decrease) when exposed to low hypoxic doses (FiO_2 ≤12.0% [54]). To negate this, implementing fixed SpO_2 values may minimise the number of 'non-responding' participants to a given hypoxic stimulus. Costalat et al. (8) recently investigated individualised intermittent passive exposure to hypoxia (SpO_2 ~80%), including normoxia phases (re-oxygenation to ~95%), in overweight and obese individuals.

569 However, this investigation was not included in this review due to a lack of a
570 control/normoxic condition.
571
572 **6. Perspectives and significance**
573 Multiple reviews investigating the effects of reduced inspired O_2 levels on those
574 whom are obese and/or overweight have been published within the last decade. However, our
575 paper is the first to highlight the beneficial effects of passive and active HC in both obese
576 animals and humans on a variety of physiological, metabolic, hormonal and cardiovascular
577 responses. These novel findings may be pivotal in improving the health and well being of
578 these individuals. The rapid development of HC devices offers significant potential for real-
579 world application as a therapeutic, cost-effective and accessible treatment.
580
581 **7. Where next?**
582 Due to the consideration of HC as a treatment for obesity being relatively new, there
583 are many avenues for future mechanistic and performance-led research to be conducted to
584 improve cardio-metabolic health and promote weight loss.
585
586 *7.1. Exercise intensity*
587 A number of studies in this review mention a reduced workload of participants
588 carrying out moderate-intensity, continuous exercise in hypoxia compared to those in
589 normoxia (46, 76), which has also been proposed elsewhere when clamping the metabolic
590 demand (20). It would be interesting to investigate whether the cardio-metabolic responses of
591 obese populations are significantly different between relative and absolute exercise intensities
592 using direct comparisons (i.e., same participants), which may inform which exercise intensity
593 is more suitable for setting training goals in this population. For example, cycling at 100

watts in hypoxic conditions will create a greater physiological strain (increased heart rate, cardiac output) on the human body compared to the same absolute intensity in normoxic conditions; thus inducing a higher internal (physiological) load for a matched external (power output) load. When cycling at a similar relative intensity, the internal load most likely will be reduced during hypoxia to match the external load of exercising in normoxia, as demonstrated by Wiesner et al. (76). Further research of this area is required to validate this claim and differentiate the effect of adding hypoxia in comparison to the effect of exercising at different intensities. It could be that, clamping the metabolic demand (i.e., working at a given relative exercise intensity in hypoxia *versus* normoxia) may be beneficial for obese populations. Arguably, the muscoskeletal system load is likely reduced in O_2-deprived environments and thereby could prevent further damage to joints, tendons and ligamets during locomotor activities (e.g., outdoor or treadmill walking).

In line with current American College of Sports Medicine (12) and UK National Health Service recommendations (49), the reviewed literature here suggests that a moderate-intensity, continuous exercise training programme (60–75% HR_{max} for 60–90-min, 3 times per week) is the recommended method to achieve weight loss. However, a growing body of literature is indicating that implementation of high-intensity intermittent exercise (3–5 sets of high-intensity exercise periods at 75–95% HR_{max} for 2–5 min interspersed with shorter recovery periods of 2–3 min) in obese populations is beneficial (19, 54, 75). Not only is this form of exercise more time- and metabolically-efficient (36), but also would be more beneficial for weight loss compared to moderate-intensity during normoxia (9, 60, 83). In prescribing such exercise, a careful manipulation of work :rest ratios depending on the aim of the session (aerobically *versus* anaerobically-based responses) is needed.

7.2. Psychological aspect of weight loss

619 A large, and often underestimated, factor in achieving weight loss is related to
620 psychological behaviours. Exercising regularly requires motivation and enjoyment to
621 maintain adherence (31). At present, pleasure-displeasure responses of healthy populations
622 exercising at a high-intensity in normoxic conditions are varied with both positive affects
623 (43) and negative moods (48) reported. To our knowledge, this type of investigation does not
624 exist during and following HC of obese humans. Implementing such affect-perceptual
625 measurements would significantly aid levels of adherence to achieve weight loss through
626 long-term interventions. Interestingly, Ekkekakis & Linds (14) concluded that enjoyment was
627 reduced when obese populations had an imposed exercise intensity 10% greater than a self-
628 selected speed. It remains to be verified whether implementation of self-selected speeds
629 during shorter work periods in hypoxia would be more applicable in an obese population, as
630 previously reported (14, 25).
631
632 *7.3. Differences within obese populations*
633 Although this review is focused on the treatment of obese (BMI: 30–38 $kg.m^2$)
634 populations, some studies have been included with participant groups of overweight and
635 sedentary animals and humans, with a large majority of evidence derived from obese animal
636 findings. Further comparative research is warranted to investigate the responses of different
637 stages of obese populations (e.g., I, II and III [10]), males *versus* females, and young *versus*
638 older populations with or without associated complications (i.e., pre-diabetes).
639
640 *7.4. Experimental considerations*
641 Finally, determining the extent of metabolic stress associated with HC for inducing
642 clinically relevant (>3%) weight losses (66) should be a key focus area. Arguably, many
643 confounding variables likely affect determination of the optimal dose-response during HC,

644 such as food consumption, in the lead up to and following the completion of sessions. If these
645 were to be controlled, and short-term (single session) cardio-metabolic responses were to be
646 assessed in obese populations, it will be possible to implement the 'optimal' exposure
647 protocol (i.e., most beneficial dose, duration and intensity) for long-term improvements in
648 cardio-metabolic health and weight loss, as proposed recently by Serebrovskaya et al. (62).
649 Additional consideration of potential drawbacks associated with HC, such as onset of
650 obstructive sleep apnoea and acute mountain sickness, should be made to increase the
651 possibility of developing optimal passive and active HC protocols.
652
653 **8. A summary of passive and active HC protocols**
654 Table 4 states a summary of passive and active HC protocols in relation to the
655 literature presented in this review for improving cardio-metabolic health and promoting
656 weight loss in obese humans. HC-induced physiological, metabolic, cardiovascular and
657 hormonal responses are undoubtedly highly individual. Importantly, all of the animal models
658 and human participant cohorts included here were free from associated cardio-metabolic
659 complications. In reality, this may not always be the case. Therefore, we recommend full
660 general practitioner clearance to be obtained from prior to undertaking any HC, similar to the
661 process of beginning any physical activity programme/dietary intervention. Positive
662 outcomes would also likely depend on the level of hypoxia employed and careful
663 manipulation of key variables structuring the HC routine (e.g., number of cycles, duration,
664 intensity, mode of exercise and/or periodisation). Importantly, this summary should be
665 interpreted with caution and seenas a starting point only, as it is based upon the findings of a
666 small amount of evidence (passive: 7 studies; active: 8 studies). We therefore encourage
667 clinicians and researchers to refine them to reach a consensus.
668 **Table 4 near here**

669

670 **9. Conclusions**

671 The findings of this review in obese populations suggest that a) passive HC could lead
672 to reduced insulin concentrations (-37 – -22%) in animals and increased energy expenditure
673 (+12 – +16%) in humans, while active HC may reduce body weight (-4 – -2%) in both
674 animals and humans as well as blood pressure (-8 – -3%) in humans; b) inconsistent findings
675 and limited understanding still exist for determining the impact of acute and chronic HC on
676 markers such as triglycerides, cholesterol levels and fitness capacity; and c) a large majority
677 of studies include animal models exposed to severe levels of hypoxia (FiO_2 = ~5.0%) that are
678 not suitable for obese humans. Also, published findings, at present, do not clearly show
679 changes in responses of heart rate, fat and muscle mass following HC being significantly
680 larger than a matched exposure and/or exercise period in normoxic conditions. Nevertheless,
681 the promising findings need larger cohorts, more mechanistic measures and real-world
682 applications of findings to improve the potential clinical impact of this novel intervention.
683 Finally, the industrial and technological advancement, including miniaturised equipment for
684 home use and accessibility to environmental chambers, will certainly contribute to the
685 expansion in the use of these methods.

686

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947

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951

952 **Figure caption**

953 Fig. 1: Flow chart of literature search results; OSA = obstructive sleep apnoea.

954 **Table 1:** Experimental details of studies included in this review that have investigated passive and active hypoxic conditioning.

	Participants							Intervention			
Study	Type	Age	Gender	BMI ($kg.m^2$)	Groups	Exposure type	Approach	Protocol	Mode	Duration	Level of hypoxia (FiO_2, %)
Brancon-Marjollet et al. (2016)	Zucker rats	9 w	48 M	NM	Obese hypoxia (n = 12)	Passive	N/a	Intermittent (30 s :30 s) 8 h.d	N/a	2 w	5.0
					Lean hypoxia (n = 12)						
					Obese control (n = 12)			N/a			N/a
					Lean control (n = 12)						
Chen et al. (2011)	Zucker rats	14 w	56 M	NM	Obese exercise (n = 7)	Active	LHTL	90 mins daily exercise	Swimming	6 w	N/a
					Lean exercise (n = 7)						
					Obese hypoxia (n = 7)	Passive		Sustained 8 h d	N/a		14
					Lean hypoxia (n = 7)						
					Obese exercise and hypoxia (n = 7)	Active		90 mins daily exercise sustained 8 h.d	Swimming		20.9, 14.0
					Lean exercise and hypoxia (n = 7)						
					Obese controls (n = 7)	N/a		N/a	N/a		N/a
					Lean controls (n = 7)						
Gatterer et al. (2015)	Humans	51.4 y	22 F, 10 M	37.1	Hypoxia (n = 16)	Combined	LLTH	90 mins moderate (65-70% HR_{max}) intensity exercise, 90 mins rest	Cycling, running, cross trainer	2x w, 8 m	14.0, 12.0
					Control (n = 16)						N/a
Kong et al. (2013)	Humans	21.1 y	8 F, 10 M	34.3	Hypoxia (n = 10)	Active	LLTH	Moderate (60-70% HR_{max}) intensity exercise, strength (40-50% 1 rep max, 3 sets of 15 reps, 2 – 3 min rest periods) training	Running, stepping, cycling, strength exercising	22 h w, 4 w	15.0
					Control (n = 8)						N/a
Ling et al. (2008)	Kunming mice	NM	80 F	NM	Hypoxia-normal diet (n = 20)	Passive	N/a	Intermittent (15 min :5 – 10 min)	N/a	8x d, 40 d	14.3
					Hypoxia-fatty diet (n = 20)						
					Control-normal diet (n = 20)			N/a			N/a

					Control-fatty diet (n = 20)						
Lu et al. (2014)	Sprague Dawley rats	3 w	20 M	NM	Hypoxia (n = 10) Control (n = 10)	Combined N/a	LHTH	1 h exercise 6 d.w, lived in hypoxia N/a	Running N/a	4 w	13.6 N/a
Morishima et al. (2014)	Humans	31y	20M	NM	Hypoxia (n = 9) Control (n = 11)	Active	LLTH	60 mins moderate (55 % VO_{2max}) intensity cycling	Cycling	3x w, 4 w	15.0 N/a
Netzer et al. (2008)	Humans	47.8 y	10 F, 22 M	33.1	Hypoxia (n = 10) Control (n = 10)	Active	LLTH	90 mins moderate (60% HR_{max}) exercise	Stepping, running, cycling	3x w, 8 w	15.0 N/a
Olea et al. (2014)	Wister rats	24 w	160 M	NM	Hypoxia obese (n = 40) Hypoxia control (n = 40) Obese (n = 40) Control (n = 40)	Passive N/a	N/a	Intermittent (40s :80 s) 8 h.d N/a	N/a	2 w	5.0 N/a
Polotsky et al. (2003)	Mice	NM	74 M	NM	Obese short-term hypoxia (n = 15) Lean short-term hypoxia (n = 16) Obese long-term hypoxia (n = 7) Lean short-term controls (n = 15) Obese short-term controls (n = 14) Controls (n = 7)	Passive N/a	N/a	Intermittent (30 s :30 s) 16 h.d Intermittent (30 s:30 s) 12 h.d N/a	N/a	5 d 12 w 5 d	4.8-5.0 N/a
Rodriguez et al. (2014)	Mice	10 w	82 M	NM	Obese hypoxia (n = 10) Lean hypoxia (n = 11) Obese controls (n = 10) Lean controls (n = 11) Obese hypoxia (n = 9) Lean hypoxia (n = 10)	Passive N/a Passive	N/a	Intermittent (30 s:30 s) 12 h.d N/a Sustained 24 h.d	N/a	4 w	5.0 N/a 10

					Obese controls (n = 10) Lean controls (n = 11)	N/a		N/a			N/a
Wang et al. (2007)	Humans	24 y	30 M	22.2	Severe hypoxia (n = 10) Moderate hypoxia (n = 10) Control (n = 10)	Passive N/a	N/a	Sustained 1 h d N/a	N/a	4 w	12.0 15.0 N/a
Wiesner et al. (2009)	Humans	42.2 y	27 F, 18 M	30	Hypoxia (n = 24) Control (n = 21)	Active	LLTH	60 mins moderate (65% VO_{2max}) intensity running	Running	3x w, 4 w	15.0 N/a
Workman & Basset (2012)	Humans	28 y	15 M	27	Acute hypoxia (n = 11) Short-term hypoxia (n = 6) Control (n = 4)	Passive N/a	N/a	Sustained 3 h N/a	N/a	1d 1x d, 1 w N/a	Target SpO2: 80% N/a
Wu et al. (2013)	Zucker rats	14 w	56 M	NM	Obese exercise (n = 7) Lean exercise (n = 7) Obese hypoxia (n = 7) Lean hypoxia (n = 7) Obese exercise and hypoxia (n = 7) Lean exercise and hypoxia (n = 7) Obese controls (n = 7) Lean controls (n = 7)	Active Passive Active N/a	LHTL	90 mins daily swimming Sustained 8 h.d 90 mins daily swimming, sustained 8 h.d N/a	Swimming N/a Swimming N/a	6 w	N/a 14 20.9, 14.0 N/a

BMI = body mass index; d = day(s); F = female(s); FiO_2 = fraction of inspired oxygen; h = hour(s); HR_{max} = heart rate maximum; LHTH = live-high train-high; LHTL = live-high train-low; LLTH = live-low train-high; M = male(s); mins = minutes; m = months; n = number; N/a = not applicable; NM = not measured; rep max = repetition maximum; s = seconds; VO_{2max} = maximal oxygen uptake; w = week(s); y = years.

Table 2: A summary of the findings for animal studies included in this review.

Study	Condition	Measures								
		Glucose	Insulin	Cholesterol	HDL	LDL	Triglycerides	Leptin	BP	Body weight
Briancon-Marjollet et al. (2016)	Obese hypoxia	↑	→	→				↑		
	Lean hypoxia			→				↑		
	Obese control			→				↑		
	Lean control			→				↑		
Chen et al. (2011)	Obese exercise									→
	Obese exercise and hypoxia	↓	↓							→
	Obese controls									↑
Ling et al. (2008)	Hypoxia-normal diet	↓		↓						
	Hypoxia-fatty diet							↑		↑
	Control-normal diet			↓						
	Control-fatty diet									↑
Lu et al. (2014)	Hypoxia			↓		↓	↓			↓
	Control				↓					
Olea et al. (2014)	Hypoxia obese	→	→	↑			↑	↑	↑	
	Hypoxia control			↑				↑		
	Obese						↑	↑	↑	
	Control							↑		
Polotsky et al. (2003)	Obese short-term hypoxia	↓	↑							→
	Lean short-term hypoxia									→
	Obese long-term hypoxia	→	↑							→
	Lean short-term control									→
	Obese short-term control									→
	Control									→
Rodriguez et al. (2014)	Obese hypoxia									↑
	Lean hypoxia									↑
Wu et al. (2013)	Obese exercise	↓								→
	Obese exercise and hypoxia	↓	↓							→
	Obese control									↑

BP = blood pressure; HDL = high-density lipoprotein cholesterol; LDL = low-density lipoprotein cholesterol; ↑ = increase; ↓ = decrease; → = maintenance.

957 Table 3: A summary of the findings for human studies included in this review.

Study	Condition	Measures												
		Glucose	Insulin	Cholesterol	HDL	Triglycerides	EE	Lipid metabolism	Glycogen metabolism	HR	BP	La^*	BMI	Body weight
Gatterer et al. (2015)	Hypoxia					↓					↓			↓
	Control					↓					↓			↓
Kong et al. (2013)	Hypoxia									↓	↓		↓	↓
	Control									↓	↓		↓	↓
Morishima et al. (2014)	Hypoxia	↓	↓	→	→	→				→			→	
	Control	↓	↓	→	→	→				→			→	↓
Netzer et al. (2008)	Hypoxia			↑	↑	↑							↓	↓
Wang et al. (2007)	Severe hypoxa										→			
Wiesner et al. (2009)	Hypoxia		↓	→	→	→				→	↓	↓		→
	Control		↓	→	→	→				→	↓	↓		
Workman & Basset (2012)	Acute hypoxia						↑	↑	↓		→			→
	Short-term hypoxia						↑	↑	↓					

BMI = body mass index; BP = blood pressure; EE = energy expenditure; HDL = high-density lipoprotein cholesterol; HR = heart rate; La^+ = lactate accumulation; ↑ = increase; ↓ = decrease; → = maintenance.

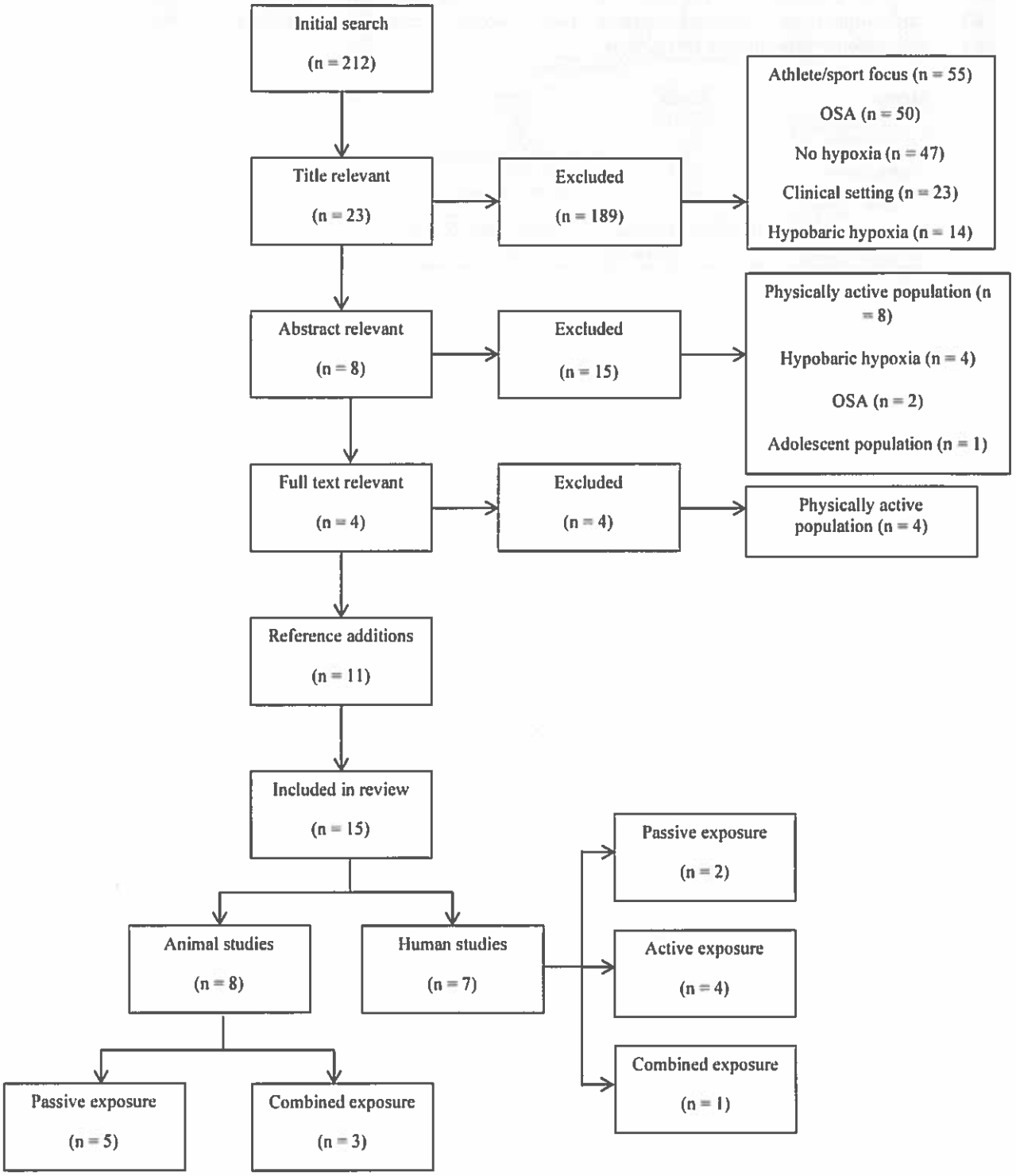
958

959 Table 4: A summary of the passive and active hypoxic conditioning protocols for improving
960 cardio-metabolic health and promoting weight loss of overweight or obese humans, based on
961 the evidence presented in this review.

	Type of exposure	
Variable	**Passive**	**Active**
Level of hypoxia (FiO_2%)	10.0–12.0	13.0–14.0
Number of cycles	5–15	N/a
Intensity	N/a	55–65% $VO2_{max}$ / 60–70% HR_{max}
Duration (hours)	1–1.5	1–1.5
Frequency	Daily	2–3 times per week
Periodisation (weeks)	2–4	4–6

FiO_2 = fraction of inspired oxygen; HR = heart rate; N/a = not applicable; VO_{2max} = maximal oxygen uptake.

962


Initial search
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Title relevant
(n = 23)
Excluded
(n = 189)
Athlete/sport focus (n = 55)
OSA (n = 50)
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Clinical setting (n = 23)
Hypobaric hypoxia (n = 14)
Abstract relevant
(n = 8)
Excluded
(n = 15)
Physically active population (n = 8)
Hypobaric hypoxia (n = 4)
OSA (n = 2)
Adolescent population (n = 1)
Full text relevant
(n = 4)
Excluded
(n = 4)
Physically active population (n = 4)
Reference additions
(n = 11)
Included in review
(n = 15)
Animal studies
(n = 8)
Human studies
(n = 7)
Passive exposure
(n = 2)
Active exposure
(n = 4)
Combined exposure
(n = 1)
Passive exposure
(n = 5)
Combined exposure
(n = 3)

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ИНТЕРВАЛЬНЫЕ ГИПО-ГИПЕРОКСИЧЕСКИЕ ТРЕНИРОВКИ В ЛЕЧЕНИИ МЕТАБОЛИЧЕСКОГО СИНДРОМА

Глазачев О. С.[1], Звенигородская Л. А.[2], Дудник Е. Н.[1], Ярцева Л. А.[1], Мищенкова Т. В.[2], Платоненко А. В.[3], Спирина Г. К.[3]

[1] Московская медицинская академия имени И. М. Сеченова

[2] Центральный научно-исследовательский институт гастроэнтерологии, Москва

[3] ООО «ВНИИМИ», Москва

Глазачев Олег Станиславович
127473, Москва, ул. Селезневская, 30, корп. 2, кв. 18
Тел.: 8 (495) 692 4158, (916) 115 5729
E-mail: glazachev@mail.ru

РЕЗЮМЕ

Цель работы — исследование возможности применения нового метода — интервальных гипо-гипероксических тренировок (ИГГТ) — в коррекции индивидуальных компонентов метаболического синдрома.

В исследовании приняли участие 35 пациентов с метаболическим синдромом (алиментарное ожирение 1–3-й ст. (ИМТ более 30 кг/м^2), нарушение толерантности к углеводам или сахарный диабет II типа, артериальная гипертензия и дислипидемия), случайным порядком разделенных на три группы: контрольную (11 чел., базовая терапия), опытную 1 (13 чел., прошедших курс из 12 процедур ИГГТ) и опытную 2 (11 чел., прошедших курс ИГГТ параллельно с системной гипертермией и вибрационным аппаратным массажем). Продолжительность курса составила в среднем 21 день. До курса процедур ИГГТ и на 3–4-й день по их завершении все пациенты проходили комплексное обследование, включающее сбор анамнеза, оценку пищевого режима; психометрическое тестирование, консультации психологом с целью определения типа пищевого поведения; антропометрические измерения; бодиимпедансометрию, биохимическое исследование крови с определением уровня ОХС, ЛПВП, ЛПНП, ТГ, глюкозы плазмы натощак (ГПН), тест 6-минутной ходьбы для оценки физической работоспособности. Установлено, что применение гипо-гипероксических тренировок (изолированно или в сочетании с системной гипертермией и аппаратным вибромассажем) приводит к значимому снижению массы тела пациентов преимущественно за счет уменьшения жировой массы, что сопровождалось снижением уровня ОХС, ЛПНП, ГПН, оптимизацией артериального давления, повышением гипоксической устойчивости, физической выносливости, улучшением психологического статуса. При индивидуальном подборе структуры курса, дозировании гипоксических воздействий, сочетании с другими физиотерапевтическими процедурами метод имеет определенные перспективы в комплексном лечении и реабилитации пациентов с метаболическим синдромом.

Ключевые слова: метаболический синдром; немедикаментозные методы; гипо-гипероксические тренировки; кардиоваскулярные факторы риска.

SUMMARY

Aim — to investigate the possibility of a new method — interval hypo-hyperoxic training (IHHT) — in the correction of the individual components of metabolic syndrome.

Materials and Methods: The study included 35 patients with metabolic syndrome (alimentary obesity 1-3rd stage [BMI over 30 kg/m2], violation of carbohydrate tolerance or diabetes mellitus type II, hypertension and dyslipidemia). All patients were random separated into three groups: control (11 pers., basic therapy), trial 1 (13 pers. who have undergone 12 procedures of the IHHT) and trial 2 (11 pers. who have undergone IHHT in parallel with systemic hyperthermia and vibrating massage hardware). Course duration was 21 days average. Prior to the course procedures IHHT and the 3-day 4 upon completion all patients were fully examined. This examination included history taking, assessment of diet, psychometric testing, counseling psychologist to determine the type of feeding behavior, anthropometric measurements; body impendansemetria, biochemical study of blood determining the level of total cholesterol, HDL, LDL, TG, fasting plasma glucose (GP), 6-minute walk test to assess physical performance.

Was established that the use of hypo-hyperoxic exercise (alone or in combination with systemic hyperthermia and hardware vibratory) leads to a significant reduction in body weight. It mainly arise by reducing fat mass accompanied by a reduction of total cholesterol, LDL, GPN, optimization of blood pressure, increased hypoxic stability, physical endurance, improved mental status. At individual selection of the course structure, dosing of hypoxic effects, combined with other physiotherapy method has some promise in treatment and rehabilitation of patients with metabolic syndrome.

Keywords: metabolic syndrome, non-drug methods; hypo-hyperoxic exercise, cardiovascular risk factors.

ВВЕДЕНИЕ

Метаболический синдром (МС) как «кластер» взаимосвязанных нарушений, включая инсулинорезистентность, висцеральное ожирение, дислипидемию, гипертензию, представляет чрезвычайно актуальную клиническую проблему [1; 2]. Индивидуальное сочетание компонентов МС является фактором высокого риска развития диабета 2-го типа, морбидного ожирения, в значительной степени ускоряет развитие и прогрессирование атеросклеротических сосудистых заболеваний [3; 4].

В то же время, по мнению ряда авторов, МС является обратимым состоянием [3], следовательно, при ранней диагностике и начале лечения можно добиться значительной редукции выраженности основных его проявлений. В этом плане существенную роль в лечении МС и ожирения играют немедикаментозные методы — дозированные физические нагрузки, диета, различные физиотерапевтические процедуры при их индивидуальном подборе и комбинировании [1; 4–6], а также гипоксические тренировки [7]. Эффективность различных режимов гипоксических тренировок исследована в программах комплексного лечения и реабилитации пациентов с ожирением [8], системной гипертензией [9], диабетом 2-го типа [10]. Показана возможность модуляции метаболических и кардиоваскулярных факторов риска у практически здоровых людей при сочетанном применении физических нагрузок с одновременным дыханием гипоксическими газовыми смесями [11; 12].

В России большую популярность приобрел метод интервальной гипоксической тренировки (ИГТ): дыхание через маску гипоксической газовой смесью короткими интервалами — 5–8 минут, прерываемыми 3–4-минутными нормоксическими паузами [3, 13]. Установлено, что при курсовом применении ИГТ развивается комплекс компенсаторных ответов: оптимизация функционирования симпато-адреналовой системы; увеличение мощности системы транспорта, захвата и утилизации кислорода и субстратов энергообеспечения; изменение метаболизма липидов и липопротеидов за счет активации ключевых ферментов, катализирующих эстерификацию холестерина и регулирующих образование липопротеидов высокой плотности, а также за счет активации цитохромной системы печени, ответственной за окисление холестерина в желчные кислоты; снижение синтеза инсулина и уменьшение инсулиновой реакции на введение глюкозы за счет активации синтеза инсулиновых рецепторов и повышения чувствительности тканей к инсулину; снижение синтеза ренина и некоторое понижение АД [13; 14]. Такой системный ответ организма является весьма важным у лиц с инсулинорезистентностью, метаболическим синдромом и нарушением толерантности к углеводам.

Как показано в работе [15], важным моментом в случае применения ИГТ является чередование периодов дозированной гипоксии и реоксигенации. В этом случае периоды реоксигенации индуцируют продукцию активных форм кислорода (АФК), которые запускают сигнальные каскады синтеза защитных внутриклеточных факторов, в том числе с антиоксидантной функцией. Эффективность ИГТ удалось повысить чередованием коротких гипоксических экспозиций гипероксическими

«импульсами». В экспериментальных работах показано, что в курсе процедур комбинации периодов умеренных гипоксии и гипероксии эффективность адаптации повышается за счет повышения интенсивности редокс-сигнала без углубления гипоксии, а режим тренировки «гипоксия/гипероксия» более эффективно предупреждает развитие АФК-индуцированных, стрессорных нарушений и повышает физическую выносливость животных по сравнению с режимом «гипоксия/нормоксия» [15; 16]. Причем тренирующие эффекты ИГТ при режиме «гипоксия/гипероксия» развиваются быстрее.

Нами разработан новый способ ИГТ человека, в котором для потенцирования ее эффектов используется дыхание гипоксическими газовыми смесями, чередующееся с дыханием гипероксическими (30% O_2) газовыми смесями, — метод интервальной гипо-гипероксической тренировки (ИГГТ) [17].

Цель выполненной работы — исследование возможности применения нового метода — ИГГТ — в коррекции индивидуальных компонентов метаболического синдрома.

МАТЕРИАЛ И МЕТОДЫ ИССЛЕДОВАНИЯ

В исследовании приняли участие 35 пациентов, мужчины и женщины в возрасте 19–64 лет, с метаболическим синдромом, включающем алиментарное ожирение 1–3-й ст. (ИМТ более 30 кг/м^2), нарушение толерантности к углеводам (или сахарный диабет II типа), артериальную гипертензию и дислипидемию. Пациенты получали базовую однотипную терапию с учетом сопутствующей патологии без использования фармакологических препаратов коррекции пищевого поведения и массы тела. В период прохождения курса рекомендовалась редуцированная диета (1600–1800 ккал/сутки). Все пациенты случайным порядком были разделены на три группы: контрольную (11 чел.), опытную 1 (13 чел., прошедших курс из 12 процедур ИГГТ) и опытную 2 (11 чел., прошедших курс ИГГТ параллельно с системной гипертермией и вибрационным аппаратным массажем). Продолжительность курса составила в среднем 21 день.

Процедуры ИГГТ отпускались в режиме 3–4 раза в неделю с применением модифицированной установки «Эдельвейс-А» (ЗАО «НВФ Метакс»). Для подбора оптимального режима тренировок предварительно проводился 10-минутный гипоксический тест (ГТ). Процедуры ИГГТ начинали с подачи через маску гипоксической смеси с 11% O_2 (5–7 минут), затем 2–3 минуты подавали гипероксическую газовую смесь с 30% O_2. Длительность гипоксического воздействия и последующей гипероксии зависела от индивидуальной гипоксической чувствительности пациента в ГТ, а их переключение осуществлялось автоматически по специальным алгоритмам (биообратная связь) [17]. В течение процедуры проводили 6–7 таких циклов. Во время процедур пациенты опытной группы 1 находились в положении лежа в капсуле «Альфа-Спа» («Сибаритик Инк», США) при температуре 25–26 °С, а пациенты опытной группы 2 одновременно проходили процедуры гипертермии и вибромассажа (температура в капсуле повышалась до индивидуально переносимой — 35–50 °С с последующим градуальным снижением). Пациенты контрольной группы в те же сроки прошли по 12 имитационных процедур в капсуле (без информирования их о характере отличий в параметрах воздействий).

До курса процедур ИГГТ и на 3–4-й день по их завершении все пациенты проходили комплексное обследование, включавшее:

— сбор анамнеза, оценку пищевого режима (первичное анкетирование для оценки фактического питания, пищевой дневник);

— психометрическое тестирование (опросник качества жизни SF-36, шкалы уровня ситуативных проявлений гнева (УСГ), депрессии (УСД) и тревожности (УСТ) [18], консультации психологом с целью определения типа пищевого поведения;

— антропометрические измерения (масса тела, ИМТ);

— бодиимпедансометрию (анализатор состава тела АВС-01, НТЦ «Медасс») с определением активной клеточной массы (АКМ, кг и %), мышечной массы (ММ, кг и %) и жировой массы (ЖМ, кг и %);

— биохимическое исследование крови с определением уровня общего холестерина (ОХС), ЛПВП, ЛПНП, триглицеридов (ТГ), индекс атерогенности, глюкозы плазмы натощак (ГПН) и гликозилированного гемоглобина;

— тест 6-минутной ходьбы для оценки физической работоспособности (6MWT) с измерением пройденной дистанции, значений АД и ЧСС до и после нагрузки.

Статистическая обработка полученных данных проведена с использованием программы *Statistica for Windows 6.0*. Для оценки достоверности различий средних использовали непараметрические критерии Манна — Уитни и Вилкоксона.

РЕЗУЛЬТАТЫ ИССЛЕДОВАНИЯ И ИХ ОБСУЖДЕНИЕ

Установлено, что курс ИГГТ самостоятельно или в сочетании с физиотерапевтическими воздействиями в капсуле «Альфа Спа» в целом способствует снижению массы тела у пациентов с метаболическим синдромом и нормализации их эмоционального, вегетативного и биохимического статуса. Не выявлено существенных различий в динамике регистрируемых клинических и биохимических показателей между пациентами опытных групп 1 и 2, поэтому в дальнейшем представлены данные сравнения объединенной опытной и контрольной групп.

В среднем снижение массы тела у пациентов опытной группы в курсе ИГГТ составило 2,5 – 3,5 кг прежде всего за счет снижения процента жировой массы *(табл. 1)*. При анализе показателей комплексной биоимпедансометрии установлено достоверное снижение значений ИМТ, значимое уменьшение процента жировой массы при повышении тощей массы. В контрольной группе каких-либо существенных сдвигов в компонентном составе и массе тела пациентов не произошло при тенденции к снижению ИМТ и ЖМ.

Снижение массы тела у пациентов опытной группы в курсе ИГГТ сопровождалось существенными сдвигами в липидном профиле и значимым снижением уровня глюкозы плазмы крови натощак *(табл. 2)*. После курса процедур у пациентов выявлено достоверное снижение исходно существенно повышенных значений уровня общего холестерина ($p < 0,001$), триглицеридов ($p < 0,001$), а также липопротеидов низкой плотности ($p < 0,0001$). У пациентов контрольной группы значимой динамики изучаемых параметров не отмечено.

Текущее эмоциональное состояние пациентов обеих групп исходно характеризовалось выраженным комплексом негативных проявлений: высоким уровнем ситуативной тревожности, ситуативной депрессии, то есть практически по всем рассматриваемым показателям представители этой подгруппы находятся в зоне повышенного риска *(табл. 3)*. После окончания курса процедур ИГГТ отмечено значимое снижение уровня ситуативной депрессии и тревоги. В самоотчетах пациенты отмечали значительное релаксирующее воздействие отпускаемых процедур, снижение дисфорических признаков, более «живое», приподнятое настроение к концу курса ИГГТ, что способствовало перестройке пищевого поведения с эмоциогенных и экстернальных типов на ограничительное. У пациентов контрольной группы, несмотря на «прохождение процедур» с использованием того же оборудования, значимой динамики показателей психологического статуса не отмечено.

Большинство пациентов хорошо переносили курс ИГГТ, лишь у 6 из 24 первые процедуры

Таблица 1

ДИНАМИКА ПОКАЗАТЕЛЕЙ КОМПОНЕНТНОГО СОСТАВА ТЕЛА У ПАЦИЕНТОВ ОСНОВНОЙ И КОНТРОЛЬНОЙ ГРУПП (M ± m)

Показатель	Опытная группа (1 + 2)		Контрольная группа	
	до ИГГТ	после	до ИГГТ	после
ИМТ	34,8 ± 1,4	32,3 ± 1,3*	33,3 ± 1,0	32,8 ± 1,0
ТМ, кг	60,9 ± 2,6	64,0 ± 2,8*	64,0 ± 4,1	66,2 ± 4,2
ЖМ, кг	37,7 ± 3,8	33,0 ± 3,4*	31,0 ± 2,3	28,3 ± 2,4
ЖМ, %	36,7 ± 2,3	32,7 ± 2,3*	32,9 ± 2,5	29,4 ± 2,6
АКМ, кг	43,5 ± 2,8	43,0 ± 3,2	40,7 ± 2,7	48,4 ± 5,4
АКМ, %	71,8 ± 3,7	66,3 ± 2,9	64,8 ± 5,2	72,7 ± 6,2

*Примечание: Здесь и далее представлены данные до начала и в конце курса процедур; * — достоверность различий при $p < 0,05$ и выше по отношению к исходным данным в одной группе.*

Таблица 2

ДИНАМИКА ОСНОВНЫХ ПОКАЗАТЕЛЕЙ ЛИПИДНОГО ПРОФИЛЯ И ГЛЮКОЗЫ ПЛАЗМЫ НАТОЩАК (M ± m)

Показатель, ммоль/л	Опытная группа (1 + 2)		Контрольная группа	
	до ИГГТ	после	до ИГГТ	после
ГПН	6,88 ± 0,50	5,44 ± 0,03*	6,43 ± 0,27	5,85 ± 0,19
ОХС	5,65 ± 0,21	4,74 ± 0,23* **	5,75 ± 0,23	5,74 ± 0,37
ЛПНП	3,58 ± 0,19	2,62 ± 0,17* **	3,81 ± 0,18	3,75 ± 0,37
ЛПВП	1,33 ± 0,12	1,34 ± 0,08	1,06 ± 0,08	0,97 ± 0,05
ТГ	2,04 ± 0,15	1,74 ± 0,14*	2,29 ± 0,31	2,13 ± 0,18

*Примечание: ** — значимость различий при $p < 0,05$ и выше по отношению к аналогичным данным в группе контроля.*

Таблица 3

ДИНАМИКА ПСИХОЛОГИЧЕСКИХ ПОКАЗАТЕЛЕЙ У ПАЦИЕНТОВ ОСНОВНОЙ И КОНТРОЛЬНОЙ ГРУПП (M ± m)				
Показатель	Опытная группа (1 + 2)		Контрольная группа	
	до ИГГТ	после	до ИГГТ	после
УСТ, балл	47,3 ± 1,3	43,6 ± 0,8*	46,1 ± 1,8	45,0 ± 1,3
УСГ, балл	36,9 ± 0,4	36,7 ± 0,5	36,4 ± 0,1	36,5 ± 0,2
УСД, балл	44,5 ± 1,4	40,0 ± 1,0*	43,9 ± 1,2	42,8 ± 1,1
Сит. дискомфорт	51,4 ± 1,0	49,4 ± 1,0	53,1 ± 1,1	50,8 ± 1,0

Таблица 4

ДИНАМИКА КЛИНИКО-ФИЗИОЛОГИЧЕСКИХ ПОКАЗАТЕЛЕЙ У ОБСЛЕДУЕМЫХ ОПЫТНОЙ И КОНТРОЛЬНОЙ ГРУПП (M ± m)				
Показатель	Опытная группа (1 + 2)		Контрольная группа	
	до ИГГТ	после	до ИГГТ	после
Дистанция в 6-минутном тесте, м	482,6 ± 17,2	517,7 ± 16,8* **	449,2 ± 19,7	466,10 ± 26,8
САД, мм рт. ст.	129,9 ± 2,5	126,7 ± 2,5	128,8 ± 4,4	127,3 ± 3,8
ДАД, мм рт. ст.	82,0 ± 1,8	77,8 ± 2,1*	78,3 ± 1,7	77,7 ± 2,7
ЧСС покоя, уд/мин	74,2 ± 1,5	71,5 ± 2,1	78,0 ± 2,7	75,2 ± 2,2
SaO_{2min}, % в ГТ	77,3 ± 1,9	81,6 ± 0,7*	v	v
$ЧСС_{max}$, уд/мин в ГТ	89,5 ± 1,4	86,5 ± 2,0	v	v

Примечание: v — *гипоксический тест (ГТ) у пациентов контрольной группы не проводился.*

сопровождались жалобами на чувство нехватки воздуха, затруднения вдоха, некоторым эмоциональным возбуждением. После незначительного снижения гипоксической экспозиции и соответствующих разъяснений все пациенты продолжили курс тренировок. Некоторые пациенты отмечали сомногенное влияние процедур ИГГТ, а к концу курса — улучшение переносимости физических нагрузок.

У пациентов опытной группы тренирующие эффекты ИГГТ проявлялись также в устойчивой тенденции к снижению значений диастолического артериального давления покоя, а также в повышении устойчивости к дозированной гипоксии в ГТ *(табл. 4)*. Так, при повторном проведении ГТ отмечены значимо меньшие прирост ЧСС ($ЧСС_{max}$) и степень снижения насыщения крови кислородом ($SaO2_{min}$). Как итоговый результат, увеличилась физическая выносливость пациентов — при повторном тестировании они проходили достоверно большую дистанцию как в сравнении с исходными данными, так и с результатами выполнения теста 6-минутной ходьбы пациентами контрольной группы.

Таким образом, применение метода интервальных гип-гипероксических тренировок (изолированно или в сочетании с системной гипертермией и аппаратным вибромассажем) в комплексном лечении пациентов с метаболическим синдромом сопровождается значимым уменьшением выраженности отдельных компонентов и проявлений заболевания, а также повышением устойчивости к острой дозированной гипоксии, физической работоспособности и выносливости. Применение предложенного метода в определенной степени снижает стрессовое воздействие редуцированных диет, а также повышает психологическую мотивацию пациентов сохранить результаты лечения и их вовлеченность в долгосрочные реабилитационно-профилактические программы, особенно на начальных этапах, при значительных затруднениях начать выполнение рекомендованных физических упражнений и (или) ограничить прием пищи.

При индивидуальном подборе структуры курса, дозировании гипоксических воздействий, сочетании с другими физиотерапевтическими процедурами метод имеет определенные перспективы в комплексном лечении и реабилитации пациентов с метаболическим синдромом. Особое значение такие процедуры могут иметь на амбулаторном этапе реабилитации пациентов с метаболическим синдромом, когда необходимо удержать и закрепить достигнутые результаты редукции массы тела, снижения уровня холестерина, артериального давления.

ВЫВОДЫ

1. Разработанный метод индивидуально дозированных интервальных гипо-гипероксических тренировок является эффективным в коррекции и минимизации отдельных компонентов метаболического синдрома, профилактике развития метаболических и кардиоваскулярных осложнений.

2. Применение гипо-гипероксических тренировок (изолированно или в сочетании с системной гипертермией и аппаратным вибромассажем) приводит к значимому снижению массы тела пациентов преимущественно за счет уменьшения жировой массы, что сопровождалось снижением уровня общего холестерина, глюкозы плазмы, оптимизацией артериального давления, повышением физической выносливости, улучшением психологического статуса.

3. Использование метода гипо-гипероксических тренировок в сочетании с возможностями физиотерапевтических комбайнов «Альфа Спа» позволяет получать клинически значимые результаты в коррекции проявлений метаболического синдрома и морбидного ожирения, что повышало психологическую мотивацию пациентов сохранить результаты лечения и их вовлеченность в долгосрочные реабилитационно-профилактические программы.

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ORIGINAL ARTICLE

Effects of intermittent hypoxia training on leukocyte pyruvate dehydrogenase kinase 1 (PDK-1) mRNA expression and blood insulin level in prediabetes patients

Tetiana V. Serebrovska[1] · Alla G. Portnychenko[1,2] · Vladimir I. Portnichenko[1,2] · Lei Xi[3,4] · Egor Egorov[5] · Ivanna Antoniuk-Shcheglova[6] · Svitlana Naskalova[6] · Valeriy B. Shatylo[6]

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Abstract

Purpose Intermittent hypoxia training/treatment (IHT) is an emerging therapeutic approach to alleviate chronic diseases, such as diabetes. The present study investigated the effects of IHT on blood leucocyte pyruvate dehydrogenase kinase 1 (PDK-1) mRNA expression and its relationship with the changes in blood insulin level.

Methods Seven adult healthy volunteers and 11 prediabetic patients participated in this study. A 3-week course of IHT consisted of a 40-min session of 4 cycles of 5-min 12% O_2 and 5-min room air breathing per day, 3 sessions per week for 3 weeks (i.e., total 9 sessions of IHT). Plasma insulin levels and leukocyte PDK-1 mRNA expression were determined at various time points either under fasting condition or following oral glucose tolerance test (OGTT). Correlation between the IHT-induced changes in PDK-1 mRNA and insulin or glucose levels in the same serological samples was analyzed.

Results At pre-IHT baseline, PDK-1 mRNA expression was two times higher in prediabetes than control subjects. IHT resulted in significant augmentation in PDK-1 mRNA expression (>twofold) in prediabetes at the end of 3-week IHT and remained elevated 1 month after IHT, which was correlated with a significantly reduced insulin release and lower blood glucose after glucose loading with OGTT.

Conclusion IHT can trigger beneficial effects in normalizing blood insulin levels in prediabetic patients under oral glucose load, which were closely correlated with an enhanced mRNA expression of PDK-1 in leukocytes. Further clinical trials are warranted to validate the utility of IHT as a non-invasive complementary therapy against diabetes-associated pathologies.

Keywords Hypoxia · Insulin · Diabetes · Pyruvate dehydrogenase kinase · Adaptation · Gene Expression

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✉ Tetiana V. Serebrovska
sereb@biph.kiev.ua

✉ Lei Xi
lxi@vcu.edu

1 Bogomoletz Institute of Physiology, Kiev 01024, Ukraine

2 ICAMER, National Academy of Sciences of Ukraine, Kiev 03680, Ukraine

3 Pauley Heart Center, Department of Internal Medicine, Virginia Commonwealth University, 1101 East Marshall Street, Room 7-020C, Richmond, VA 23298, USA

4 School of Sports Medicine and Health, Chengdu Sport University, Chengdu 610041, China

5 CELLGYM Technologies GmbH, 10623 Berlin, Germany

6 D.F. Chebotarev State Institute of Gerontology, Kiev 04114, Ukraine

Abbreviations

ANOVA	Analysis of variance
GLUT	Glucose transporter
HIF-1α	Hypoxia inducible factor 1α
IHT	Intermittent hypoxia training/treatment
INSR	Insulin receptor
OGTT	Oral glucose tolerance test
PDH	Pyruvate dehydrogenase
PDK	Pyruvate dehydrogenase kinase
PDK-1	Pyruvate dehydrogenase kinase 1
R	Correlation coefficient
SD	Standard Deviation
TCA	Tricarboxylic acid cycle

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Introduction

Intermittent hypoxia training/treatment (IHT), which was originated in 1930s in the former Soviet Union for the training of pilots, is now increasingly used both in sports practice and for the prevention and treatment of certain chronic diseases, such as cardiovascular diseases (Mallet et al. 2018; Serebrovskaya and Xi 2016), diabetes (Camacho-Cardenosa et al. 2018; Mackenzie et al. 2012; Morishima et al. 2015; Serebrovskaya 2002), and many others (Navarrete-Opazo and Mitchell 2014). In a recent publication, we described the positive effect of IHT on glucose homeostasis, hypoxia tolerance and some leukocyte mRNA gene expression, i.e., hypoxia inducible factor 1α (HIF-1α), insulin receptor (INSR), facilitated glucose transporter - solute carrier family-2 (SLC2), and potassium voltage-gated channel subfamily J (KCNJ8), in prediabetic patients (Serebrovska et al. 2017). However, the previous study also raised additional questions about how IHT lead to blood glucose reduction as far as 1 month after the end of IHT. Therefore, we decided to use the remaining serological samples collected from the same participants of this small clinical study for further analysis, focusing on the effects of IHT on the pyruvate dehydrogenase kinase 1 (PDK-1) mRNA expression in leukocytes and blood insulin levels in this group of prediabetes patients.

Pyruvate dehydrogenase kinase (PDK) is perhaps the best described mitochondrial metabolic gene target of hypoxia-inducible factor 1 (HIF-1), which controls metabolic flexibility and plays a crucial role in adaptation to hypoxia (Hollinshead and Tennant 2016; Kim et al. 2006). PDK plays a gatekeeper role for the tricarboxylic acid (TCA) cycle controlling quantity of pyruvate feeding into cells via inhibition of pyruvate dehydrogenase complex activity (Nguyen et al. 2016). PDK actively regulates mitochondrial function in hypoxic condition by shunting pyruvate toward lactate, thus permitting continued glycolysis (Huang et al. 2002; Kim et al. 2006; Minchenko et al. 2004; Papandreou et al. 2006). Four known PDK isoforms (PDK-1 to PDK-4) differ in their catalytic activity and responsiveness to the modulators such as NADH and acetyl-CoA, as well as tissue-specific expression (Ferriero et al. 2015). In mammalian species, these isoenzymes of PDK have different binding affinity, phosphorylation site specificity and tissue distribution. Only PDK-1 is capable to phosphorylate all phosphorylation sites, while other isoenzymes can only phosphorylate one site with different rates (Zhou et al. 2016). PDK-1 is present preferentially in the pancreatic islets, heart and skeletal muscles.

PDK-1 is also considered as a potent suppressor of pyruvate dehydrogenase (PDH), especially when blood glucose levels are low and pyruvate can be conserved for gluconeogenesis. Under diabetic conditions, an elevation of PDK gene expression has been implicated in the increased gluconeogenesis in the liver and the decreased glucose utilization in the peripheral tissues (Lee 2014; Peters et al. 2001). Therefore, suppression of PDKs expression (mainly PDK-2 and PDK-4) was considered as a potential mechanism for alleviating the diabetic states (Ferriero et al. 2015; Khan et al. 2017; Kim et al. 2006; Kulkarni et al. 2012).

On the other hand, the transcription of genes involved in glycolysis and its regulation is influenced by insulin. Hypoxia potentiates the glycolytic effect of insulin shifting the balance of the high energy phosphates towards AMP. In addition, this process further limits gluconeogenesis, since the synthesis of glucose is ATP-dependent (Minchenko et al. 2004). It was proved that intermittent hypoxia increases blood insulin levels via inhibition of the islet destruction and promotion of new beta-cell formation in acinar tissue (Kolesnyk et al. 1994, 2013). There is a close relationship between insulin and PDK. In obese and type 2 diabetic animals, both fat and glucose regulate PDK gene and protein expression in islet cells (Xu et al. 2006). Hyperglycemia and hyperlipidemia may contribute to the decline in diabetic islet PDH activity by increasing mRNA and protein expression of PDK. Therefore, the current notion is that, to optimize glucose-stimulated insulin secretion, a low PDK-1 activity has to be maintained to keep PDH in a dephosphorylated and active state (Krus et al. 2010). Interestingly, a more recent study showed that 4-week sustained hypoxia led to decreased blood glucose and increased insulin levels, which were associated with a significantly elevated PDK-2 mRNA expression in mouse liver (Nam et al. 2016).

It was shown in human investigation that acute intermittent exposure to hypoxia decreased insulin sensitivity in healthy adult humans (Peltonen et al. 2012). Other investigators who have used the models simulating obstructive sleep apnea (i.e., very short hypoxic periods under very low oxygen) showed that such mode of intermittent hypoxia leads to impairments in glucose metabolism and causes reductions in insulin sensitivity (Carreras et al. 2012). To the contrary, whereas acute exposure to continuous or short repetitive severe intermittent hypoxia could cause metabolic dysfunction, chronic exposure to moderate intermittent hypoxia may be associated with normalization, or even an enhancement of whole body metabolic function (Lee et al. 2013). To shed light on this intricate question, the present study focused at investigating whether the previously reported beneficial regime of IHT influences HIF-1α–dependent PDK-1 mRNA expression and analyzing possible correlation between PDK-1 expression levels and blood glucose and insulin in prediabetes patients (Serebrovska et al. 2017).

Methods

Characteristics of subjects

Seven healthy volunteers (44–68 years) and 11 prediabetic patients (48–70 years) participated in the current study. The subjects in the healthy control group had no cardiovascular, respiratory, endocrine or central nervous system disorders and their fasting glucose concentration was less than 5.6 mmol/L, and less than 7.8 mmol/L 2 h after a standard glucose tolerance test. Selection of subjects in the prediabetes group was carried out in accordance with recommendations of the expert committee on the diagnosis and classification of diabetes mellitus (Genuth et al. 2003), which include: (1) Impaired fasting blood glucose level (from 5.6 to 6.9 mmol/L); (2) Impaired glucose tolerance, i.e., blood glucose level after 2 h of standard glucose tolerance test (oral intake of 75 g glucose) was from 7.8 to 11.1 mmol/L; and (3) Combined impairments with both elevated fasting blood glucose level and glucose intolerance defined above.

The research protocols, patient health information and informed consent forms were approved by the Ethics Committee of Chebotarev Institute of Gerontology, Kiev, Ukraine. This human study was performed in accordance with the ethical standards according to the 1964 Declaration of Helsinki. All subjects underwent measurements of several anthropometric variables (Table 1), which indicated no significant difference in age and height between *Healthy* and *Prediabetes* groups, whereas the body weight, body mass index, and waist circumference were higher in the prediabetes patients than those of healthy control subjects.

Experiment protocols

All sessions of the present study were conducted in a quiet room at comfortable temperature within a clinical research center of the Chebotarev Institute of Gerontology. Measurement sessions were performed during 2 days before IHT course, 2 days and 1 month after the termination of IHT. For determination of PDK-1 mRNA expression in blood leukocytes, venous blood samples were collected again next day after 1-week IHT course. Patient examination included: (1) anthropometric measurements; (2) determination of PDK-1 mRNA; (3) standard oral glucose tolerance test (OGTT) with plasma insulin determination.

In the morning of the first experiment day, after 3-day routine hospital diet (250–300 g carbohydrates) and normal physical activity, a venous blood sample was drawn under fasting condition from the median antecubital vein for measurement of fasting insulin level as well as genetic analysis. Thereafter, a standard OGTT was conducted according to Ryden et al. (Ryden et al. 2007), which used 75 g glucose mixed in 250 mL water. Venous blood samples were drawn at 120 min after the oral glucose ingestion (2 h post-OGTT). Plasma insulin levels were measured by immunoenzyme method using DRG Insulin ELISA kit (DRG Instruments GmbH, Germany). We also reused the individual blood glucose data from the same subjects reported in our previous article (Serebrovska et al. 2017) for further analyzing if there is any correlation between the changes in blood glucose and leukocyte PDK-1 mRNA expression.

From the next morning, after a light breakfast, all participated subjects received total of nine sessions of IHT (i.e., three times a week for the subsequent 3 weeks). Each session consisted of four cycles of 5-min hypoxia (12% inspired O_2) followed by 5-min normoxia (room air breathing). The normobaric hypoxia was administered to the subjects in sitting position, using a hypoxic apparatus—Hypotron® (Kiev Polytechnic Institute, National Technical University of Ukraine). Next day and 1 month after the end of 3-week IHT, the post-IHT examinations were conducted in the same manner as the pre-IHT ones.

In addition, blood lactate level was determined at two time-points (i.e., Pre-IHT baseline and after the 3-week sessions of IHT) using a SUPER GL lactate analyzer (Dr. Müller Gerätebau GmbH, Freital, Germany).

Measurement of gene expression

mRNA expression of PDK-1 was determined in circulating blood leukocytes collected in various time points using

Table 1 Anthropometric characteristics of the participants

Groups	Gender (female/male)	Age (year)	Height (cm)	Weight (kg)	BMI (kg/m^2)	Waist (cm)
Healthy	5/2	58.7 ± 11.8	170 ± 15	76.1 ± 17.3	27.2 ± 6.4	93.7 ± 9.2
Prediabete	7/4	66.4 ± 5.2	167 ± 10	90.2 ± 9.9	33.2 ± 5.6	99.7 ± 8.9
Healthy versus prediabetes		NS	NS	$p < 0.05$	$p < 0.05$	$p = 0.05$

Data are Mean ± Standard Deviation (SD); Student *t* test was used to determine the statistical significance of the differences between *Healthy* and *Prediabetes* groups

BMI body mass index, *Waist* waist measurements, *NS* no significant difference

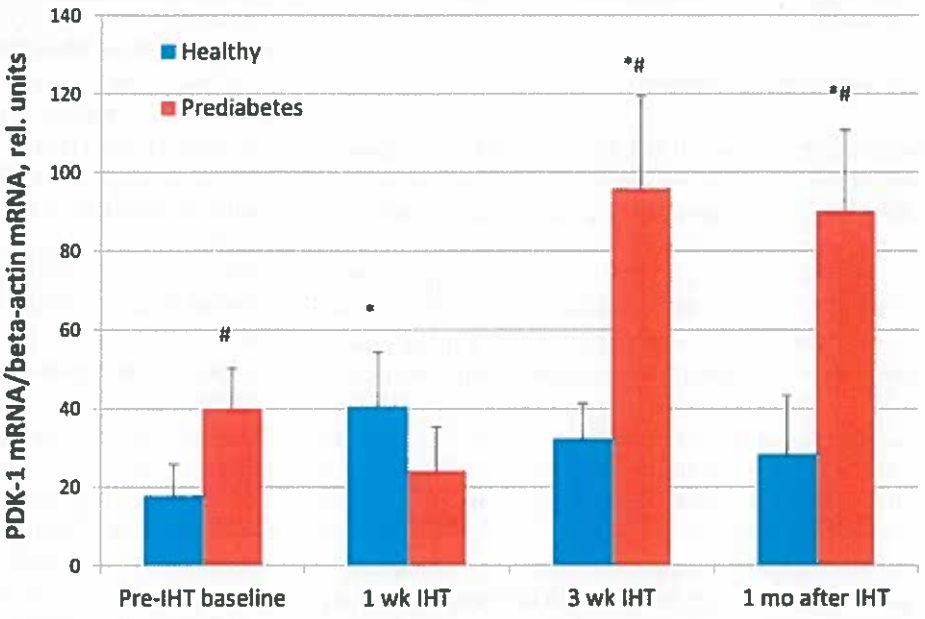

Fig. 1 Time-dependent effects of intermittent hypoxia training (IHT) on pyruvate dehydrogenase kinase 1 (PDK-1) mRNA expression in blood leukocytes. Data are Mean ± SD and were analyzed with two-way ANOVA with repeated measures. Symbols indicate: *$p < 0.05$ versus Pre-IHT baseline; #$p < 0.05$ versus *Prediabetes* group. Group Main Effect (*Healthy* versus *Prediabetes*): $F = 7.185$; $p = 0.017$. Time Effect (3 time-points): $F = 5.837$; $p = 0.029$. Group + Time Effect: $F = 3.822$; $p = 0.068$

real-time polymerase chain reaction (RT-PCR) assay. Blood leukocytes were obtained by centrifuging the blood samples at 1500*g* for 1.5 min. After centrifugation, supernatant with interphase fraction was collected and transferred in new tube. After a secondary centrifugation (3000*g* for 3 min) the supernatant was removed, the precipitate was used for RNA isolation using phenol–chloroform extraction after homogenization with guanidine isothiocyanate (Trizol RNA Prep 100 Kit, Russian Federation). Total RNA concentration was determined with a spectrophotometer (Model ND1000, NanoDrop Technologies Inc., USA). cDNA was synthesized from 5 µg of total RNA by reverse transcription with 10 mM Tris–HCl (pH 9.0), 5 mM $MgCl_2$; 1 mM dNTPs; 20 U RiboLock, Random hexamer primers (0.5 µg $µl^{-1}$) and 200 U RevertAid H Minus M-MuLV Reverse Transcriptase. PCR was performed using an Applied Biosystems 2700 (PerkinElmer, USA).

Gene expression of PDK-1 (Hs00176853_m1) was determined using TaqMan® Gene Expression Assay (Applied Biosystems, USA). The pairs of forward and reverse primers for PDK-1 and the TaqMan® probes for the target mRNA were designed by Applied Biosystems based on the human mRNA sequence. Gene expression in each probe was normalized with β-actin, using a TaqMan® human β-actin control reagent. The thermal cycles of PCR amplification consisted of initial denaturation step at 95 °C for 20 s, followed by treatment at 95 °C for 3 s, and at 60 °C for 30 s and for 50 cycles using 7500 Fast Real-time PCR equipment (Applied Biosystems, USA). The cycle threshold is defined as the number of cycles required for the fluorescence signal to exceed the detection threshold. The expression level of target gene was calculated relative to the housekeeping gene (β-actin) as the difference between the threshold values of the two genes. Each PCR step was performed in duplicate and the calculations were done using the 7500 Fast System SDS software (Applied Biosystems, USA).

Statistical analyses

All data were analyzed using SPSS software version 21.0 (SPSS Inc., USA). Data are expressed as Mean ± Standard Deviation (SD). Statistical significance of the differences between the means of the variables at different time points of IHT was calculated by two-way analysis of variance (ANOVA) with repeated measures. Student *t* test was used for comparing anthropometric characteristics of the participants between *Healthy* and *Prediabetes* groups. Pearson product-moment correlation coefficient (*R*) was calculated to show the degree of linear relationship between blood insulin or glucose and PDK-1 mRNA expression in the fasting and 2 h post-OGTT conditions. The level of statistical significance was set at $p < 0.05$.

Results

In general, all subjects well tolerated the entire process of medical examination and IHT sessions. No subjective discomforts and/or any other adverse effects were reported.

PDK-1 mRNA expression in leukocytes

Initial level of PDK-1 mRNA expression was in two times higher in *Prediabetes* group comparable to *Healthy*

subjects (Fig. 1, $p<0.05$). During the first week of training it increased in two times in *Healthy* group with a gradual return to the baseline by the end of the training period. In *Prediabetes* group, this increase occurred with a delay until the end of IHT, where PDK-1 mRNA expression increased > twofolds and remained at an elevated level (145%) 1 month after the end of training.

Blood insulin and lactate levels

Figure 2 demonstrates the effects of IHT on blood insulin level in *Healthy* subjects and *Prediabetes* patients. At pre-IHT baseline, fasting insulin in prediabetes patients did not differ significantly from healthy subjects, but 2 h post-OGTT showed triple excess in patients in comparison with healthy volunteers ($p<0.01$). One day after IHT termination fasting insulin increased by 86% in Healthy group ($p<0.05$). In Prediabetes patients only the tendency to increase was registered because of the large interindividual dispersion.

One month after IHT the fasting insulin remained at around 66% higher level in *Healthy* group, whereas it returned to the initial level in *Prediabetes* group. Meanwhile, 2 h post-OGTT insulin returned to the baseline in both groups, while the difference in the insulin level under glucose load between the groups remained 2.6 times higher in *Prediabetes* patients ($p<0.05$). Two-way ANOVA test has shown the statistical difference for fasting insulin in time effect only ($p<0.01$) and for 2 h post-OGTT insulin level in group main effect ($p<0.05$), wherein in the group + time effect the difference was close to statistical significance ($p=0.07$).

Figure 3 provides the evidence that blood lactate concentration was not significantly different between *Healthy* and *Prediabetes* groups either at the pre-IHT baseline or immediately after the end of 3-week IHT, indicating the long-term 9 sessions of IHT (40 min each session) did not activate the cellular signaling cascades leading to enhanced production of lactate via anaerobic glycolysis.

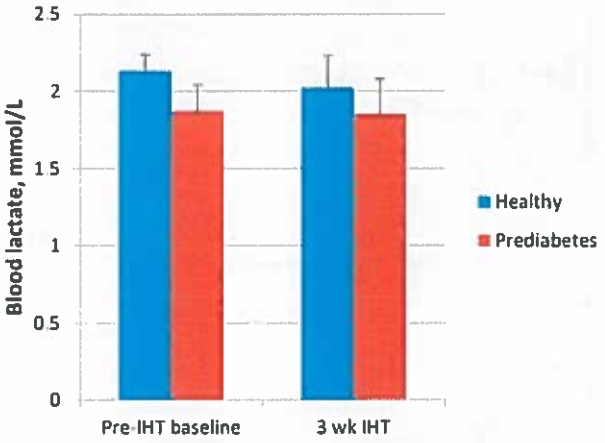

Fig. 3 Blood lactate levels before and after 3-week sessions of intermittent hypoxia training (IHT). Data are Mean ± SD. Student *t* test indicates no significant difference in blood lactate between the end of 3-week IHT versus Pre-IHT baseline as well as between *Healthy* and *Prediabetes* groups at either of these time-points

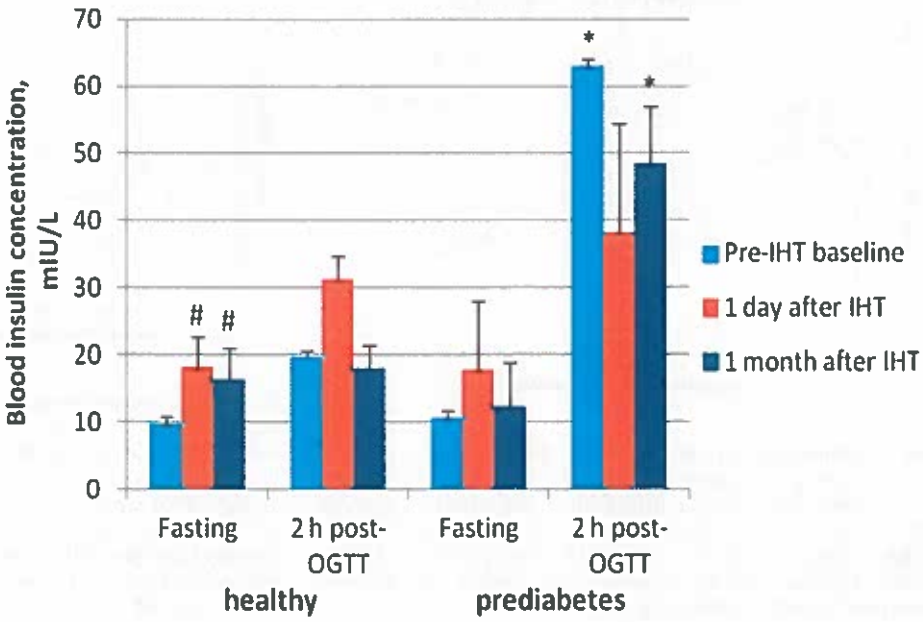

Fig. 2 Blood insulin levels following oral glucose tolerance test (OGTT) at pre-IHT time-point and right after the 3-week sessions of IHT. Data are Mean ± SD and were analyzed with two-way ANOVA with repeated measures. Symbols indicate: *$p<0.05$ versus Pre-IHT baseline; #$p<0.05$ versus *Healthy* group

Correlation analysis

Investigation of the links between PDK-1 and other indices showed that the 1 day after IHT, mRNA expression of PDK-1 (the point of the greatest increase in *Prediabetes* group) is positively associated with fasting insulin in this point for all participants (*Prediabetes* group $r=0.55$, $p<0.01$; *Healthy group* $r=0.53$; $p=0.05$; Fig. 4a) and negatively—with the level of 2 h post-OGTT glucose (*Prediabetes* group $r=-0.47$, $p<0.05$; *Healthy group* $r=-0.46$; $p=$NS; Fig. 4b). It means that the subjects with higher PDK-1 expression after hypoxic training had higher level of fasting insulin and lower level of blood glucose after glucose load. This fact is confirmed by the data in Fig. 4c, i.e., the higher the level of PDK-1 to the end of training course, the more the level of 2 h post-OGTT glucose decreases in comparison with the basal point (*Prediabetes* group $r=-0.55$; $p<0.01$; *Healthy group* $r=-0.59$, $p<0.05$).

Figure 5 shows the relationships between changes in PDK-1 mRNA expression in human leukocytes 1 month after the termination of 3-week IHT and blood insulin levels under glucose load at 2-h post-OGTT time-point. We observed that in the prediabetic patients, the increased expression in PDK-1 mRNA was positively correlated with more glucose-stimulated insulin secretion at baseline point (Fig. 5a, $r=0.66$, $p<0.01$). Conversely, at 1 month after

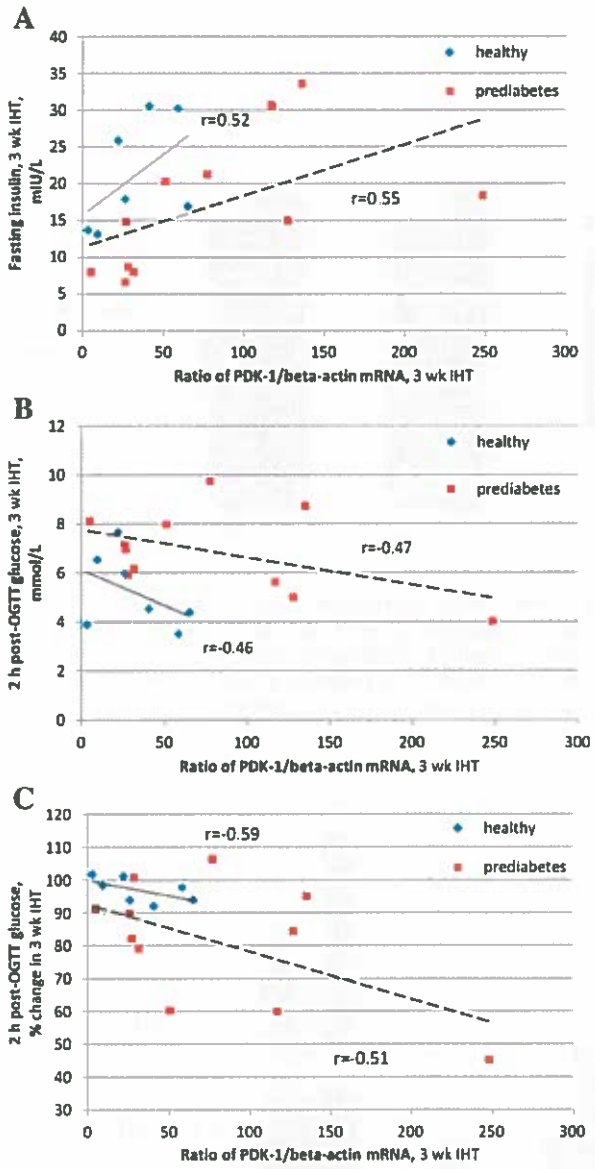

Fig. 4 Relationships between pyruvate dehydrogenase kinase 1 (PDK-1) mRNA expression in human leukocytes after 3-week sessions of intermittent hypoxia training (IHT) and other parameters. a PDK-1 mRNA expression and fasting blood insulin levels. b PDK-1 mRNA expression and 2 h post-OGTT glucose levels. c PDK-1 mRNA expression and % changes in 2 h post-OGTT glucose levels between the pre-IHT baseline and after 3-week IHT

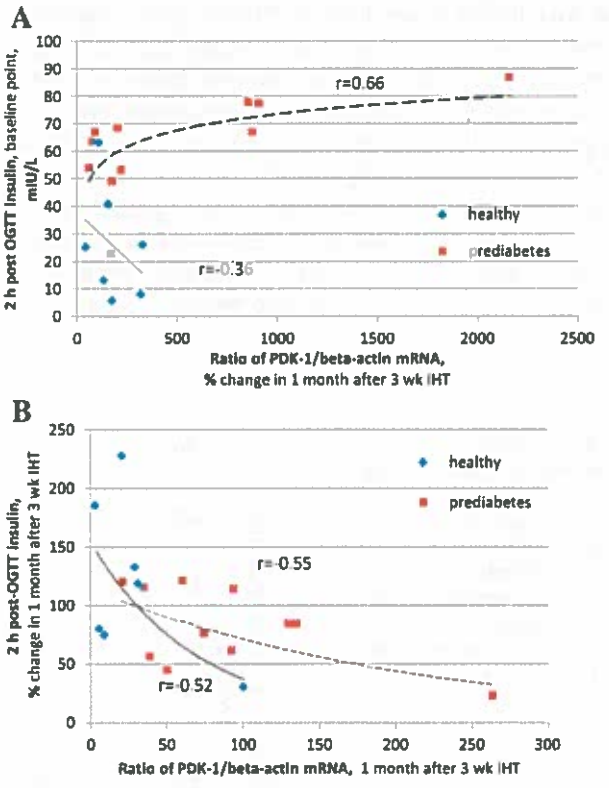

Fig. 5 Relationships between changes in pyruvate dehydrogenase kinase 1 (PDK-1) mRNA expression in human leukocytes 1 month after IHT termination and blood insulin levels at baseline and under glucose load. a Correlation between the % changes in leukocyte PDK-1 mRNA expression 1 month after 3-week IHT and blood insulin levels at baseline time-point and 2 h post-OGTT. b Correlation between leukocytes PDK-1 mRNA expression 1 month after 3-week IHT and % changes of 2 h post-OGTT blood insulin levels at 1 month after 3-week IHT

IHT time-point, the prediabetes patients with higher PDK-1 mRNA expression had lower insulin release in response to glucose load (Fig. 5b, $r = -0.55$, $p < 0.05$), suggesting PDK-1 inhibits insulin secretion in beta cells.

Discussion

The present study is an expanded analysis for a new target in the same serological samples obtained from the same groups of subjects included in our recent publication (Serebrovska et al. 2017), in which we observed that 3-week IHT reduced fasting and 2-h post-OGTT blood glucose in prediabetes patients, significantly increased their tolerance to acute hypoxia, and upregulated HIF-1α mRNA expression as well as several HIF-1α-regulated genes in blood leukocytes. The most salient finding of the current study is the identification of the involvement of PDK-1, which plays an important role in regulation of post-OGTT blood insulin and glucose levels, the good indicators of glucose tolerance.

The beneficial effects of IHT observed in our present study in prediabetes appear to be conceptually in agreement with other recently published works with various modes of IHT in normal sedentary subjects (Morishima et al. 2015) or elderly cardiac patients with comorbidities (Dudnik et al. 2018). Concerning the possible mechanistic explanations of the benefits of IHT, it was previously reported that acute severe hypoxia, as well as hyperglycemia, induced HIF-1α and PDK-1 protein in pancreas cells of experimental animals and modified glucose metabolism (Nguyen et al. 2016). Another mouse study also showed that 4-week continuous hypoxia resulted in decreased blood glucose level and increased insulin levels, along with a significantly elevated PDK-2 mRNA expression in liver (Nam et al. 2016).

It is noteworthy that our results on the IHT-induced increase in PDK-1 mRNA expression and alleviation of hyperglycemia along with improved insulin sensitivity in prediabetic patients appear to be contradictory with several studies suggesting increased PDK activity would lead to a deterioration in metabolic disorders. These authors suggested that PDK family members phosphorylate PDH complex preventing the incorporation of pyruvate into oxidative phosphorylation process, and in turn leading to elevated anaerobic glycolysis and decreased cellular respiration (Kim et al. 2006; Sutendra and Michelakis 2013; Park et al. 2018; Wu et al. 2018). Inactivation of PDH results in pyruvate conversion to lactate. This glycolytic metabolic shift has been outlined in diverse pathological conditions, including diabetes. It was assumed that the inhibition of PDKs could be a beneficial approach in treating metabolic diseases (Jeoung 2015; Wu et al. 2018). Inhibition of PDKs augmented usage of the glycolysis-produced pyruvate in the mitochondria and in turn increased oxidative phosphorylation (Khan et al. 2017).

To the contrary, a favorable role of upregulated PDK under hyperglycemic conditions was also suggested (Sugden et al. 2001), in which PDK-1 expression may be important for the intensified utilization of glucose and lipids by pancreatic islets. PDK activity may be important under hypoxic conditions, when the energy metabolism mainly shifts to the use of lipid substrates (Portnichenko et al. 2012b). The importance of PDK-1 functionality was also supported by the fact that PDK-1 inhibitors could have negative effects on hemopoiesis and skeletal muscle function (Halvarsson et al. 2017; Nguyen et al. 2016). Nevertheless, the function of PDK-1 isoform has been much less understood either in the development of diabetes, or in adaptation to hypoxia. Previous studies mostly focused on PDK-2 and PDK-4, which were elevated in patients with type 2 diabetes and the exact molecular mechanisms remain unclear (Kim et al. 2006; Kulkarni et al. 2012). Our present investigation indicated that mRNA expression of PDK-1 in patients with prediabetes was > twofold higher than healthy controls (Fig. 1). Following IHT, PDK-1 gene expression in healthy subjects increased at the 3rd session of hypoxic exposure and subsequently returned to the baseline, which is paralleled with the change pattern of HIF-1α described in our recent publication (Serebrovska et al. 2017). On the other hand, PDK-1 in prediabetic patients had a delayed and more persistent elevation up to 1-month post-IHT. Again, this change in PDK-1 was in accordance with the previously described the pattern of changes in other HIF-1α target genes, namely insulin receptor (INSR) and potassium voltage-gated channel subfamily J (KCNJ8) (Serebrovska et al. 2017). Other studies in animals reported that intermittent hypoxia coincidentally increased protein levels of PDK-1 and HIF-1 in skeletal muscle (Nguyen et al. 2016). In addition, Costalat et al. recently showed that single IHT session altered the intensity of glycolysis, increased blood lactate and decreased glucose level in health men (Costalat et al. 2018). The post-IHT hypoglycemic effect (Serebrovska et al. 2017) might be attributable to increased glycolytic processes, the so-called Pasteur effect (Sakata et al. 2000). However, we found no changes in blood lactate levels in the present study (Fig. 3), which refutes such an explanation. Nevertheless, a short-term activated glycolysis in various tissues during the brief hypoxic episodes of IHT may not be completely ruled out, but this process cannot be extended to the subsequent steady-state normoxic condition when we measured the blood lactate samples under air breathing at the next day after 3-week course of IHT.

Regarding the changes in blood insulin under hypoxic conditions, there is a wide range of contradictory opinions. An increased synthesis of insulin in the pancreas at moderate high altitudes has been considered as a possible mechanism for the development of hypoglycemia in

non-adapted organisms (Essop 2007; Roberts et al. 1996). During long-term adaptation to hypoxia, an enhanced glucose metabolism is likely provided through the induction of glucose transporter 1 (GLUT-1) and stress-reactive regulation (Portnichenko et al. 2012a). Most recent report showed that long-term hypoxic exposures of similar magnitude and duration, but consisting of different patterns (i.e., sustained hypoxia versus intermittent hypoxia) elicited discrepant effects on visceral white adipose tissues insulin sensitivity in mice, which may reflect different trajectories of HIF-1a transcriptional activity (Gozal et al. 2017). On the other hand, in response to short-term hypoxia, a decrease in plasma glucose response for glucose loading in healthy people was reported, likely due to a shift in the hormonal milieu that increased glucose utilization, not insulin per se (Hao et al. 2015; Kelly et al. 2010). Mackenzie et al. investigated the effect of single 60-min hypoxic exposure (~ 14.7% O_2) in combination with exercise in the patients with type 2 diabetes and showed improvement in fasting insulin resistance index at 24- and 48-h time points after the hypoxic exposure. In addition, Tian et al. investigated type-2 diabetic rats underwent the adaptation to simulated altitude of 5000 m (6 h per day for 28 days) (Tian et al. 2016) and they observed significant anti-diabetes effects by this type of IHT through ameliorating insulin resistance via hepatic HIF-insulin signaling pathway. Another study compared the effects of different duration of IHT (2 weeks versus 4 weeks) on glucose metabolism (Morishima et al. 2015) and the authors found the area under the curve for serum insulin concentrations after glucose ingestion significantly decreased after 4-week IHT suggesting a longer period of IHT afforded greater improvement in insulin sensitivity.

However, prolonged exposures to severe intermittent hypoxia (from 12.5 to 5% O_2, 8 h/day for 12 weeks) reduced the insulin/proinsulin ratio in the pancreatic tissue, and caused pancreatic tissue lesions and cells apoptosis in a hypoxia dose-dependent manner (Wang et al. 2017). In healthy human volunteers, treatment with 5-h intermittent hypoxia simulating obstructive sleep apnea decreased insulin sensitivity and glucose effectiveness (Louis and Punjabi 2009). However, another human study with 3-h exposure to severe intermittent hypoxia, simulating sleep apnea (25-s exposures to 5% O_2 followed by 2-min normoxia) failed to find any change in insulin sensitivity using OGTT (Newhouse et al. 2017).

It is also noteworthy that hypoxia-enhanced glucose transport via cellular pathways independent of insulin often produces a false impression of insulin resistance (Mackenzie and Watt 2016), since hypoxia may decrease insulin signaling but may not induce whole body insulin resistance. Our present study did not observe significant changes in fasting blood insulin following 3-week IHT, but insulin release in response to glucose loading significantly decreased (Fig. 2). Moreover, the subjects with higher level of PDK-1 expression after IHT had lower post-OGTT blood glucose concentration (Fig. 4c). These data suggested a possible role of PDK-1 as a negative regulator in regulating glucose-stimulated insulin secretion by pancreatic beta cells in patients with prediabetes. The prolonged expression of PDK-1 may gradually achieve normalization of insulin secretion in response to glucose load. Conversely, induction of PDK-1 in healthy individuals was not maintained 1 month after the termination of IHT, since they had no defective glucose-stimulated insulin secretion. Thus, the hypoxic induction of PDK-1 and possibly other PDK isoform(s) may play an important role in regulating not only the cellular processes of glucose utilization, but also the insulin-dependent and insulin-independent glucose transport into cells.

Based on these initial and limited pieces of evidence, we postulate that in patients with prediabetes, there are disturbed insulin-dependent regulation of glucose and lipid metabolism and substrate deficiency for synthesis of high-energy phosphates. Such disorders would modify the cellular metabolism with the possible transition to glycolysis or the preferential use of lipid substrates. Assuming the induction of PDK-1 by IHT is found most pronounced in muscle tissues that ensure the possible emergency transition to the glycolytic pathway of ATP synthesis, if severe hypoxia has developed in the tissues. As we previously reported, even under moderate hypoxia, a switch to more efficient use of lipid substrates for cells may take place (Portnichenko et al. 2012b). The initial metabolic change may include enhanced glucose utilization via induction of insulin-dependent glucose transporter 4 (GLUT-4) and insulin-like growth factor 1 (IGF-1), whereas during prolonged exposure to hypoxia, insulin-independent glucose transporter 1 (GLUT-1) may also be upregulated (Portnichenko et al. 2009), which regulates energy metabolism even under the conditions of impaired insulin sensitivity and/or insulin synthesis. Reduction of glucose-stimulated insulin response observed after IHT may be mediated by PDK-1 induction in pancreatic cells and its negative regulatory action on insulin secretion under hyperglycemia.

Conclusion

Our present study provided novel evidence showing 3-week IHT can trigger beneficial effects in lowering post-OGTT blood insulin level in prediabetes patients, indicating an improved insulin sensitivity. In their leucocytes, IHT-induced activation of HIF-1 led to further increase in PDK-1, a HIF-1 target gene that may be responsible for glucose metabolism with a long-lasting manner after the termination of IHT. The increased PDK-1 following IHT was insufficient for marked glycolysis activation and significant lactate

production and accumulation. Further studies are needed to validate the current findings obtained from the small number of patients in a single center and it is also warranted to determine the most effective mode and dose of hypoxia for an ultimate use of IHT as a non-invasive, complementary preventive and/or therapeutic approach against diabetes.

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Author contributions All authors participated in the design and interpretation of the studies, data analysis, review, and final approval of the manuscript. TVS designed the study and wrote the manuscript. TVS, AGP, VIP, EE, IAS, SN, and VBS elaborated the study protocols and performed statistical analyses of the results. LX critically edited and final-assembled the manuscript. VBS provided the enrollment and clinical examination of the subjects as well as general research management.

Compliance with ethical standards

Conflict of interest LX is a co-founder of Xiamen Innovo Medical Technology Co. Ltd., Xiamen, China and EE is an owner of CellGym Technologies GmbH, Berlin, Germany. All other authors declare no potential conflicts of interest with respect to the research, authorship, and publication of this article.

Ethical approval The research protocols, patient health information and informed consent forms were approved by the Ethics Committee of Chebotarev Institute of Gerontology, Kiev, Ukraine. This human study was performed in accordance with the ethical standards according to the 1964 Declaration of Helsinki.

Informed consent Informed consent was obtained from all individual participants included in this study.

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Humane kontrollierte Studie

Original Research

Intermittent hypoxia training in prediabetes patients: Beneficial effects on glucose homeostasis, hypoxia tolerance and gene expression

Tetiana V Serebrovska[1], Alla G Portnychenko[1,2], Tetiana I Drevytska[1,2], Vladimir I Portnichenko[1,2], Lei Xi[3,4], Egor Egorov[5], Anna V Gavalko[6], Svitlana Naskalova[6], Valentina Chizhova[6] and Valeriy B Shatylo[6]

[1]Bogomoletz Institute of Physiology, Kiev 01024, Ukraine; [2]ICAMER, National Academy of Sciences of Ukraine, Kiev 03680, Ukraine; [3]Pauley Heart Center, Department of Internal Medicine, Virginia Commonwealth University, Richmond, VA 23298, USA; [4]School of Sports Medicine and Health, Chengdu Sport University, Chengdu 610041, China; [5]CELLGYM Technologies GmbH, Berlin 10623, Germany; [6]D.F. Chebotarev State Institute of Gerontology, Kiev 04114, Ukraine

Corresponding authors: Lei Xi. Email: lxi@vcu.edu; Tetiana V Serebrovska. Email: sereb@biph.kiev.ua

Impact statement

The present study investigated the beneficial effects of intermittent hypoxia training (IHT) in humans under prediabetic conditions. We found that three-week moderate IHT induced higher HIF-1α mRNA expressions as well as its target genes, which were positively correlated with higher tolerance to acute hypoxia and better glucose homeostasis in both middle-aged healthy and prediabetic subjects. This small clinical trial has provided new data suggesting a potential utility of IHT for management of prediabetes patients.

Abstract

The present study aimed at examining beneficial effects of intermittent hypoxia training (IHT) under prediabetic conditions. We investigate the effects of three-week IHT on blood glucose level, tolerance to acute hypoxia, and leukocyte mRNA expression of hypoxia inducible factor 1α (HIF-1α) and its target genes, i.e. insulin receptor, facilitated glucose transporter–solute carrier family-2, and potassium voltage-gated channel subfamily J. Seven healthy and 11 prediabetic men and women (44–70 years of age) were examined before, next day and one month after three-week IHT (3 sessions per week, each session consisting 4 cycles of 5-min 12% O_2 and 5-min room air breathing). We found that IHT afforded beneficial effects on glucose homeostasis in patients with prediabetes reducing fasting glucose and during standard oral glucose tolerance test. The most pronounced positive effects were observed at one month after IHT termination. IHT also significantly increased the tolerance to acute hypoxia (i.e. SaO_2 level at 20th min of breathing with 12% O_2) and improved functional parameters of respiratory and cardiovascular systems. IHT stimulated HIF-1α mRNA expression in blood leukocytes in healthy and prediabetic subjects, but in prediabetes patients the maximum increase was lagged. The greatest changes in mRNA expression of HIF-1α target genes occurred a month after IHT and coincided with the largest decrease in blood glucose levels. The higher expression of HIF-1α was positively associated with higher tolerance to hypoxia and better glucose homeostasis. In conclusion, our results suggest that IHT may be useful for preventing the development of type 2 diabetes.

Keywords: Intermittent hypoxia, diabetes, hypoxia inducible factor-1, hypoxia inducible factor-1-regulated genes, adaptation, hyperglycemia

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Introduction

The method of intermittent hypoxia training (IHT) is an emerging therapeutic modality for treatment and prevention of various human diseases and has gained increasing attention. The mechanisms underlying the beneficial effects of IHT have been investigated at the multiple biological levels, from systemic physiological reactions to genomic regulation.[1–5] The potential therapeutic uses of IHT in treating cerebrovascular and cardiovascular disorders have been the focal areas of extensive research.[6,7]

Despite these advances, the effects of IHT on diabetes mellitus, especially type 2 diabetes, one of the most prevalent pathological conditions in the current world population, are much less investigated.[8] In the mid-1990s, Ukrainian scientists first demonstrated in diabetic animals that IHT could reduce vascular risk factors and increases blood insulin levels via inhibition of the islet destruction

and promotion of new beta-cell formation in acinar tissue.[9] These authors recently confirmed that two-week IHT led to an increase in the area of pancreatic islets and the number of β-cells in diabetic rats, mainly due to a significant reduction of β-endocrine cells apoptosis. The positive effect was maintained for at least 10 days.[10] These findings in preclinical animal studies suggested possible utilization of IHT in control or treatment of type 2 diabetes and its associated insulin resistance. It is notable that the favorable effects of IHT on glucose metabolism were also suggested.[11,12] In particular, it was shown that hypoxic training increased glycolytic enzyme activities, enhanced the number of mitochondria in skeletal muscles, and improved insulin sensitivity as well.[13,14]

Glucose-lowering effects of IHT were also previously reported in diabetic patients[15,16] and the beneficial effect is particularly important in elderly population with higher risks in developing diabetes.

Moderate levels of intermittent hypoxia mobilize genome that in turn activates a cascade of intracellular signaling transduction, which involves various receptors, mitochondrial respiratory chain, key intracellular regulatory systems, early genes, superfamilies of the inducible and activation transcription factors, which are sequentially engaged in the processes of initiation and induction of hypoxic tolerance. One of the key regulators of oxygen homeostasis under hypoxic conditions is hypoxia inducible factor (HIF), which initiates transcriptional activation of numerous target genes to improve oxygen delivery and utilization[17] as well as glucose homeostasis.[12,18,19] Delicate balance exists between HIF-1 level and optimal metabolic functions.[20,21] Malfunction of these relations leads to hyperglycemia and type 2 diabetes. Based on these results, we have suggested that the use of IHT for treatment of patients with prediabetic abnormalities can improve carbohydrate metabolism and lead to prevention of diabetes development.

Among the HIF-1α target genes, energy-independent facilitative glucose transporter-1 (GLUT-1; encoded by solute carrier family-2 gene SLC2A1) is one of most important for regulating glucose metabolism which predominates in many types of human cells[22] and is the only vehicle that transports glucose into the brain.[23] GLUT-1 mediates glucose uptake increasing intracellular glucose levels to be used by glycolysis and other metabolic pathways.[24,25] GLUT-1 is upregulated under hypoxia, and its activity depends of the severity of hypoxic impact.[26,27]

Another HIF-1α target gene important for glucose homeostasis is insulin receptor (INSR). Insulin exerts its physiological effects through this member of tyrosine kinase family of transmembrane signaling proteins encoded by a single gene INSR.[28] Increase of INSR level could alleviate insulin resistance. Recent studies indicated that INSR is expressed at higher levels under hypoxic stress[29] and overexpression of INSR improves obese and diabetic phenotypes in mice.[30]

ATP-sensitive potassium (K_{ATP}) channels are also involved in the regulation of insulin secretion in the β-cells of pancreas. It couples cell metabolism to electrical activity of the plasma membrane by regulating membrane K^+ fluxes.[31,32] K_{ATP} channels also play important role in adaptation to intermittent hypoxia.[33,34] One of the pore forming subunits of K_{ATP} channels is encoded as KCNJ8 (potassium inwardly-rectifying channel, subfamily J), also known as KIR6.1.[35]

Under the context, the present study was designed to investigate the effects of a three-week session of IHT on blood glucose and hypoxic tolerance in healthy humans and patients with prediabetes. Furthermore, we focused on the effects of IHT on mRNA expression of HIF-1α and its targeted genes, such as INSR, SLC2A1, and KCNJ8.

Materials and methods

Characteristics of participants

Seven healthy volunteers (44–68 years, 3 males and 4 females) and 11 prediabetic patients (48–70 years, 5 males and 6 females) participated in the current study. The prediabetes patients were diagnosed using the criteria issued by the American Diabetes Association. We included patients who had an elevated fasting glucose level (5.6 to 6.9 mmol/L), impaired glucose tolerance (i.e. plasma glucose level of 7.8 to 11.0 mmol/L 2 h after an oral dose of 75 g glucose challenge), or their combination. Subjects in the healthy control group had no cardiovascular, respiratory, endocrine or central nervous system disorders and their fasting glucose concentration was less than 5.6 mmol/L and less than 7.8 mmol/L 2 h after a standard glucose tolerance test.

This clinical study was conducted under the state laws of Ukraine and the ethical principles of the 1964 Declaration of Helsinki. The research protocols, patient health information and informed consent forms were approved by the Ethics Committee of Chebotarev Institute of Gerontology, Kiev, Ukraine. All subjects received detailed information of the study process and a written informed consent was obtained from each of the participated subjects. All participants were nonsmokers and did not take any medication two weeks prior to and during the sessions of the study. They had no infections during the past month and no major cardiovascular or respiratory complications. All subjects underwent measurements of several anthropometric variables (Table 1), which indicated no significant difference in age and height between healthy and prediabetes groups, whereas the body weight, body mass index, and waist circumference were higher in the prediabetes patients than those of healthy control subjects. All participants were informed about the strict observance of lifestyle one month prior to, during, and one month after IHT (including levels of physical activity, caloric content of daily diet, consumption of coffee and tea, abstinence of alcohol, etc.). They kept diaries in which they noted any lifestyle changes, if occurred. Any violation of the regime by the subjects resulted in exclusion from the study.

Experiment protocols

All sessions of the present study were conducted in a quiet room at a temperature of 22–23°C within a clinical research center of the Chebotarev Institute of Gerontology.

Table 1 Anthropometric characteristics of the participants[a]

Groups	Gender (female/male)	Age (year)	Height (cm)	Weight (kg)	BMI (kg/m^2)	Waist (cm)
Healthy	5/2	58.7 ± 11.8	170 ± 15	76.1 ± 17.3	27.2 ± 6.4	93.7 ± 9.2
Prediabetes	7/4	66.4 ± 5.2	167 ± 10	90.2 ± 9.9	33.2 ± 5.6	99.7 ± 8.9
Healthy vs. prediabetes		NS	NS	$P < 0.05$	$P < 0.05$	$P = 0.05$

BMI: body mass index; waist: waist measurements; NS: no significant difference.
[a]Data are mean ± SD. Student's *t*-test was used to evaluate the statistical significance of the differences between healthy and prediabetes groups.

Table 2 Experimental timetable of the procedures, sample collections, and functional tests before, during, and after the IHT sessions

	Procedures and tests				
Date of investigation	Venous blood sampling for mRNA assays	Fasting blood glucose and OGTT	Cardio-vascular parameters	Acute hypoxic test	IHT sessions
Monday (week 1, 2 days before IHT start)	+	+			
Tuesday (week 1, 1 day before IHT start)			+	+	
Wednesday (week 1), Friday (week 1), Monday (week 2)					+++
Tuesday (week 2, 24 hours after IHT)	+				
Wednesday, Friday (week 2), Monday, Wednesday, Friday (week 3), Monday (Week 4)					++++++
Tuesday (week 4, 24 hours after IHT)	+	+			
Wednesday (week 4)			+	+	
Tuesday (1 month after IHT)	+	+			
Wednesday (1 month after IHT)			+	+	

IHT: intermittent hypoxic training; OGTT: oral glucose tolerance test.

The logistic plan and timetable of the studies are summarized in Table 2. Measurement sessions were performed during two days before IHT course, one day and one month after the termination of IHT. For determination of HIF-1α mRNA expression and its target genes in blood leukocytes, venous blood samples were collected again next day after one-week IHT course. Patient examination included: (1) anthropometric measurements; (2) determination of HIF1α mRNA and its target genes; (3) standard oral glucose tolerance test (OGTT) with plasma glucose determination; and (4) acute hypoxic test (AHT) with measurements of routine cardiovascular parameters.

In the morning of the first experiment day, after three-day routine hospital diet (250–300 g carbohydrates) and normal physical activity, a venous blood sample was drawn under fasting condition from the median antecubital vein for measurement of fasting glucose level as well as genetic analysis. Thereafter, a standard OGTT was conducted according to Ryden *et al.*,[36] which used 75 g of glucose mixed in 250 mL of water. Venous blood samples were drawn at 120 min after the oral glucose ingestion. Plasma glucose concentrations were analyzed by glucose oxidase method in normoxic conditions on semi biochemical analyzer BTS-330 using reagents "Glucose", Bio LATEST Lachema Diagnostica.

Next day, after a light breakfast, the baseline cardiovascular parameters of the subjects were measured in a relaxing sitting position with spontaneous breathing of room air. Arterial blood oxygen saturation (SaO_2) and heart rate (HR) were recorded using a patient vital sign monitor UM 300-12 (UTAS, Ukraine, http://www.utasco.com). Systolic (SBP) and diastolic (DBP) blood pressure values were measured on brachial artery with a mercury sphygmomanometer (Erkameter 3000, Germany). After all the baseline tests, the participants were connected to an open breathing circuit through a mask to perform an AHT[37]: breathing a gas mixture with 12% O_2 for 20 min while monitoring the changes in the subject's cardiovascular parameters and SaO_2. This study analyzed the indices at the 20th min of the test.

From the next morning, after a light breakfast, all participated subjects received the sessions of IHT three times a week for the subsequent three weeks, i.e. each subject received total of nine sessions of IHT. Each session consisted of four cycles of 5-min hypoxia (12% inspired O_2) followed by 5-min normoxia (room air breathing). The normobaric hypoxia was administered to the subjects in sitting position, using a hypoxic apparatus—Hypotron® (Kiev Polytechnic Institute, National Technical University of Ukraine). The subjects' SBP, DBP, HR, and SaO_2 were

continuously monitored and recorded. Next day and one month after the end of three-week IHT, the post-test examinations were conducted in the same manner as the pre-test ones (Table 2).

Determination of gene expression

mRNA expression of HIF-1α, INSR, SLC2A1, and KCNJ8 was determined in circulating blood leukocytes collected in various time points using real-time polymerase chain reaction (RT-PCR) assay. Blood leukocytes were obtained by centrifuging the blood samples at 1500 g for 1.5 min. After centrifugation, supernatant with interphase fraction was collected and transferred in new tube. After a secondary centrifugation (3000 g for 3 min) the supernatant was removed, the precipitate was used for RNA isolation using phenol–chloroform extraction after homogenization with guanidine isothiocyanate (Trizol RNA Prep 100 Kit, Russian Federation). Total RNA concentration was determined with a spectrophotometer ND1000 (NanoDrop Technologies Inc., USA). cDNA was synthesized from 5 μg of total RNA by reverse transcription with 10 mmol/L Tris-HCl (pH 9.0), 5 mmol/L $MgCl_2$; 1 mmol/L dNTPs; 20 U Ribo-Lock, Random hexamer primers (0.5 $\mu g \cdot \mu L^{-1}$) and 200 U RevertAid H Minus M-MuLV Reverse Transcriptase. PCR was performed using an Applied Biosystems 2700 (PerkinElmer, USA).

Gene expression of HIF-1α (Assay ID: Hs00153153_m1), SLC2A1 (Hs00892681_m1), INSR (Hs00961554_m1), and KCNJ8 (Hs00958961_m1) was determined using TaqMan® Gene Expression Assay (Applied Biosystems, USA). The pairs of forward and reverse primers for genes above mentioned and the TaqMan® probes for the target mRNAs were designed by Applied Biosystems based on the human mRNA sequence. Gene expression in each probe was normalized with β-actin, using a TaqMan® human β-actin control reagent. The thermal cycles of PCR amplification consisted of initial denaturation step at 95°C for 20 s, followed by treatment at 95°C for 3 s, and at 60°C for 30 s and for 50 cycles using a 7500 Fast Real-time PCR equipment (Applied Biosystems). The cycle threshold is defined as the number of cycles required for the fluorescence signal to exceed the detection threshold. The expression level of each target gene was calculated relative to the housekeeping gene (β-actin) as the difference between the threshold values of the two genes. Each PCR step was performed in duplicate and the calculations were done using the 7500 Fast System SDS software (Applied Biosystems).

Statistical analysis

All data were analyzed using SPSS software version 21.0 (SPSS Inc., USA). Student's *t*-test was used to test anthropometric differences between healthy and prediabetes groups (Table 1). To evaluate the changes of blood glucose concentration over time in both groups (Table 3), two-way analysis of variance (ANOVA) with repeated measures was used followed by Bonferroni post hoc test to determine both group main effect (healthy vs. prediabetes) and time effect (for 3 time-points: Pre-IHT baseline, 1 day after IHT, 1 month after IHT). To assess the differences between physiological parameters at 20th min of acute hypoxia test before and after IHT (Table 4), three-way ANOVA with repeated measures and Bonferroni post hoc test was used to analyze both the group main effect, time effect, and hypoxia effect for each of dependent variables. Pearson product-moment correlation coefficient (*r*) was calculated to show the degree of linear relationship between variables. The level of statistical significance was set at $P < 0.05$. Data are expressed as mean ± SD.

Results

All subjects well tolerated the entire process of medical examination and IHT sessions. No subjective discomforts and/or any other adverse effects were reported.

Blood glucose level

Table 3 demonstrates the effects of IHT on blood glucose level in healthy subjects and prediabetic patients. Prior to

Table 3 Glucose blood serum concentration during oral glucose tolerance test (OGTT) before and after the three-week sessions of IHT[a]

	Healthy ($n = 7$)	Prediabetes ($n = 11$)	Group main effect healthy vs. prediabetes	Time effect 3 time-points	Group + time effect
Fasting glucose (mmol/L)					
Pre-IHT baseline	4.6 ± 0.4	5.6 ± 0.6	$F = 25.967$	$F = 0.845$	$F = 1.084$
1 day after IHT	4.5 ± 0.3	5.4 ± 0.7	$P = 0.000$	$P = 0.434$	$P = 0.348$
1 month after IHT	4.6 ± 0.5	5.2 ± 0.4*			
2 h post-OGTT glucose (mmol/L)					
Pre-IHT baseline	5.3 ± 1.5	7.9 ± 1.5	$F = 25.757$	$F = 2.590$	$F = 9.570$
1 day after IHT	5.0 ± 1.2	7.0 ± 1.9*	$P = 0.000$	$P = 0.102$	$P = 0.007$
1 month after IHT	4.9 ± 1.2	6.4 ± 1.0**			

IHT: intermittent hypoxic training; SaO_2: blood oxygen saturation; HR: heart rate; SBP: systolic blood pressure.
[a]Data are mean ± SD and were analyzed with two-way ANOVA with repeated measures.
*$P < 0.05$ versus pre-IHT baseline; **$P < 0.01$ versus pre-IHT baseline.

Table 4 Arterial blood oxygen saturation and cardiovascular indices at 20th min of acute hypoxia test (12% inspired O_2) before and after the three-week sessions of IHT[a]

	Healthy		Prediabetes						
	Normoxia	Hypoxia	Normoxia	Hypoxia	Group main effect	Time effect	Hypoxia effect	Time + Hypoxia Effect	Group + hypoxia + time effect
SaO_2 (%)									
Pre-IHT baseline	98.8 ± 0.6	80.9 ± 3.1	98.6 ± 0.5	80.3 ± 3.0	$F = 0.030$	$F = 20.247$	$F = 1536.695$	$F = 28.454$	$F = 0.038$
1 day after IHT	98.9 ± 0.5	86.1 ± 3.3*	98.7 ± 0.5	85.0 ± 2.8*	$P = 0.834$	$P = 0.000$	$P = 0.000$	$P = 0.000$	$P = 0.848$
1 month after IHT	98.8 ± 0.6	84.5 ± 3.2*	98.8 ± 0.6	83.8 ± 2.5*					
HR (beat per min)									
Pre-IHT baseline	65.4 ± 3.9	74.1 ± 12.2	69.8 ± 3.4	75.5 ± 4.6	$F = 1.354$	$F = 5.098$	$F = 15.328$	$F = 0.466$	$F = 0.018$
1 day after IHT	63.0 ± 2.9	68.4 ± 8.2*	66.4 ± 3.4	68.1 ± 7.6*	$P = 0.262$	$P = 0.020$	$P = 0.001$	$P = 0.666$	$P = 0.982$
1 month after IHT	64.5 ± 4.2	68.9 ± 7.4*	67.4 ± 8.4	69.4 ± 10.4*					
SBP (mmHg)									
Pre-IHT baseline	128 ± 21	146 ± 18	132 ± 20	154 ± 18	$F = 0.932$	$F = 17.264$	$F = 31.718$	$F = 26.126$	$F = 0.360$
1 day after IHT	124 ± 16	134 ± 19*	123 ± 14*	136 ± 15*	$P = 0.350$	$P = 0.001$	$P = 0.000$	$P = 0.000$	$P = 0.704$
1 month after IHT	125 ± 20	138 ± 21	128 ± 13	140 ± 17					

IHT: intermittent hypoxic training; SaO_2: arterial blood oxygen saturation; HR: heart rate; SBP: systolic blood pressure.
[a]Data are mean ± SD and were analyzed with three-way ANOVA with repeated measures.
*$P < 0.05$ vs. pre-IHT baseline.

IHT, the fasting glucose level was within the normal range in both groups, but in healthy group it was significantly lower (~18%) than prediabetes group. The results of OGTT indicated that 2 h after 75 g glucose ingestion the plasma glucose concentration increased by 15% in the healthy subjects, but by 41% in the prediabetic patients ($P < 0.05$).

Two-way ANOVA results indicated that main group effect (healthy vs. prediabetes) was significant ($P < 0.01$). On the other hand, although the general time effect was not significant due to the absence of IHT influence on healthy subjects, a separate Bonferroni post-hoc analysis in the prediabetic patients showed a significant difference between fasting glucose pre-IHT baseline and one month after IHT ($P < 0.05$), between 2-h post-OGTT glucose pre-IHT baseline and one day after IHT ($P < 0.05$) as well as one month after IHT ($P < 0.01$). Both group main effect and group + time effect were statistically significant for 2-h post-OGTT glucose (Table 3).

Tolerance to acute hypoxia

Table 4 demonstrates the values of blood oxygen saturation and cardiovascular indices during AHT before and after the three-week sessions of IHT. Under acute hypoxia (12% inspired O_2 for 20 min), SaO_2 (a non-invasive indicator of hypoxic tolerance) dropped at the initial stage by 18% in healthy group and 19% in prediabetes group. No statistical difference between the two groups was observed (group main effect $F = 0.030$, $P = 0.834$). At the end of three-week IHT, SaO_2 fell much less (by 13% or 14%, respectively) suggesting the body has gained an increased tolerance to hypoxia. This effect maintained at one month after IHT completion (time effect $F = 20.247$, $P = 0.001$; time + hypoxia effect $F = 28.454$, $P = 0.001$). The indices of cardiovascular response to acute hypoxia also showed an increased tolerance to acute hypoxia. For examples, HR was significantly lower during 20-min hypoxic load in both groups at the end of or one month after the three-week IHT sessions (time effect $F = 5.098$, $P = 0.02$; hypoxia effect $F = 15.328$; $P = 0.001$). Similarly, the hypoxia-triggered increases in SBP were reduced right after the end of IHT (time effect $F = 17.264$, $P = 0.001$; hypoxia effect $F = 31.718$, $P = 0.001$; time + hypoxia effect $F = 26.126$, $P = 0.001$). Three-way ANOVA test did not show significant common effect group + hypoxia + time effect, which demonstrates the absence of differences between groups in adaptive reactions of cardiovascular indices to hypoxia.

HIF-1α mRNA expression

Initial level of HIF-1α mRNA expression was comparable in the healthy group and prediabetes group (Figure 1(a)). IHT resulted in approximately four-fold (Healthy) and five-fold (Prediabetes) increase during the first week of IHT. In the next two weeks HIF-1α expression returned to the baseline level in the healthy subjects. However, in the prediabetic patients HIF-1α expression continued to increase, exceeding the initial level for 6.5 times and remained two-fold higher one month after the end of IHT.

SLC2 mRNA expression

mRNA expression of SLC2—the insulin-independent glucose transporter (Figure 1(b)) was not different significantly between the two groups prior to IHT. At the end of three-week IHT sessions, SLC2 mRNA expression increased significantly in the healthy participants with subsequent 80-fold augmentation in a month after IHT termination, but no changes were observed throughout the test period in prediabetes group, indicating a prediabetes-related defect in this transporter in response to intermittent hypoxia.

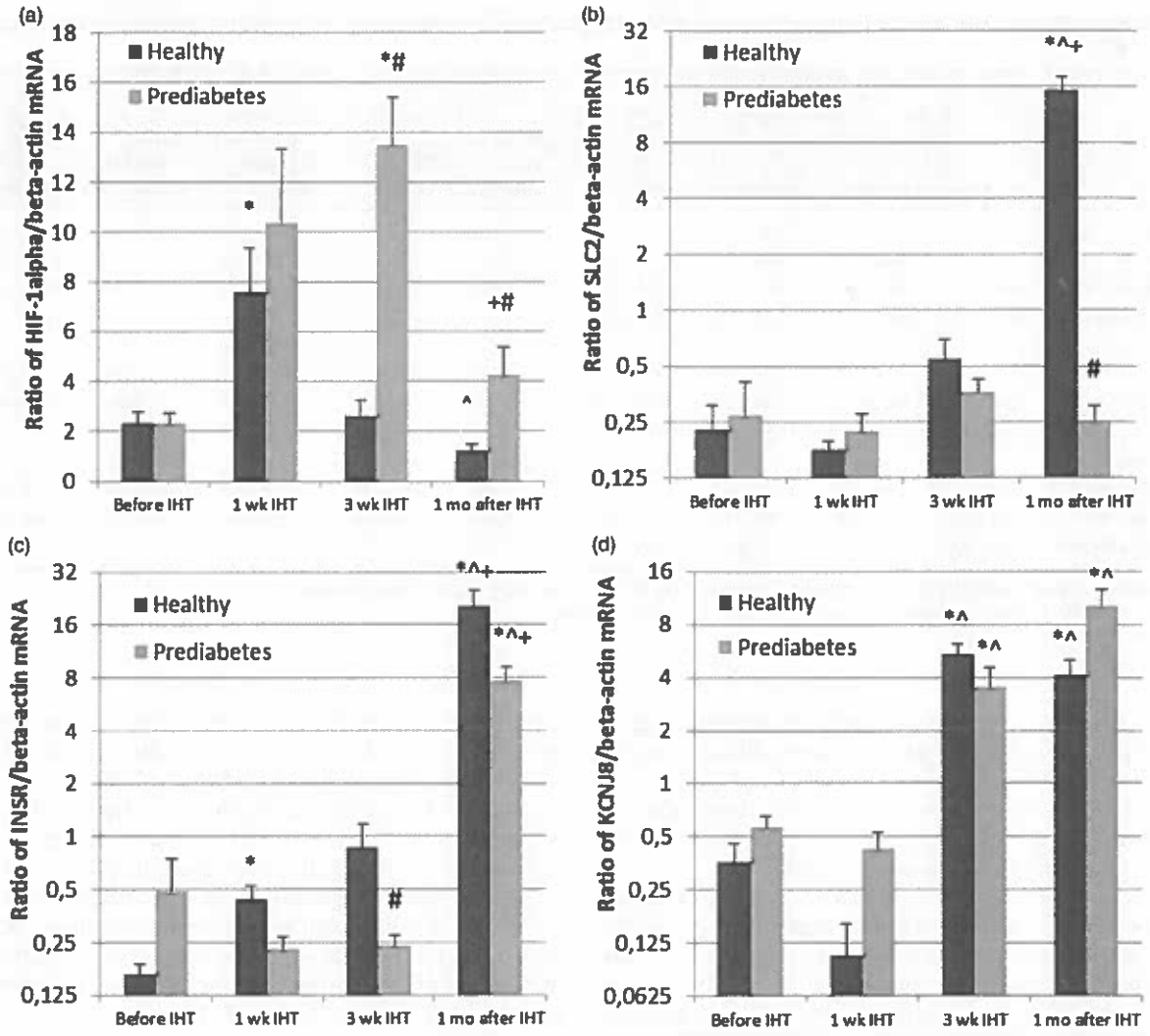

Figure 1 Effect of IHT on mRNA expression of hypoxia inducible factor 1α (HIF-1α) (a), facilitated glucose transporter-solute carrier family-2 (SLC2) (b), insulin receptor (INSR) (c), and potassium voltage-gated channel subfamily J (KCNJ8) (d) in healthy subjects and prediabetic patients. Data are presented as mean ± SD. $^{*}P < 0.05$ vs. pre-IHT baseline; $^{\wedge}P < 0.05$ vs. 1 wk IHT; $^{+}P < 0.05$ vs. 3 wk IHT; and $^{\#}P < 0.05$ vs. healthy group. IHT: intermittent hypoxia training

INSR mRNA expression

Basal INSR mRNA expression was not statistically different between the two groups, mainly due to large individual variance among the prediabetes patients, from 0.1 to 12 units (Figure 1(c)). During IHT this parameter gradually increased in the healthy subjects reaching a five-fold increase by the end of IHT and continued to increase in the coming month, exceeding more than 100 times above the baseline value. Meanwhile, in the prediabetic patients, IHT caused slight decrease in INSR mRNA expression during training period, and only in a month after IHT termination this parameter increased to a level of 16-folds above the baseline, suggesting a remarkable delay in response to IHT.

KCNJ8 mRNA expression

The pre-IHT basal mRNA expression of KCNJ8 potassium channels was identical in both groups (Figure 1(d)). During the first week of IHT no significant changes was observed, but at the end of three-week IHT sessions KCNJ8 increased about 15-folds in healthy group and six-folds in prediabetes group. Interestingly, one month after IHT termination, the augmented level of KCNJ8 was maintained in healthy group, but showed more pronounced increase in prediabetes group.

Correlation analysis

Figure 2 demonstrates the relationship between SaO_2 at 20th minute of acute hypoxic test (the marker of tolerance to acute hypoxia) and several measured parameters. Although no significant correlation was found between the levels of SaO_2 and baseline fasting blood glucose (Figure 2(a)) or 2-h post-OGTT blood glucose (Figure 2(b)) in healthy group, a strong negative correlation was identified in prediabetes group. In these prediabetes patients, the lower SaO_2 under acute hypoxic test, the higher fasting glucose (Figure 2(a), $r = -0.75$; $P < 0.01$) and 2-h post-OGTT

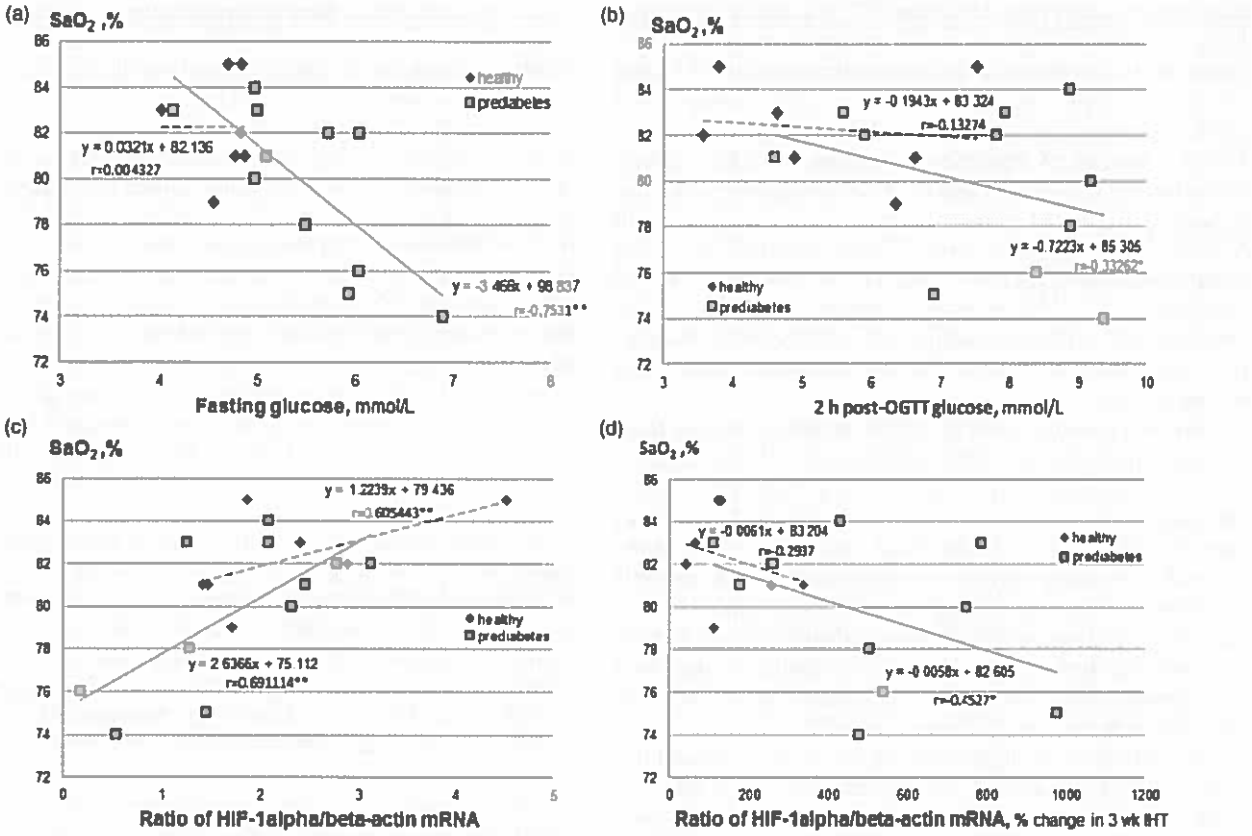

Figure 2 Relationships between baseline SaO_2 at 20th minute of acute hypoxic test (AHT, breathing with 12% of oxygen) and baseline fasting glucose (a), baseline 2 h post-OGTT glucose (b), baseline mRNA expression of HIF-1α (c), and % changes in mRNA expression of HIF-1α between the pre-IHT baseline and post-IHT values (d). HIF: hypoxia inducible factor; OGTT: oral glucose tolerance test. Symbols of correlation coefficient (*$P < 0.05$ or **$P < 0.01$) indicate a significant degree of linear relationship between the variables

glucose level (Figure 2(b), $r = -0.33$, $P < 0.05$). In addition, we also found a significant positive correlation between SaO_2 and baseline HIF-1α mRNA expression in both healthy ($r = 0.61$; $P < 0.01$) and prediabetes groups ($r = 0.69$; $P < 0.01$) (Figure 2(c)). Besides, a negative correlation between SaO_2 and % changes in mRNA expression of HIF-1α between baseline and the post-IHT examination in prediabetes group (Figure 2(d), $r = -0.42$, $P < 0.05$). These data indicate that the subjects with lower tolerance to hypoxia had initially higher blood glucose level and greater increase in HIF-1α mRNA expression under IHT.

Discussion

The present study revealed the following salient findings: (1) three-week sessions of IHT afforded beneficial effects on glucose homeostasis in patients with prediabetes (Table 3), particularly IHT significantly reduced fasting and 2-h post-OGTT blood glucose levels with the most pronounced beneficial effects observed at one month after the end of IHT; (2) IHT significantly increased the body's tolerance to acute hypoxia and improved cardiovascular function under hypoxia in both healthy and prediabetic individuals (Table 4); (3) subjects with higher initial level of blood glucose had lower tolerance to hypoxia (Figure 2(a) and (b)); (4) IHT stimulated HIF-1α mRNA expression in blood leukocytes in a bi-phasic manner, which showed early activation during the first week of IHT and subsequently returned to the pre-IHT basal level in healthy men, but in prediabetic patients the maximum response to IHT was observed in a delayed manner at the end of IHT (Figure 1(a)); (5) HIF-1α regulated genes such as INSR, SLC2, and KCNJ8 were differentially affected by IHT in the healthy and prediabetic individuals; the greatest changes in mRNA expression of the target genes occurred one month after termination of IHT and coincided with the largest decrease in blood glucose levels, both fasting and under hyperglycemia load (Figure 1(b) to (d)); and (6) the higher expression of HIF-1α was positively associated with higher tolerance to hypoxia and better glucose homeostasis in both healthy and prediabetes subjects and interestingly the greater increase in HIF-1α mRNA expression under IHT was observed in the subjects with lower resistance to hypoxia.

The abovementioned findings are conceptually supportive to the notion of anti-diabetes effect of IHT, which was first demonstrated by Kolesnyk *et al.* in rats[9] and subsequently confirmed in other animal and human studies using different IHT models[13,38–42] as well as during the high altitude hypoxia adaptation.[43,44] However, it is notable that severe intermittent hypoxia, such as those found in patients with obstructive sleep apnea (OSA) may cause various negative effects, including the suggested association

between OSA and type 2 diabetes.[45–47] It is increasingly realized that different patterns of intermittent hypoxia could result in divergent effects on metabolic function[21,48–52] and the specific mode of hypoxia, including depth, duration, and cyclic frequency, can be critical for determining the healing or harmful results of intermittent hypoxia.[2,53] Our present investigation provided direct evidence suggesting a moderate and non-invasive protocol of four short cycles of 5-min hypoxic breathing (12% O_2) and 5-min normoxic breathing (room air), three times a week for three weeks is sufficient to reduce fasting and 2-h post-OGTT hyperglycemia in prediabetic patients, while increasing their resistance to acute hypoxia with improved cardiovascular functional parameters under hypoxia.

Hypoxia is well known to initiate an adaptive gene transcription program via HIFs, among which HIF-1 triggers hypoxia-dependent gene expression in regulating many metabolic processes for the improvement of O_2 transport capacity.[11,17,20,21,54] Reduced levels of HIF-1α have been found in the cells or tissues collected from diabetic animals or patients, indicating an inhibitory effect of hyperglycemia on HIF-1α expression.[18,55–59] This diabetes-blunted HIF-1α response to hypoxic conditions may result in impaired angiogenesis and inability to upregulate glycolytic ATP generation in the type 2 diabetic heart.[60]

The selection of leukocytes to be studied on cellular response to hypoxia had several rationales. The primary rationale to study leukocytes is their cellular lifespan (2–10 days), which allows observing the cell phenotypic changes under intermittent hypoxia sessions. In fact, leukocytes are the only nucleated fraction in blood cells, in which the changes in gene expression under hypoxic stimuli can be non-invasively quantified and it has been thought to reflect better the processes of genetic activation in cells than the mRNAs extracted from blood plasma. For example, a previous study by Tissot van Patot *et al.*[61] using leukocytes demonstrated that HIF-1 DNA binding activity was enhanced *in vivo* in response to acute hypoxia in 14 men exposed to hypobaric hypoxia (4300 m or equivalent to 12% O_2) in a hypoxic chamber for 8 h, both HIF-1 DNA binding and HIF-1α protein levels in leukocytes were elevated, in association with plasma and urinary markers of hypoxic stress. To our best knowledge this is the first time to show HIF-1α mRNA increased following IHT sessions. The present study also showed that the maximum increase in HIF-1α expression induced by IHT occurred much earlier in healthy people than those in prediabetic patients, which may indicate the inhibitory effect of higher blood glucose levels on HIF-1α response to IHT in the prediabetic patients. In supporting this notion, recent study by Xiao *et al.*[59] demonstrated that whereas hyperglycemia upregulates HIF-1α signaling in some cell types, high glucose can also inhibit HIF-1α and its target genes. Regarding the mechanisms of HIF-1α impairment under diabetic conditions, the negative effects of various diabetes-associated factors include overproduction of reactive oxygen species, increased sensitivity to Von Hippel-Lindau (VHL) machinery, and altered osmolarity and proteasome activity, which could deactivate HIF-1α.

Our present study further investigated mRNA expression of several main target genes of HIF-1α, which are likely participating in insulin reception (INSR), facilitated glucose transport (SLC2A1), and regulation of insulin secretion in the β-cells (KCNJ8). It is known that as a part of adaptive response to hypoxia, there is upregulated expression of several genes encoding glycolytic enzymes.[27] However, to our best knowledge, an IHT-induced change in mRNA expression of these genes has not been reported prior to our current study. Our present results showed that whereas no significant difference in baseline expression of the examined genes between prediabetes and healthy subjects, the changes in HIF-1α-regulated gene expression in response to IHT was subsequent to those of HIF-1α mRNA in the healthy group and the maximum response was observed one month after the end of IHT (Figure 1(b) to (d)). Notably the prediabetes group exhibited different temporal profiles.

It is well recognized that physiological effects of insulin implemented mainly through INSR by binding to α subunit of INSR and stimulating the intrinsic kinase activity of β subunit of INSR.[28] Increased levels of INSR could alleviate insulin resistance, because overexpression of INSR improved obese and diabetic phenotypes in rodents.[62] KCNJ8 is one of the subunits of K_{ATP} channels and presents in many tissues, including pancreatic islet cells, therefore it is considered as metabolic sensors via coupling cellular metabolic status to cell membrane potential.[32,35,63] Functional and structural defects in K_{ATP} channels impair insulin secretion leading to the onset of diabetes.[64] Our present study demonstrated an increased KCNJ8 mRNA expression at the end of IHT in both healthy and prediabetic subjects and more pronounced effect at one month after IHT in prediabetic patients. Based on the observed similarity in KCNJ8 mRNA expression among healthy and prediabetes subjects under IHT, we postulate that KCNJ8 channels may be less vulnerable during the development of diabetes. Such a hyperactive KCNJ8 following IHT may lead to the increase in insulin secretion by β-cells. Previous laboratory animal studies suggested an organ-protective role of vascular K_{ATP} channel under diabetic condition, via suppressing diabetic oxidative stress.[32] Cardioprotective and antiarrhythmic effect of adaptation to intermittent hypoxia is also mediated via activation of K_{ATP} channels.[34]

GLUT-1 is one of the key components of the HIF-1α-mediated hypoxia response.[24,65] It is responsible for basal glucose uptake and expressed in virtually all tissues under normal conditions.[66] HIF-1α accelerates the expression and activation of GLUT-1 and induces glucose uptake and glycolysis,[26] which in turn induces HIF-1α degradation[67] and GLUT-1 mutations that reduce its function are associated with reduced glucose uptake.[68] HIF-1α and GLUT-1 levels increased significantly in the cells exposed to chronic intermittent hypoxia, suggesting a transcriptional activation and adaptive response to intermittent hypoxia.[24,65] Alterations in glucose transporter genes are also associated with major pathologies, e.g. Alzheimer's disease.[69] GLUT-1 itself is a relatively stable protein as compared with HIF-1α[70] and therefore its changes in response to hypoxia-reoxygenation should have a more sustained profile. In our present study,

IHT elicited no change in SCL2A1 mRNA expression in the prediabetes group. The exact molecular mechanism underlying the loss of ability of IHT to upregulate GLUT-1 in prediabetic patients requires further investigation.

Notably, the most significant changes in mRNA expression of HIF-1α target genes were observed a month after IHT termination and were coincided with the largest decrease in fasting blood glucose and 2-hour post-OGTT glucose levels in the prediabetic patients (Table 3). Such long-term cause-and-effect relationships are not fully understood. Our previous studies in healthy people have also shown that the most pronounced changes in circulating hematopoietic stem and progenitor cell counts were observed not during IHT but two weeks after the completion of IHT.[71] Mechanism underlying the much delayed augmentation mRNA expression of HIF-1α target genes by IHT remains unclear and should be elucidated in future studies.

In addition, it was suggested that glucose sensing in the carotid bodies may play a role in metabolism.[72,73] We previously described in prediabetic patients the relationships between the tolerance to hypoxia and cardiorespiratory response to acute hypoxia as well as the severity of glucose metabolism disorder.[37] Our current study also found that prediabetic patients with impaired glucose homeostasis had lower tolerance to hypoxia (Table 4, Figure 2). The higher expression of HIF-1α was positively associated with higher tolerance to hypoxia and better glucose homeostasis in both healthy and prediabetes subjects. Besides, the greatest increase in HIF-1α mRNA expression under IHT was observed in the subjects with lower tolerance to hypoxia (Figure 2(d)). This observation is in accordance with previous findings from the rats with high or low resistance to hypoxia,[74,75] which reported higher increase in HIF-1 mRNA in the brain tissue of low tolerant animals following adaptation to intermittent hypoxia.

Taken together, the close relationships between the IHT-reduced blood glucose levels and the IHT-enhanced tolerance to hypoxia, which are also associated with the IHT-enhanced HIF-1α expression and its target genes, clearly suggested a better normalization of carbohydrate metabolism during IHT.

Nevertheless, several limitations of the present study include that we only investigated the transcription of HIF-1α as a possible mechanism of potentiation of the "weak links" of the HIF-1α-mediated responses in diabetic patients. At the same time, the obtained results about the induction of target genes indirectly indicate the effectiveness of HIF-1α protein stabilization and its transcriptional activity. Undoubtedly, future studies focusing on posttranslational modifications of HIF-1α protein would be highly warranted and informative.

Conclusion

The present study elucidated the poorly understood molecular mechanism underlying the beneficial effects of IHT under prediabetic conditions. We found that three-week moderate IHT induced higher HIF-1α and its target genes mRNA expressions, which were positively correlated with higher tolerance to acute hypoxia and better glucose homeostasis in both middle-aged healthy and prediabetic subjects. Our results suggest a potential utility of IHT as an effective non-pharmacologic preventive therapy for management of prediabetes patients.

Author contributions: All authors participated in the design and interpretation of the studies, data analysis, review, and final approval of the manuscript. TVS designed the study and wrote the manuscript. TVS, AGP, and VBS elaborated the study protocols. AGP and TID performed and interpreted genetic analyses. VIP, EE, AVG, SN, VC, and VBS conducted physiological investigations and performed statistical analyses of the results. LX critically edited the manuscript. VBS provided the enrollment and clinical examination of the subjects as well as general research management.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Review

Additive stress of normobaric hypoxic conditioning to improve body mass loss and cardiometabolic markers in individuals with overweight or obesity: A systematic review and meta-analysis

Domingo J. Ramos-Campo[a,*], Olivier Girard[b], Andrés Pérez[c], Jacobo Á. Rubio-Arias[a]

[a] Department of Physical Activity and Sports Sciences, Faculty of Sports, UCAM, Catholic University San Antonio, Murcia, Spain
[b] Murdoch Applied Sport Science Laboratory, Murdoch University, Perth, Australia
[c] UCAM Research Centre for High Performance Sport, Catholic University San Antonio, Murcia, Spain

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ABSTRACT

We performed a systematic review and meta-analysis to determine if hypoxic conditioning, compared to similar training near sea level, maximizes body mass loss and further improves cardiometabolic markers in overweight and obese individuals. A systematic search of PubMed, Web of Science and the Cochrane Library databases (up to January 2019) was performed. This analysis included randomized controlled trials with humans with overweight or obesity assessing the effects of HC on body mass loss or cardiometabolic markers. A subgroup analysis was performed to examine if HC effects differed between individuals with overweight or obesity. 13 articles (336 participants) qualified for inclusion. HC significantly decreased body mass ($p = .01$), fat mass ($p = .04$), waist/hip ratio ($p < .001$), waist ($p < .001$), LDL ($p = .01$), diastolic ($p < .01$) and systolic blood pressure ($p < .01$) with these effects not being larger than equivalent normoxic interventions. There were trends towards higher triglycerides decrement ($p = .06$) and higher muscle mass gain in hypoxic ($p = .08$) compared with normoxic condition. Also, the two BMI categories displayed no difference in the magnitude of the responses. Compared to normoxic equivalent, HC provides greater reductions in triglycerides and greater muscle growth, while body mass changes are similar. In addition, HC responses were essentially similar between individuals with overweight or obesity.

1. Introduction

Obesity is the pandemic of the 21st century. It is characterized by excessive fat mass accumulation and chronic systemic inflammation, which likely predisposing individuals with obesity to metabolic diseases [1]. Obesity is generally defined as a body mass index (BMI) of 30 kg/m^2 and above, while overweight is defined as a BMI between 25 and 30 kg/m^2 [2]. Although it arises from a multifactorial etiology (e.g., genetics, lifestyle, socioeconomic status) [1] being obesity or overweight typically is caused by a positive energy balance, which results from an increased food intake, a decreased energy expenditure, or both [3]. Obesity is associated with increased risk of premature mortality and other comorbidities such as dyslipidemia, type 2 diabetes mellitus, hypertension, cancer, stroke and coronary heart diseases [4,5]. Carrying additional weight also produces excessive joint loads, eventually leading to the development of musculoskeletal pathologies (i.e osteoarthritis) that in turn limit functional capabilities [6].

There is an urgent need for effective interventions to treat obesity. During the last decades, caloric restriction and exercise interventions have been primarily implemented as treatment for obesity [7]. Current exercise recommendations suggest that individuals with obesity should undertake 30–60 min of moderate-intensity physical activity on most, if not all, days of the week [8]. However, adherence to exercise often declines over time, and perhaps even more so in diseased populations [9]. This may lead to a plateau of body mass loss with a partial or total recovery of lost body mass only 6 months after the start of the nutrition and/or exercise interventions [9]. Today, it is imperative that innovative, non-pharmacological approaches are developed for individuals with overweight or obesity to match current exercise recommendations [5].

Several recent studies have used hypoxic exposure as a new therapeutic strategy to improve the symptoms of a range of cardiovascular, metabolic and pulmonary diseases including obesity [3,9,10]. Hypoxia is defined as a reduced O_2 supply to tissues caused by decreases in O_2

* Corresponding author at: UCAM Universidad Católica San Antonio de Murcia, Campus de los Jerónimos, N° 135 Guadalupe, 30107 Murcia, Spain.
E-mail address: djramos@ucam.edu (D.J. Ramos-Campo).

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saturation of arterial blood [11]. Hypoxic conditioning (HC) relates to passive (i.e., during rest) or active (i.e., during exercise) recurrent exposure to systemic (whole body) and/or local (tissue) hypoxia. By decreasing arterial O_2 availability, HC has the potential to further improve cardio-metabolic health, functional performance and well-being of individuals with chronic diseases and/or sustaining acute musculo-skeletal injuries [12]. HC that activates the hypoxia-inducible factor (HIF) may play an essential role in effective metabolism regulation (i.e. body mass maintenance, glucose homeostasis and liver metabolism) and thereby in the prevention of obesity [1]. Reportedly, passive and active hypoxia stimulate HIF-1 production [13], improving glucose intake and transport, glycolysis, lactate production to provide ATP [14] and oxygen transport and satiety [15] among others. Also, lipid metabolism can be further enhanced when exercise training is conducted in O_2-deprived environments [16]. However, other HC studies failed to demonstrate similar positive results on lipid metabolism [17] and body mass loss management [17,18]. Thus, there is conflicting evidence in relation to the effectiveness of HC as a tool to improve body mass loss and lipid oxidation in individuals with overweight or obesity.

To date, several studies have analyzed the effect of low intensity training (55–65% of maximum oxygen uptake (VO_2max)) [16,18–21] in hypoxia on body mass loss and cardiometabolic markers in individuals with overweight or obesity. Compared to normoxia, hypoxic training at low intensity in patients with obesity can induce higher increases in noradrenaline levels, peripheral vasodilatation, number of mitochondria, glycolytic enzyme activity, insulin sensitivity and/or reduction of leptin levels [9]. Other positive effects of HC have been observed on blood pressure [21] and metabolic markers such as triglycerides [22,23] or cholesterol [23], which were not found (or to a lower extent) with equivalent normoxic training. However, other studies did not found any additional effect of HC on blood pressure [16,24], tryglicerides [17,20] or blood glucose [23,25]. To date, contradictory findings exist in the literature about the additional effect of low-intensity training HC on improvement of cardiometabolic markers.

During the past few years, new training paradigms under hypoxic conditions have been introduced. It is well established that high intensity training (HIT) in hypoxia can improve cardiorespiratory function (i.e VO_2max) and performance (i.e best and mean sprint during an repeat sprint ability test) [26] in athletic populations, while the usefulness of this training modality in patients as a tool to improve body mass loss and cardiometabolic markers is more recent [10,27]. Recent evidence suggests that HIT in hypoxia is more effective at increasing lean mass than normoxic exercise in women with overweight and obesity [28]. For instance, additional body mass loss and body fat reduction with also a concomitant increase in muscle mass have been reported after 12 weeks of HIT in hypoxia [10]. However, another HIT study [29] failed to report a positive change in body composition after a 5-wk training period. Contradictory findings exist regarding whether or not HC facilitates body mass loss compared to equivalent normoxic training.

Previous literature reviews (mainly narrative in nature) have critically discussed the potential of passive and active HC as a therapeutic intervention to loose body mass and improve health-related markers [3,5,9,30–32] in individuals with obesity. Limitations of this previous work include the analysis of the effect of HC on some other diseases (i.e pulmonary and cardiovascular), inclusion of non-randomized controlled trials and an analysis period up to until 2017. Only in 2018, six additional randomized controlled trials [10,17,22,23,25,28] have been published that represent half of the total number of studies that were available until then. Remarkably, only one of these reviews is a systematic review [5], while it included both animals and humans (6 randomized controlled trials (RCT)) research. This 2017 systematic review that featured inconsistent findings for triglycerides and cholesterol markers. A potential limitation of a systematic review is that it does not include a data synthesis and statistical analysis to determine summary effect of the intervention on the outcomes measures. This implies that the results obtained in the literature review by Hobbins et al [5] could be oversized without a specific statistical analysis that offers a more accurate and general picture of the HC effects on body composition and health markers. Taken as a whole, this clearly demonstrates the growing interest around HC potential and the need to conduct new analysis.

Previous studies found that body composition and physiological adaptations to training may differ between individuals with normoweight, with overweight or with obesity [33]. For instance, a recently study [23] has shown a positive effect of low intensity training in hypoxia in overweight, but not normoweight, individuals on cardiometabolic markers such as triglycerides or high-density lipoprotein (HDL). To our knowledge, no meta-analysis study exists that specifically analyzed the influence of participant background on the magnitude of body mass loss and cardiometabolic health responses.

Therefore, our aim was to perform a systematic review and meta-analysis to determine if hypoxic conditioning, compared to similar training near sea level, maximizes body mass loss and further improves cardiometabolic markers in overweight and obese individuals.

2. Methods

2.1. Study design

The review was registered in PROSPERO International Prospective Register of Systematic Reviews (www.crd.york.ac.uk/prospero/index.asp, identifier CRD42018117868). The methodological process was based on the recommendations formulated in the PRISMA declaration [34]. For the meta-analysis, only randomized controlled trials investigating the effects of normobaric HC on body mass loss and/or cardiometabolic markers were considered.

2.2. Data sources and search profile

A comprehensive literature search was performed using PubMed-Medline, Web of Science and the Cochrane Library from database inception up to January 2019. The database searches were performed independently by two authors (AP and DJRC) and the results obtained were the same. The flow diagram of the search process is shown in Fig. 1. The following combination of terms was used: "hypoxia" or "intermittent hypoxia" or "hypoxic training" or altitude training" or "passive hypoxic exposure". The Boolean operator "AND" was used to combine these descriptors with: "obesity" or "overweight" or "weight loss".

2.3. Selection criteria

The specific inclusion criteria were: [1] original studies with a randomized controlled design; [2] human experimentation; [3] participants with overweight (BMI > 25 kg/m^2) or/and obesity (all obesity categories; BMI > 30 kg/m^2); [4] studies examining the effect of passive or active normobaric HC intervention; [5] studies assessing at least body mass of tested participants; [6] studies published in English; and [7] chronic interventions with a minimal duration of two weeks. Research studies were excluded if they: [1] only focussed on sport performance outcomes; [2] included physically active participants who performed moderate-intensity aerobic physical activity for a minimum of 30 min/d on 5 d/week or vigorous-intensity aerobic activity for a minimum of 20 min/d on 3 d/wk.; [3] were clinical studies; [4] examined the effect of hypobaric hypoxia (terrestrial altitude and hypobaric hypoxia in a climatic chamber) or used other devices that do not reduce the FiO_2 (i.e altitude training mask); [5] were reviews or assessed the effects of an acute intervention; and [6] were not an original investigation published in full.

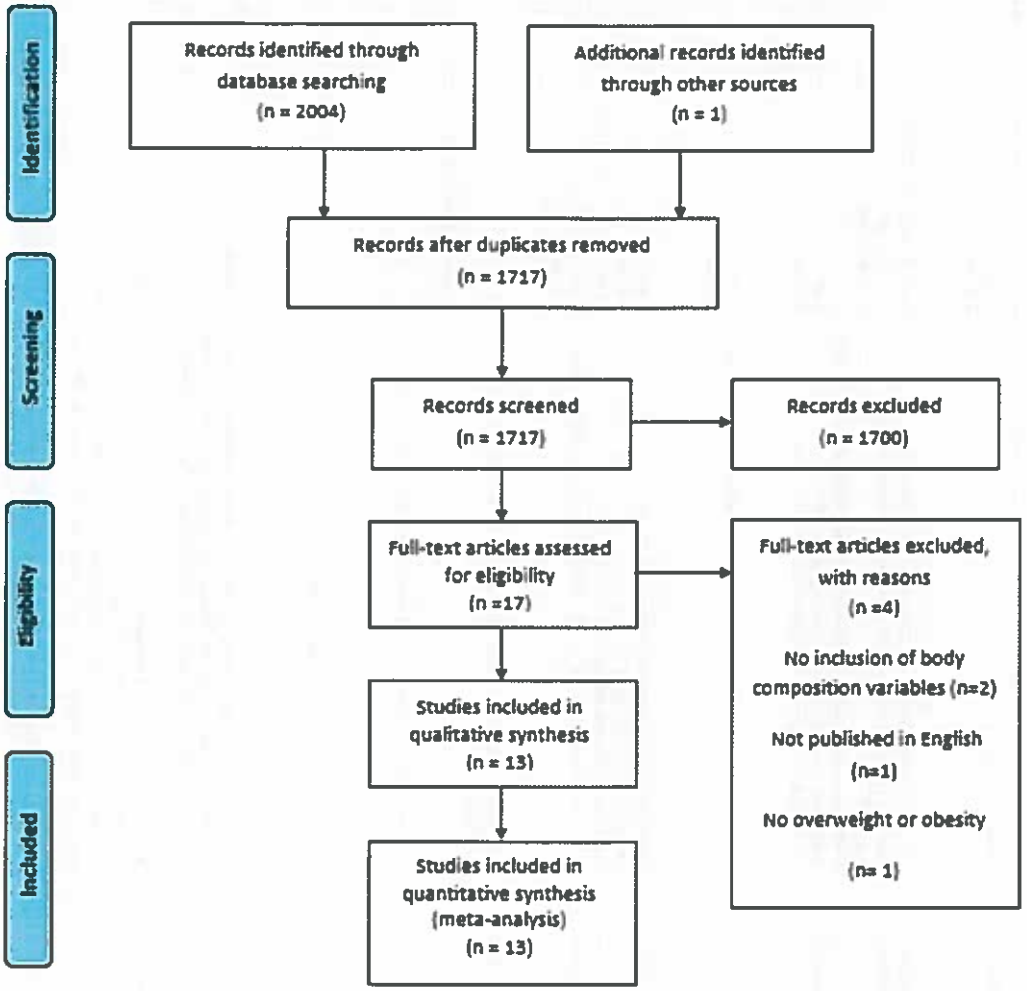

Fig. 1. Search process flow diagram.

2.4. Study selection and data extraction

Retrieved articles were reviewed independently by two authors (AP and DJRC) in order to select relevant articles. In addition to the literature search, references were scanned for further relevant articles and were included in our analysis if they met the inclusion criteria. Also, authors of selected studies were contacted for non-reported information. Two authors (AP and DJRC) independently extracted data from the included studies. The following information was extracted: authors of the paper, study design, number of participants included in each group, age, gender and BMI. Regarding the characteristics of the hypoxic intervention, the information extracted included: type of hypoxic exposure (passive, active or combination of both), protocol and training characteristics (volume, intensity, frequency, rest...), duration (number of weeks) and level of hypoxia.

2.5. Outcomes

The primary outcome was body mass loss. The secondary outcomes were: i) BMI; ii) waist circumference; iii) waist/hip (W/H) ratio; iv) muscle mass; v) fat mass; vi) Low-density lipoprotein (LDL); vii) HDL; viii) triglycerides; ix) blood glucose; x) systolic blood pressure (SBP); and xi) diastolic blood pressure (DBP).

2.6. Evaluation of the methodology of the studies selected

The methodological quality of the selected studies was assessed with the Cochrane risk-of-bias tool [35] that includes the following parameters: [1] random sequence generation (selection bias); [2] allocation concealment (selection bias); [3] blinding of participants and personnel (performance bias); [4] blinding of outcome assessment (detection bias); [5] incomplete outcome data (attrition bias); [6] selective reporting (reporting bias) and [7] other bias. For each study, each item was described as having either a low risk of bias, an unclear risk of bias or a high risk of bias. Risk of bias was assessed independently by two authors (JARA and DJRC) using the Cochrane risk-of-bias tool [35].

2.7. Data synthesis and statistical analysis

The meta-analysis and the statistical analysis were conducted using the Review Manager software (RevMan 5.2; Cochrane Collaboration, Oxford, UK). A random effects meta-analysis was conducted to determine the effect of HC on body composition (BMI, waist, W/H ratio, muscle mass, fat mass, and body mass) and cardiometabolic markers (LDL, HDL, triglycerides, blood glucose, SBP and DBP. The effects sizes of outcomes between hypoxic and normoxic conditioning as well as the differences between before and after training intervention were expressed as standard mean differences (SMD) and their 95% confidence intervals (CI). The threshold values for SMD were > 0.2 (small), > 0.6 (moderate), > 1.2 (large), and > 2.0 (very large). Also, the mean

Table 1
Main characteristics of included studies in the meta-analysis.

Study	Participants characteristics				Intervention				
	Participants	Age	Weight	BMI	Exposure type	Type of training	Protocol	Duration	FiO_2
Fernández-Menéndez et al. [17]	12 (F), 2 (M)	34.8 (4.7)	96.8 (9.5)	34.1 (2.6)	Active	Aerobic	Walking 60 min	3 wk. 3 d/wk.	14.5
	11 (F), 2 (M)	32.2 (8.4)	96.6 (9.6)	32.9 (2.7)	–			60 min/s	20.9
Kong et al. [21]	5(M), 5(F)	19.8 (2.2)	99 (19.5)	34.7 (5.3)	Active	Aerobic + strength	Aerobic: Running, Cycling. Stepping (60–70% of HRmax) Strength: Strength training at 40–50% 1R, 4–6 training motions,	4 wk.; 8 s of N (16 h) and 3 s of H (6 h) per wk	16.4/ 14.5
	5(M), 3(F)	22.3 (1.7)	103.4 (24.7)	33.8 (5.6)	–		3 × 15–20 reps Dumble: 4–6 motions,3 × 10–15 reps with light weight exercise (< 5 lb)	11 s of N (22h)	21
Shin et al. [23]	8(M)	45.6 (20.9)	78.3 (8.4)	26.8 (2.3)	Active	Aerobic	Running in treadmill at 60% of the maximum HR 50′ (Included 5′ warm-up; 40′ main set and 5′ cold down)	4 wk. 3 d/wk	14.5
	9 (M)	46.0 (20.5)	79.8 (11.0)	27.0 (3.0)	–				20.9
Netzer al. [20]	2 (M),8 (F)	50.1	89.8 (7.4)	33.4 (3.3)	Active	Aerobic	90 min at 60% of the maximum HR stepper, treadmill, bicycle ergometer	8 wk. 3 d/wk	15
	2 (M), 8 (F)	45.5	91.5 (10.7)	32.8 (7.1)	–				20.1
Yang et al. [38]	8 (M), 8 (F)	14.3 (1.4)	93.0 (15.1)	32.9 (3.5)	Passive	Aerobic	Training low: Swimming and basketball (6 MET) aerobic exercise (7.5 MET)	4 wk. 6 d/wk.	14.7
	11 (M), 8 (F)	13.9 (0.9)	86.9 (12.8)	31.5	–		Living High: Sleeping during 10 h	2 S/d	21
Wiesner et al. [16]	10 (M), 14 (F)	42.2 (0.2)	93.4 (2.6)	33.1 (0.3)	Active	Aerobic	60 min in a treadmill at HR of 65% of maximum oxygen consumption	4 wk. 3 d/wk	15
	8 (M), 13 (F)	42.1 (1.7)	87.5 (3.6)	32.5 (0.8)	–				21
Camacho-Cardenosa et al. [10,28]	13 (F)	44.4 (7.2)	80.1 (18.9)	30.0 (6.4)	Active	Aerobic	X intervals: 3 min at 90% Wmax followed by 3 min of active recovery (55–65%Wmax)	12 wk. 3d/wk.	17.2
	13 (F)	43.1 (7.7)	80.4 (16.3)	29.6 (5.2)	–			N° intervals: wk. 1–2: 3 wk. 3–5: 4	20.9
	18 (F)	37.4 (10.2)	73.7 (11.1)	27.7 (4.5)	Active	HIIT	X intervals: 30 s of all-out (130%Wmax) followed by 3 min of active recovery at 55–65% Wmax	wk. 6–8: 5 wk. 9–12: 6	17.2
	15 (F)	40 0.0 (8.7)	77.9 (11.3)	28.7 (4.8)	–				20.9
Kong et al. [29]	14 (F)	–	69.5 (5.8)	26.0 (2.4)	Active	HIIT	60 × 8 s all out in a bike ergometer with 12-s of recovery	5 wk. 4 d/wk	15
	15 (F)	–	68.2 (8.1)	25.7 (2.2)	–				21
Gatterer et al. [19]	12 (F), 4 (M)	50.3 (10.3)	105.5 (20.0)	37.9 (8.1)	Active + Passive	Aerobic	90 min at 65–70% of maximum HR (cycle ergometer, treadmill or cross trainer) and rested for additional 90 min in normobaric hypoxic chambers*	8 months 2 d/wk	14
	10 (F), 6 (M)	52.4 (7.9)	103.2 (15.1)	36.3 (4.0)	–		When treadmill or cross trainer was used, the target HR was increased by 10 beats/min		21
De Groote et al. [22]	3 (M), 4 (F)	14.3 (1.1)	104.6 (13.6)	37.9 (8.1)	Active	Aerobic + Strength	Aerobic: 12 min on a cycloergometer: S1: 2 min at 50%MAP and 10 min at 70%MAP.	6 wk. 3 d/wk.	15
	3 (M), 4 (F)	13.7 (1.39	97.5 (25.9)	36.3 (4.0)	–		S2: 2 min at 50% MAP and 5 × 1 min 80%–1 min 50% MAP S3: incremental training started at 40% MAP with an increase of 10% MAP each 2 min. Strength: abdominal. Quadriceps. and biceps muscles (15 repetitions at 50% 1RM + 4 × 6 repetitions at 70% 1RM; resting time: 2 min)	50–60 min per S	21

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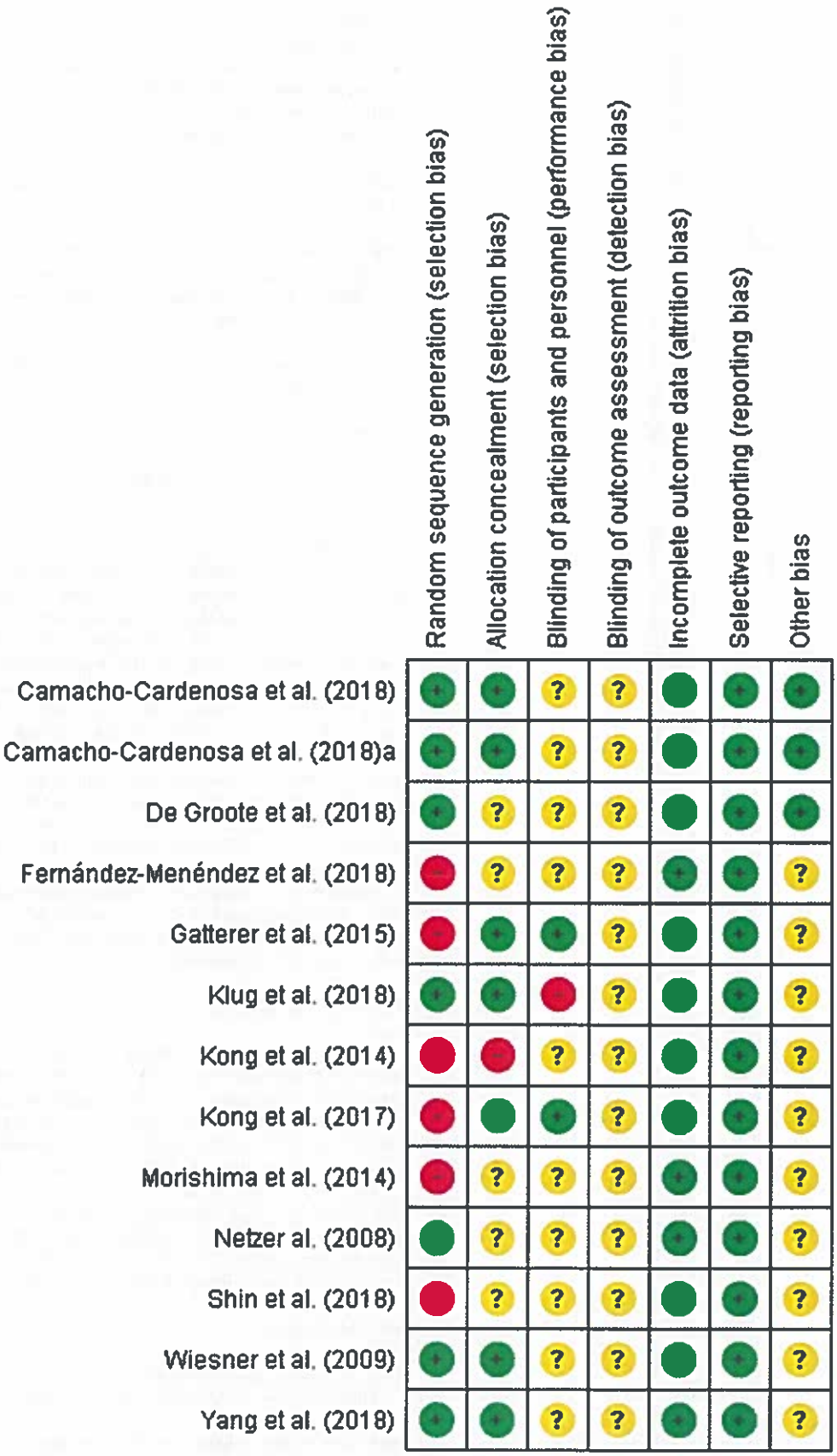

Fig. 2. Assessment of risk of bias in included randomized controlled trials.

Table 1 (*continued*)

Study	Participants characteristics				Intervention				
	Participants	Age	Weight	BMI	Exposure type	Type of training	Protocol	Duration	FiO_2
Morishima et al. [18]	9 (M)	30.0 (2.0)	74.4 (4.2)	25.6 (1.2)	Active	Aerobic	60 min cycling at 55% of the maximal oxygen uptake	4 wk. 3 d/wk.	15
	11 (M)	32.0 (3.0)	73.8 (4.0)	25.4 (0.9)	-			60 min per S	21
Klug et al. [25]	12 (M)	55.0 (2.1)	109.1 (5.2)	35.5 (1.4)	Active	Aerobic	60 min with 3 × 15 min of walking on a treadmill with 5 min of rest	6 wk. 3 d/wk.	15
	11 (M)	57.6 (2.2)	108.5 (3.0)	34.1 (0.9)				60 min per S	21

M: male; F: female; wk.: weeks; S: sessions; d: days; H: Hypoxia; N: Normoxia; FiO_2: inspired fraction of oxygen; kg: kilogram; HR: Hear rate; 1RM: one-repetition maximum; *: Only for hypoxic group; HIIT: High intensity interval training; MAP: maximal aerobic power; Wmax: maximal power achieved during the last 3 min step complete during the incremental test; Mean (standard deviation).

difference (MD) was used when all the studies assessed the same outcome and measured it in the same way. Each difference of the means was weighed according to the inverse variance method [36].

The heterogeneity between the studies was evaluated through the I^2 statistic, and between-study variance using the tau-square (Tau^2) [37]. I^2 values of 30–60% represented a moderate level of heterogeneity. A $p < .1$ value suggests the presence of substantial statistical heterogeneity. The publication bias was evaluated through an asymmetry test as estimated from a funnel plot. In addition, the Egger's test was used to assess publication bias. A $p < .05$ value was considered to be statistically significant. Finally, subgroup analyses were used to find the effects of the initial BMI (individuals with overweight versus individuals with obesity) of the individuals on the effectiveness of the HC. The cut-off value of the BMI variable was: individuals with overweight (25 kg/m^2 < BMI < 30 kg/m^2) and individuals with obesity (BMI > 30 kg/m^2). The effects were expressed as SMD and MD and their 95% of confidence intervals.

3. Results

3.1. General characteristics of studies

The initial search identified 2004 articles from databases and 1 article from other sources. After excluding duplicate articles, 1717 article abstracts were screened. Thereafter, 1700 articles were excluded and 17 were screened as full-texts. Finally, 13 articles [10,16–23,25,28,29,38] that met the inclusion criteria were left, and these were selected for the meta-analysis (Fig. 1). The effects of HC (passive and active) on body mass loss were analyzed in 13 articles and 336 participants. The analysis of secondary outcomes is based on the following number of studies: BMI = 10; waist = 7; W/H = 5; muscle mass = 10; fat mass = 11; LDL = 8; HDL = 8; triglycerides = 11; blood glucose = 8; DBP = 7; SBP = 7. The number of participants analyzed in these secondary outcomes ranged between 66 and 166 participants. All selected studies were published between 2009 and 2018.

Table 1 provides an overview of the intervention and participants characteristics of the studies included in the quantitative analysis (meta-analysis). The age and BMI ranged from 13.7 to 52.4 years and 25.7 to 38.6 kg/m^2, respectively. The exercise program duration ranged from 3 to 34 weeks and from 2 to 12 sessions per week. Also, the FiO_2 applied in normoxic and hypoxic groups ranged from 20.0 to 20.9% and from 12.2 to 17.2%, respectively.

3.2. Risk-of-bias assessment

Risk-of-bias assessment is shown in Fig. 2. Overall, the risk of bias was 'high' in all studies due to lack of random sequence of participants, the allocation concealment and the blinding of participants and researchers to assigned training conditions. The regression test funnel plot asymmetry showed no significant heterogeneity for the following body composition outcomes: BMI (Z = 0.600, $p = .549$), muscle mass (Z = 0.169, $p = .866$), fat mass (Z = 0.416, $p = .677$) and body mass (Z = 0.502, $p = .615$). However, significant heterogeneity was observed in the following cardiometabolic outcomes: triglycerides (Z = 4.504, $p \leq .001$), LDL (Z = 3.626, $p \leq .001$) and HDL (Z = 2.522, $p = .012$) and blood glucose (Z = 4.148, $p < .001$).

3.3. Meta-analysis

3.3.1. Effects on body composition

Regarding body composition variables, a significant body mass loss was found in participants who trained under normoxic (MD = −1.61, 95% CI = −2.90, −0.33, p = .01; $I^2 = 0\%$, $p = .99$) and hypoxic (MD = −1.42, 95% CI = −2.76, −0.09, $p = .04$; $I^2 = 0\%$, $p = .98$) conditions. In addition, significant decreases in fat mass were found in participants who trained under hypoxia (SMD = −0.26, 95%

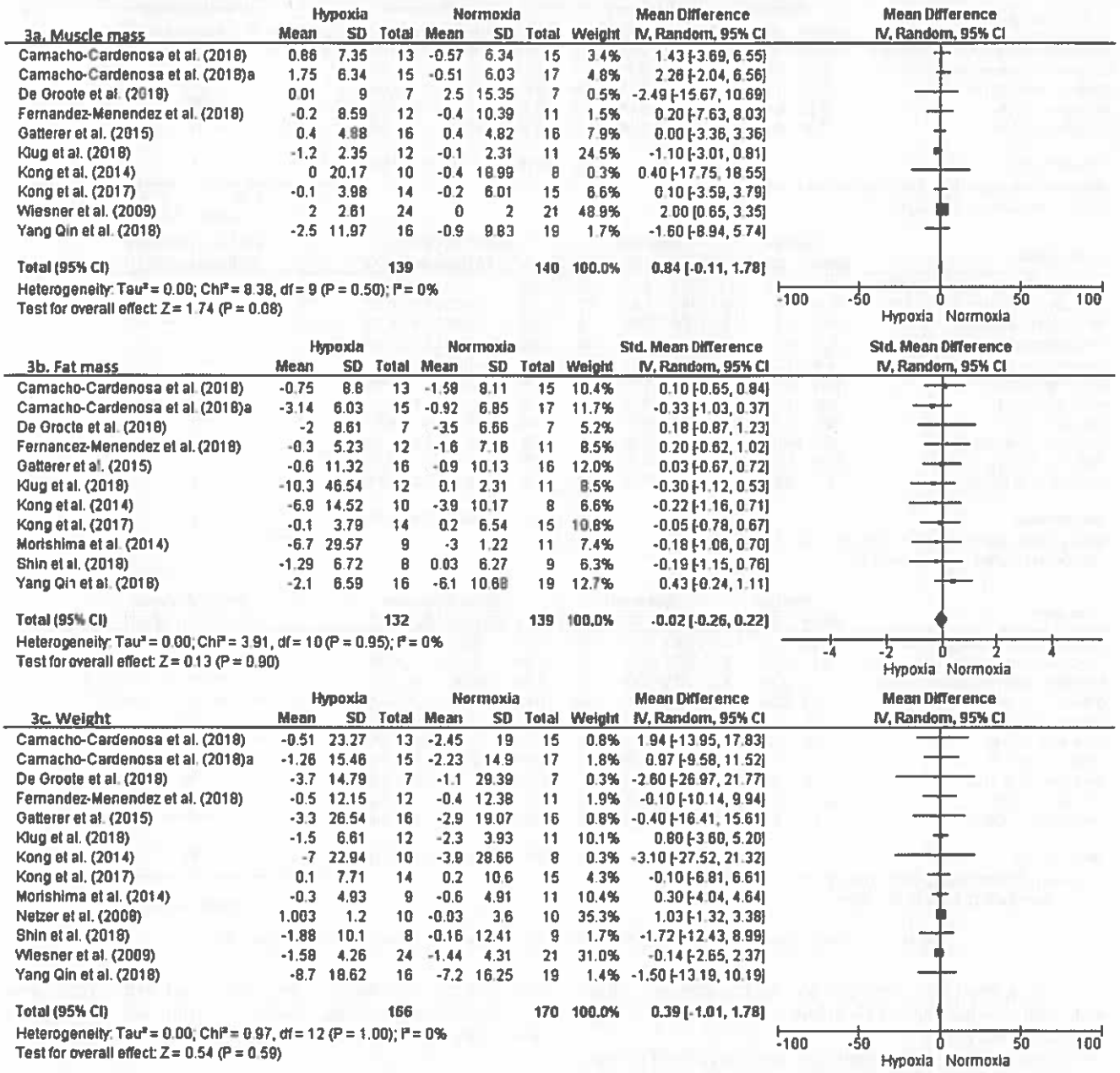

Fig. 3. Total effects of treatment on muscle mass (a), fat mass (b) and weight (c) hypoxic group vs. normoxic group.

CI = −0.50, −0.01, p = .04; I^2 = 0%, p = .99) but not in normoxia. Also, BMI (MD = −0.54, 95% CI = −1.01, −0.07, p = .03; I^2 = 0%, p = .75) decreased significantly in normoxic but not in hypoxic condition. Moreover, no significant post-training changes were observed on muscle mass in normoxic (p = .86) and hypoxic (p = .47) conditions. However, a trend towards higher muscle mass gain in hypoxic than in normoxic condition (p = .08) was observed. Furthermore, no significant differences between conditions were observed for fat mass (p = .90) and body mass changes (p = .59) (Fig. 3).

The W/H ratio decreased after both normoxic (MD = −0.02, 95% CI = −0.03, −0.01, p = .003; I^2 = 0%, p = .86) and hypoxic (MD = −0.02, 95% CI = −0.04, −0.01, p < .001; I^2 = 0%, p = .76) conditioning, yet with no statistical significant differences between conditions (Fig. 4a). Likewise, waist circumference decreased to the same extent in hypoxic (MD = −3.52, 95% CI = −4.75, −2.30, p < .001; I^2 = 0%, p = .86) and normoxic (MD = −2.09, 95% CI = −3.37, −0.81, p = .001; I^2 = 0%, p = .99) conditions (Fig. 4b). BMI decreased significantly after training under normoxic (MD = −0.50, 95% CI = −0.98, −0.03, p = .04; I^2 = 0%, p = .73) but not hypoxic condition (Fig. 4c).

3.3.2. Effect on cardiometabolic markers

A significant decrease in triglycerides was observed after training under hypoxic (SMD = −0.67, 95% CI = −1.02, −0.32, p < .001; I^2 = 41%, p = .09) and normoxic (SMD = −0.57, 95% CI = −0.98, −0.15, p = .008; I^2 = 61%, p = .006) conditions (Fig. 5a), also with a more favourable effect (p = .06; Chi^2 = 8.20, p = .61) due to hypoxia.

LDL decreased in normoxia (SMD = −0.46, 95% CI = −0.75, 0.17, p = .002; I^2 = 0%, p = .60) and hypoxia (SMD = −0.51, 95% CI = −0.9, −0.12, p = .01; I^2 = 41%, p = .12), with no difference between conditions (Fig. 5b). Compared to before, no significant HDL level differences occurred after training in either normoxic

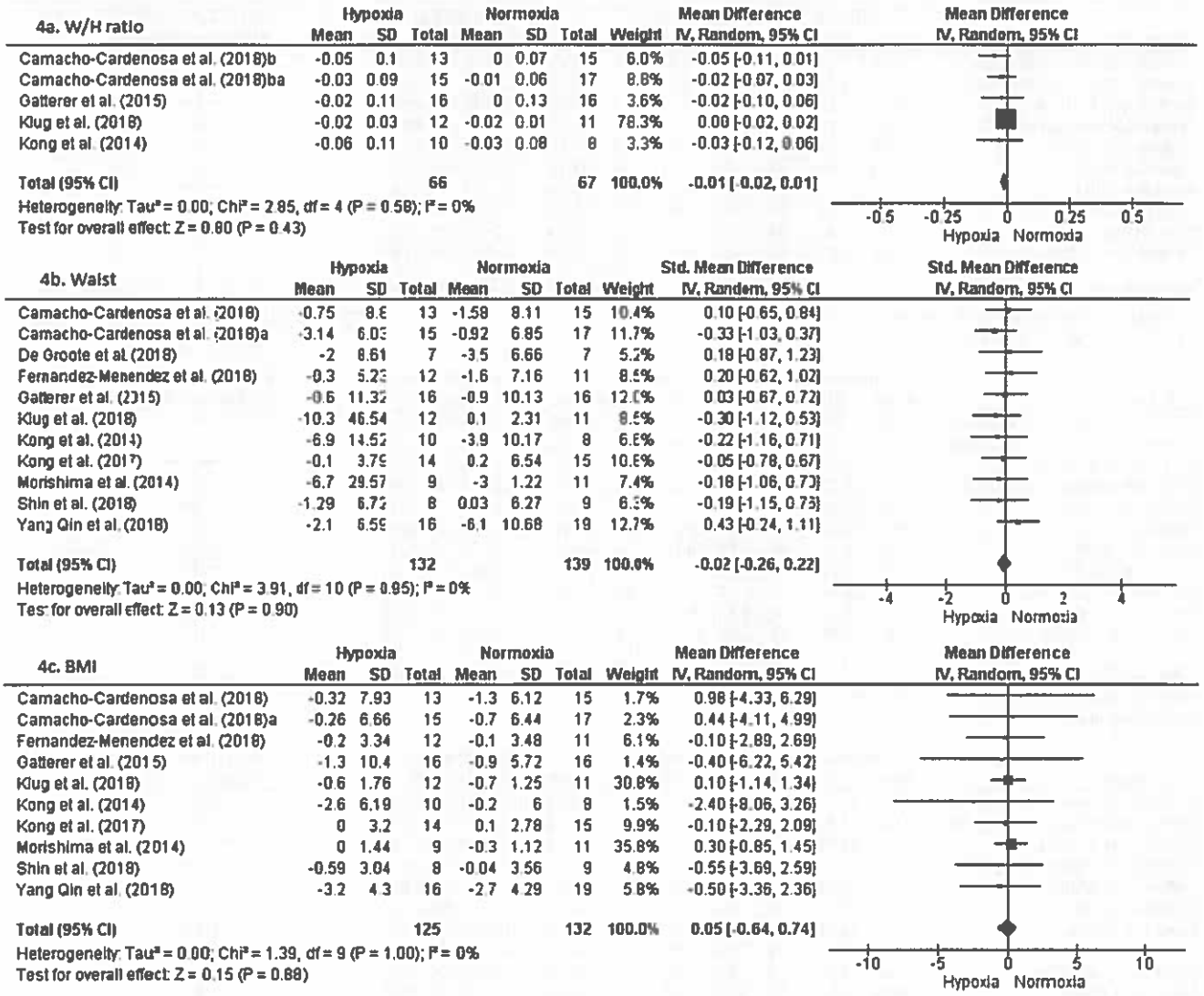

Fig. 4. Total effects of treatment on W/H ratio (a), waist (b) and BMI (c) hypoxic group vs. normoxic group.

(SMD = −0.14, 95% CI = −0.49, 0.21, p = .42; I^2 = 28%, p = .22) or hypoxic (SMD = −0.20, 95% CI = −0.66, 0.26, p = .40; I^2 = 55%, p = .04) conditions (Fig. 5c).

After training under hypoxic conditions, there was a trend towards lower blood glucose levels (SMD = −0.39, 95% CI = −0.79, 0.02, p = .06; I^2 = 48%, p = .06), while no change were observed in normoxia (SMD = −0.38, 95% CI = −0.97, 0.21, p = .21; I^2 = 77%, p < .001) (Fig. 5d).

DBP was lowered after training in normoxia (MD = −2.99, 95% CI = −5.52, −0.47, p = .02; I^2 = 58%, p = .03) and hypoxia (MD = −2.67, 95% CI = −3.59, −1.76, p < .01; I^2 = 0%, p = .61), yet with no significant differences between conditions (Fig. 6a). Similarly, SBP was similarly decreased after training in normoxia (MD = −6.08, 95% CI = −11.19, −0.97, p = .02; I^2 = 76%, p < .001) and hypoxia (MD = −4.96, 95% CI = −7.90, −2.02, p < .01; I^2 = 49%, p = .07) (Fig. 6b).

3.4. Sub-analysis

When a statistical comparison between individuals with overweight and obesity was performed, no significant differences were observed between conditions on fat mass (SMD = −0.01, 95% CI = −0.25, 0.23, p = .29; I^2 = 0%, p = .96), body mass (MD = 0.01, 95% CI = −0.20, 0.23, p = .86; I^2 = 0%, p = 1.0) and muscle mass (MD = 0.83, 95% CI = −0.1, 1.77, p = .86; I^2 = 0%, p = .49).

4. Discussion

This systematic review with meta-analysis aimed to analyse the effect of HC as a means of further reducing body mass and improving cardiometabolic markers compared to similar training near sea-level. A secondary objective was also to examine if this intervention is more effective in overweight versus obese individuals. The major findings indicate that HC significantly reduces body mass, fat mass, W/H ratio, waist circumference and improve several cardiometabolic markers (triglycerides, LDL, HDL, SBP and DBP). However, only the magnitude of triglycerides decrease and muscle mass growth were greater in hypoxic than in normoxic condition. Moreover, the sub-analysis found no significant interaction for initial BMI level indicating that HC effects were similar in overweight and obese individuals.

4.1. Effect of HC on body composition

We observed a significant positive effect on body mass loss for both

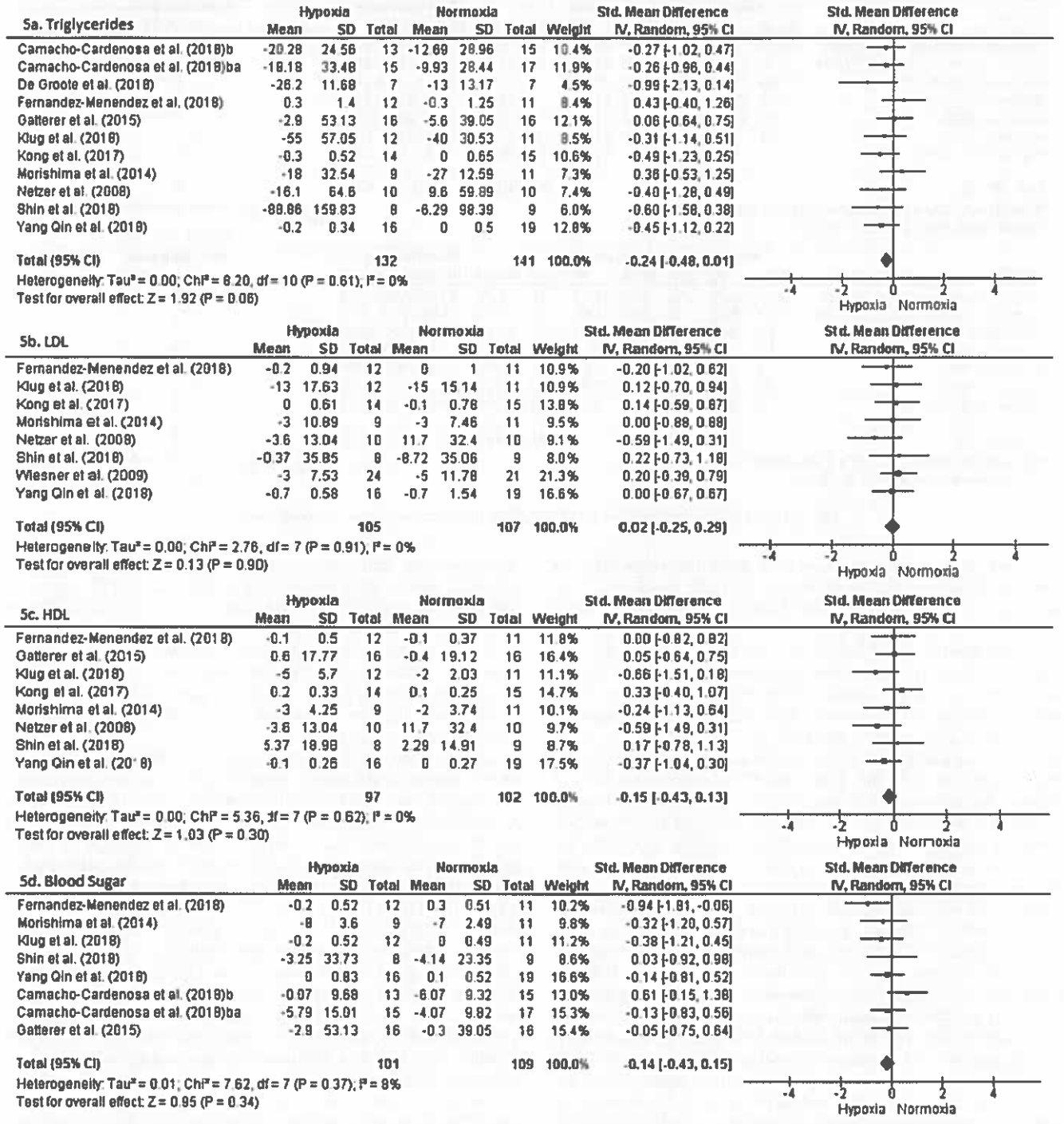

Fig. 5. Total effects of treatment on triglycerides (a), LDL (b), HDL (c) and blood glucose (d) hypoxic group vs. normoxic group.

hypoxic and normoxic conditioning, with also no significant differences between conditions (MD = 0.39, 95% CI −1.01, 1.78). A close inspection of the literature highlights four separate studies (3 with active and 1 with passive HC) reporting significantly larger body mass loss in hypoxia versus normoxia [20,21,23,38], while three other studies using active HC displayed similar body mass losses in the two conditions [19,22,25]. One possible explanation for these discrepant findings may relate to the hypoxic dose during the session and the entire HC program. In general, studies reporting no body mass loss had HC session with shorter duration [28,29] (i.e less than one hour) and/or had a lower total number of hours of hypoxic exposure during the program (i.e 9 [17] to 12 h [18]). Also, HC protocols that increase basal metabolic rate and energy expenditure likely benefit body mass loss in individuals with overweight and obesity [3]. Pending confirmatory research, this metabolic rate increment could result from an optimization of substrate utilization and mitochondrial oxidative capacity via signalling pathways that stimulate GLUT-4 transport [39]. This supports a view that prescribing HC with an appropriate dose may be relevant in individuals with overweight and obesity to lose more body mass.

Our meta-analysis showed a trend towards higher muscle mass gain in hypoxia versus normoxia (Z = 1.74; p = .08). Increased muscle growth is a positive adaptation in individuals with obesity who are

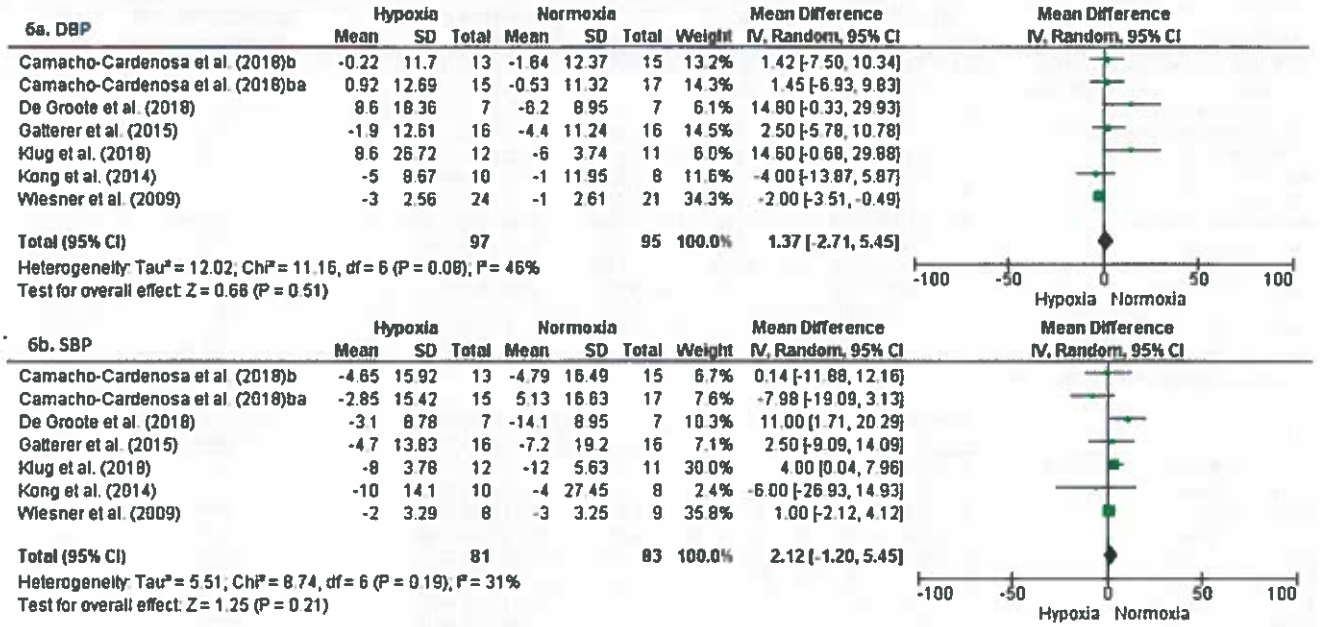

Fig. 6. Total effects of treatment on DBP (a) and SBP (b) hypoxic group vs. normoxic group.

commonly suffering from sarcopenia [40]. Regarding muscle mass, we report three separate studies displaying significant increases (2–4%) following active HC [10,16,28] or decreases (−1.5%) following passive [38] HC, while no changes occurred in normoxic condition. Another study also showed significant improvements in both normoxic and hypoxic conditions [22]. Disparate findings between studies could be due to alterations in the structure of the HC program (e.g., active or passive, intensity, FiO_2) performed. Specifically, passive HC seems to reduce muscle mass in the same terms as normoxia, while active HC would produce greater increases of muscle mass. Regarding active HC, only one study reported that four weeks of low intensity HC (65% of VO_2max) can improve fat-free mass (+2%) in hypoxia without changes in normoxic condition [16]. The most common type of exercise performed to increase muscle mass is resistance training. Reportedly, resistance training under hypoxia may lead to larger muscle gains than the same training in normoxia [41], primarily due to increases in metabolic stress and anaerobic glycolysis [41–43]. Other proposed mechanism involved in muscle growth are cellular swelling from metabolite accumulation in the cells and hypoxia-mediated increases in motor unit recruitment [41–44]. It is therefore possible that HIT may produce larger structural muscle adaptations by stimulating glucose-dependent metabolic pathways and consequently an acidic environment [45]. In fact, two of the studies with improved muscle mass [10,28] applied a HIT training of 12 weeks of duration at 17.2% FiO_2 increasing muscle mass by 2–4%. Taken as a whole, active HC at high-intensity may provide a small added benefit for muscular development over the same training performed in normoxia. An advantage of HC programs over normoxic training in patients who suffer from orthopaedic limitations is that this treatment may participate to reduce the risk of orthopaedic injury while also enhancing metabolic efficiency [28].

Previous studies reported that passive [38] or active [10,21,23,28] HC could significantly decrease fat mass. Using a meta-analytical analysis, our results showed a significant fat mass decrease in participants who trained under hypoxia but not in normoxia. This suggests a positive effect of active HC with a reduction of fat mass, which could possibly be attributed to higher post-exercise lipid oxidation [28]. In addition, a recent study [28] has shown an increase in fat oxidation at rest after 12 weeks of HIT in hypoxia, whereas an opposite trend was reported after the same training in normoxia. Thus, HIT in hypoxia likely increases lipids metabolism at rest. In addition, BMI has been significantly reduced after passive [38] or active [10,21] HC programs. However, our results indicate that training with oxygen deprivation was not more effective than in normoxia to reduce fat mass or BMI. While BMI is frequently used to estimate the prevalence of obesity [46] it does not account for variation in body fat distribution and abdominal fat mass [47]. Arguably, measurements of waist circumference and W/H ratio would be more appropriate measures of both intra-abdominal fat mass and total fat [48].

We report the original observation that waist circumference and W/H ratio decreased significantly after HC and normoxia. Interestingly, two separate studies [16,23] with decreases in waist circumference in hypoxia but not in normoxic condition, implemented a low intensity aerobic training (60 min on a treadmill at 65% of VO_2max at 14.5–15% of FiO_2). Another study [10] also demonstrated a significant decrease in waist circumference after training for 12 weeks using 30 s "all out" efforts performed at FiO_2 = 17.2%. These findings, suggest a positive effect of combined hypoxia with HIT for reducing abdominal fat, which could be attributed to higher post-exercise lipid oxidation [28]. However, a rapid plateau in the aforementioned body composition adaptations can occur if the program fails to apply an unaltered stimulus (i.e., hypoxic level, exercise intensity/duration) [19]. Such scenario has previously been reported by both Camacho-Cardeñosa et al. [10] and Gatterer et al. [19] who found similar improvements in body composition after completing either half (6 weeks and 3 months, respectively) or the entire (12 weeks and 8 months, respectively) conditioning program. Therefore, as for athletes, effective management of an HC program undoubtedly requires periodization strategies and readjusting regularly the training stimulus during the intervention.

In relation to the principle of initial value, a previous study [49] reported that the magnitude of body mass loss could largely be due to initial body composition. In support, those individuals with greater initial body fat and BMI values also were those who lost more body mass and fat after a combined exercise/diet intervention compared with those with a lower BMI [49]. In our review, overweight participants on average lost less body mass than individuals with obesity (−0.8 and −3.2 kg respectively) after HC. Similar results were obtained after low intensity HC comparing overweight vs normo-weight individuals [23]. However, the differences across the two BMI groups (individuals with obesity vs with overweight) observed in the present

review were small (no statistical differences). These results are in accordance with a previous review showing that initial BMI was not related to body mass loss during an intervention [50]. In this way, HC appears equally effective to body mass loss for individuals with overweight and obesity.

4.2. Effect of HC on cardiometabolic markers

Our study showed a higher no significant decrease of triglycerides after training under hypoxic than normoxic condition ($p = .06$). Three studies [10,23,38] found a larger decrease in this variable after HC than in normoxia. Interesting, the higher decrease was observed in Camacho-Cardeñosa's study [10]: i) -24.5% after 12 weeks of HIT in hypoxia using either 30-s "all out" efforts with 3 min of active recovery and ii) -27.5% after 12 weeks of training using 3 min at 90% of peak power with 3 min of active recovery both at 17.2% of FiO_2. The interplay of mechanisms of HC which may improve some cardiometabolic markers such as triglycerides and cholesterol levels are still being elucidated. However, exercise protocols increasing post-exercise lipid oxidation also seem to decrease triglyceride levels [51]. Similarly, the mechanism by which HC reduces triglycerides levels likely include increased lipid oxidation through the transcription coactivator PGC1α [14], which plays a key role in the regulation of muscle fatty acid oxidation [52]. Therefore, the use of high-intensity HC represents an effective method to increase post-exercise lipid oxidation and to reduce triglycerides values. In order to obtain a positive HC-related effects on lipid-related metabolic markers, interventions lasting at least 4 weeks would be required [5]. Our novel findings support this suggestion since the previous studies which demonstrated a significant improvement in triglycerides ranged between 4 [23,38] and 12 weeks [10,28] in duration.

Regarding cholesterol variables, our analysis showed significant decrease in LDL values after hypoxia and normoxia with no difference between conditions. In addition, our meta-analysis showed no significant increases in HDL after training in either normoxic or hypoxic conditions. Previous studies confirm that the increases in energy expenditure associated with aerobic intensity have been shown to positively influence in LDL and HDL [53]. In fact, only one low intensity HC study [25] reported a decrease in LDL, but similar changes were also observed after normoxic conditions. In addition, it has been reported that intense exercise is required to elicit reductions in LDL [54]. However, none of the HC studies using a high-intensity training program led to an improvement in HDL and LDL. Therefore, we conclude that active and passive HC may not promote any additional effect than the same normoxic program on both HDL and LDL levels.

Morishima et al. [18] found a significant decrease in glucose concentration after hypoxic (−8%) and normoxic (−7%) active training (60 min cycling at 55% of the maximal oxygen uptake at 15% of FiO_2). Although, no other study found a significant decrease in blood glucose after HC, our meta-analysis reports a trend towards lower glucose concentrations under hypoxic ($p = .06$) but not normoxic environments. These findings are in accordance with previous studies that reported a reduction of blood glucose [55] after passive or active HC in rats, suggesting that insulin signalling and glucose may have been upregulated following HC [9]. Thus, comparted to normoxia, HC may improve glycaemic control in individuals with overweight and obesity [9].

Regarding blood pressure, we report similar decreases in both SBP and DBP for hypoxic and normoxic conditioning. Previous studies applying active HC have shown significant improvements in SBP compared with active normoxic condition [21]. Kong et al. [21] found a significant decrease in SBP (7.6%) after 4 weeks of aerobic and strength training in hypoxia (14.5–16.4% of FiO_2) but no difference in the normoxic condition. These findings suggest that normoxic and HC have similar effectiveness to reduce blood pressure in individuals with obesity.

We found no differences in cardiometabolic markers after HC according to the baseline BMI category when expressed as a percentage from baseline. Both, individuals with overweight and with obesity demonstrated similar magnitude of improvement in tryglycerides, blood pressure, LDL, HDL and blood glucose after HC and normoxic condition. These findings are previously reported by some studies [56] which found that BMI category does not alter the benefit of body mass loss intervention on cardiometabolic markers if the results are expressed proportionally to the baseline.

4.3. Limitations, future research and practical applications

We acknowledge several limitations of this meta-analysis, which are related in part to the available RCTs and the divergent methodologies employed, including (i) the small number of studies; (ii) the number of studies using passive HC ($n = 1$); (iii) the different intensities, volume and training characteristics procedures applied in active HC studies; (iv) the lack of systematic information about the obesity related symptoms separating individuals with overweight and obesity; (v) the small number of studies using high-intensity training to obtain a more specific picture about the effect of this type of training in hypoxia on body composition and cardiometabolic markers; and (vi) the lack of longer studies to analyse the chronic effect of HC (only two studies had a program duration of > 8 weeks). In addition, we found that the available evidence has high risk of bias primarily due to low quality of available RCTs. Therefore, before a more comprehensive picture is depicted, further studies with a better quality design, analysing the effect of intervention of longer duration (< 8 weeks) and applying high-intensity HC programs are needed.

Previous studies [5] recommend the use of low intensity active HC at the commencement of the training program. During the first step of the treatment, according to the individual's characteristics, it is recommended that the HC program characteristics should include the following features: 4–6 weeks of 2–3 sessions of 60–90 min at 55–65% of VO_2max/60–70% of maximum heart rate at 13–14% of FiO_2. To avoid a body mass loss plateau it is also necessary to implement a new training stimulus by using other types of training with increased exercise intensity. Specifically, HC should be designed to elicit higher post-exercise lipid oxidation to reduce fat mass and body mass and to increase metabolic stress under hypoxia to maximize muscle growth. Considering the findings from studies [10,28,29] which have demonstrated benefits for HC, high intensity training may produce these two responses. In doing so, HIT sessions should include a duration of 30–60 min per session, using intervals of 8–30 s all-out followed by 3 min of active recovery at 55–65% of peak power performed 3–4 times per week. HIT should be undertaken in moderate level hypoxia (FiO_2 = 14–17.2%) though it is not known whether a dose–response relationship exists for the level of hypoxia on body mass loss. Finally, HIT sessions in hypoxia should be included progressively as a second step in the training program and always in combination with other sessions of aerobic training.

5. Conclusion

We conclude using a systematic review with meta-analysis that HC does result in significant reductions in body mass, fat mass, W/H ratio, waist circumference and in several cardiometabolic markers (triglycerides, LDL, HDL, SBP and DBP). However, only the magnitude of reductions in triglycerides and greater muscle growth was greater in hypoxic than in normoxic condition. In addition, the usefulness of HC was similar in individuals with overweight and obesity.

Potential conflicts of interest

None declared.

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Original Research

Intermittent hypoxia training in prediabetes patients: Beneficial effects on glucose homeostasis, hypoxia tolerance and gene expression

Tetiana V Serebrovska[1], Alla G Portnychenko[1,2], Tetiana I Drevytska[1,2], Vladimir I Portnichenko[1,2], Lei Xi[3,4], Egor Egorov[5], Anna V Gavalko[6], Svitlana Naskalova[6], Valentina Chizhova[6] and Valeriy B Shatylo[6]

[1]Bogomoletz Institute of Physiology, Kiev 01024, Ukraine; [2]ICAMER, National Academy of Sciences of Ukraine, Kiev 03680, Ukraine; [3]Pauley Heart Center, Department of Internal Medicine, Virginia Commonwealth University, Richmond, VA 23298, USA; [4]School of Sports Medicine and Health, Chengdu Sport University, Chengdu 610041, China; [5]CELLGYM Technologies GmbH, Berlin 10623, Germany; [6]D.F. Chebotarev State Institute of Gerontology, Kiev 04114, Ukraine

Corresponding authors: Lei Xi. Email: lxi@vcu.edu; Tetiana V Serebrovska. Email: sereb@biph.kiev.ua

Impact statement

The present study investigated the beneficial effects of intermittent hypoxia training (IHT) in humans under prediabetic conditions. We found that three-week moderate IHT induced higher HIF-1α mRNA expressions as well as its target genes, which were positively correlated with higher tolerance to acute hypoxia and better glucose homeostasis in both middle-aged healthy and prediabetic subjects. This small clinical trial has provided new data suggesting a potential utility of IHT for management of prediabetes patients.

Abstract

The present study aimed at examining beneficial effects of intermittent hypoxia training (IHT) under prediabetic conditions. We investigate the effects of three-week IHT on blood glucose level, tolerance to acute hypoxia, and leukocyte mRNA expression of hypoxia inducible factor 1α (HIF-1α) and its target genes, i.e. insulin receptor, facilitated glucose transporter–solute carrier family-2, and potassium voltage-gated channel subfamily J. Seven healthy and 11 prediabetic men and women (44–70 years of age) were examined before, next day and one month after three-week IHT (3 sessions per week, each session consisting 4 cycles of 5-min 12% O_2 and 5-min room air breathing). We found that IHT afforded beneficial effects on glucose homeostasis in patients with prediabetes reducing fasting glucose and during standard oral glucose tolerance test. The most pronounced positive effects were observed at one month after IHT termination. IHT also significantly increased the tolerance to acute hypoxia (i.e. SaO_2 level at 20th min of breathing with 12% O_2) and improved functional parameters of respiratory and cardiovascular systems. IHT stimulated HIF-1α mRNA expression in blood leukocytes in healthy and prediabetic subjects, but in prediabetes patients the maximum increase was lagged. The greatest changes in mRNA expression of HIF-1α target genes occurred a month after IHT and coincided with the largest decrease in blood glucose levels. The higher expression of HIF-1α was positively associated with higher tolerance to hypoxia and better glucose homeostasis. In conclusion, our results suggest that IHT may be useful for preventing the development of type 2 diabetes.

Keywords: Intermittent hypoxia, diabetes, hypoxia inducible factor-1, hypoxia inducible factor-1-regulated genes, adaptation, hyperglycemia

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Introduction

The method of intermittent hypoxia training (IHT) is an emerging therapeutic modality for treatment and prevention of various human diseases and has gained increasing attention. The mechanisms underlying the beneficial effects of IHT have been investigated at the multiple biological levels, from systemic physiological reactions to genomic regulation.[1–5] The potential therapeutic uses of IHT in treating cerebrovascular and cardiovascular disorders have been the focal areas of extensive research.[6,7]

Despite these advances, the effects of IHT on diabetes mellitus, especially type 2 diabetes, one of the most prevalent pathological conditions in the current world population, are much less investigated.[8] In the mid-1990s, Ukrainian scientists first demonstrated in diabetic animals that IHT could reduce vascular risk factors and increases blood insulin levels via inhibition of the islet destruction

and promotion of new beta-cell formation in acinar tissue.[9] These authors recently confirmed that two-week IHT led to an increase in the area of pancreatic islets and the number of β-cells in diabetic rats, mainly due to a significant reduction of β-endocrine cells apoptosis. The positive effect was maintained for at least 10 days.[10] These findings in preclinical animal studies suggested possible utilization of IHT in control or treatment of type 2 diabetes and its associated insulin resistance. It is notable that the favorable effects of IHT on glucose metabolism were also suggested.[11,12] In particular, it was shown that hypoxic training increased glycolytic enzyme activities, enhanced the number of mitochondria in skeletal muscles, and improved insulin sensitivity as well.[13,14]

Glucose-lowering effects of IHT were also previously reported in diabetic patients[15,16] and the beneficial effect is particularly important in elderly population with higher risks in developing diabetes.

Moderate levels of intermittent hypoxia mobilize genome that in turn activates a cascade of intracellular signaling transduction, which involves various receptors, mitochondrial respiratory chain, key intracellular regulatory systems, early genes, superfamilies of the inducible and activation transcription factors, which are sequentially engaged in the processes of initiation and induction of hypoxic tolerance. One of the key regulators of oxygen homeostasis under hypoxic conditions is hypoxia inducible factor (HIF), which initiates transcriptional activation of numerous target genes to improve oxygen delivery and utilization[17] as well as glucose homeostasis.[12,18,19] Delicate balance exists between HIF-1 level and optimal metabolic functions.[20,21] Malfunction of these relations leads to hyperglycemia and type 2 diabetes. Based on these results, we have suggested that the use of IHT for treatment of patients with prediabetic abnormalities can improve carbohydrate metabolism and lead to prevention of diabetes development.

Among the HIF-1α target genes, energy-independent facilitative glucose transporter-1 (GLUT-1; encoded by solute carrier family-2 gene SLC2A1) is one of most important for regulating glucose metabolism which predominates in many types of human cells[22] and is the only vehicle that transports glucose into the brain.[23] GLUT-1 mediates glucose uptake increasing intracellular glucose levels to be used by glycolysis and other metabolic pathways.[24,25] GLUT-1 is upregulated under hypoxia, and its activity depends of the severity of hypoxic impact.[26,27]

Another HIF-1α target gene important for glucose homeostasis is insulin receptor (INSR). Insulin exerts its physiological effects through this member of tyrosine kinase family of transmembrane signaling proteins encoded by a single gene INSR.[28] Increase of INSR level could alleviate insulin resistance. Recent studies indicated that INSR is expressed at higher levels under hypoxic stress[29] and overexpression of INSR improves obese and diabetic phenotypes in mice.[30]

ATP-sensitive potassium (K_{ATP}) channels are also involved in the regulation of insulin secretion in the β-cells of pancreas. It couples cell metabolism to electrical activity of the plasma membrane by regulating membrane K^+ fluxes.[31,32] K_{ATP} channels also play important role in adaptation to intermittent hypoxia.[33,34] One of the pore forming subunits of K_{ATP} channels is encoded as KCNJ8 (potassium inwardly-rectifying channel, subfamily J), also known as KIR6.1.[35]

Under the context, the present study was designed to investigate the effects of a three-week session of IHT on blood glucose and hypoxic tolerance in healthy humans and patients with prediabetes. Furthermore, we focused on the effects of IHT on mRNA expression of HIF-1α and its targeted genes, such as INSR, SLC2A1, and KCNJ8.

Materials and methods

Characteristics of participants

Seven healthy volunteers (44–68 years, 3 males and 4 females) and 11 prediabetic patients (48–70 years, 5 males and 6 females) participated in the current study. The prediabetes patients were diagnosed using the criteria issued by the American Diabetes Association. We included patients who had an elevated fasting glucose level (5.6 to 6.9 mmol/L), impaired glucose tolerance (i.e. plasma glucose level of 7.8 to 11.0 mmol/L 2 h after an oral dose of 75 g glucose challenge), or their combination. Subjects in the healthy control group had no cardiovascular, respiratory, endocrine or central nervous system disorders and their fasting glucose concentration was less than 5.6 mmol/L and less than 7.8 mmol/L 2 h after a standard glucose tolerance test.

This clinical study was conducted under the state laws of Ukraine and the ethical principles of the 1964 Declaration of Helsinki. The research protocols, patient health information and informed consent forms were approved by the Ethics Committee of Chebotarev Institute of Gerontology, Kiev, Ukraine. All subjects received detailed information of the study process and a written informed consent was obtained from each of the participated subjects. All participants were nonsmokers and did not take any medication two weeks prior to and during the sessions of the study. They had no infections during the past month and no major cardiovascular or respiratory complications. All subjects underwent measurements of several anthropometric variables (Table 1), which indicated no significant difference in age and height between healthy and prediabetes groups, whereas the body weight, body mass index, and waist circumference were higher in the prediabetes patients than those of healthy control subjects. All participants were informed about the strict observance of lifestyle one month prior to, during, and one month after IHT (including levels of physical activity, caloric content of daily diet, consumption of coffee and tea, abstinence of alcohol, etc.). They kept diaries in which they noted any lifestyle changes, if occurred. Any violation of the regime by the subjects resulted in exclusion from the study.

Experiment protocols

All sessions of the present study were conducted in a quiet room at a temperature of 22–23°C within a clinical research center of the Chebotarev Institute of Gerontology.

Table 1 Anthropometric characteristics of the participants[a]

Groups	Gender (female/male)	Age (year)	Height (cm)	Weight (kg)	BMI (kg/m^2)	Waist (cm)
Healthy	5/2	58.7 ± 11.8	170 ± 15	76.1 ± 17.3	27.2 ± 6.4	93.7 ± 9.2
Prediabetes	7/4	66.4 ± 5.2	167 ± 10	90.2 ± 9.9	33.2 ± 5.6	99.7 ± 8.9
Healthy vs. prediabetes		NS	NS	$P < 0.05$	$P < 0.05$	$P = 0.05$

BMI: body mass index; waist: waist measurements; NS: no significant difference.
[a]Data are mean ± SD. Student's *t*-test was used to evaluate the statistical significance of the differences between healthy and prediabetes groups.

Table 2 Experimental timetable of the procedures, sample collections, and functional tests before, during, and after the IHT sessions

	Procedures and tests				
Date of investigation	Venous blood sampling for mRNA assays	Fasting blood glucose and OGTT	Cardio-vascular parameters	Acute hypoxic test	IHT sessions
Monday (week 1, 2 days before IHT start)	+	+			
Tuesday (week 1, 1 day before IHT start)			+	+	
Wednesday (week 1), Friday (week 1), Monday (week 2)					+++
Tuesday (week 2, 24 hours after IHT)	+				
Wednesday, Friday (week 2), Monday, Wednesday, Friday (week 3), Monday (Week 4)					++++++
Tuesday (week 4, 24 hours after IHT)	+	+			
Wednesday (week 4)			+	+	
Tuesday (1 month after IHT)	+	+			
Wednesday (1 month after IHT)			+	+	

IHT: intermittent hypoxic training; OGTT: oral glucose tolerance test.

The logistic plan and timetable of the studies are summarized in Table 2. Measurement sessions were performed during two days before IHT course, one day and one month after the termination of IHT. For determination of HIF-1α mRNA expression and its target genes in blood leukocytes, venous blood samples were collected again next day after one-week IHT course. Patient examination included: (1) anthropometric measurements; (2) determination of HIF1α mRNA and its target genes; (3) standard oral glucose tolerance test (OGTT) with plasma glucose determination; and (4) acute hypoxic test (AHT) with measurements of routine cardiovascular parameters.

In the morning of the first experiment day, after three-day routine hospital diet (250–300 g carbohydrates) and normal physical activity, a venous blood sample was drawn under fasting condition from the median antecubital vein for measurement of fasting glucose level as well as genetic analysis. Thereafter, a standard OGTT was conducted according to Ryden *et al.*,[36] which used 75 g of glucose mixed in 250 mL of water. Venous blood samples were drawn at 120 min after the oral glucose ingestion. Plasma glucose concentrations were analyzed by glucose oxidase method in normoxic conditions on semi biochemical analyzer BTS-330 using reagents "Glucose", Bio LATEST Lachema Diagnostica.

Next day, after a light breakfast, the baseline cardiovascular parameters of the subjects were measured in a relaxing sitting position with spontaneous breathing of room air. Arterial blood oxygen saturation (SaO_2) and heart rate (HR) were recorded using a patient vital sign monitor UM 300-12 (UTAS, Ukraine, http://www.utasco.com). Systolic (SBP) and diastolic (DBP) blood pressure values were measured on brachial artery with a mercury sphygmomanometer (Erkameter 3000, Germany). After all the baseline tests, the participants were connected to an open breathing circuit through a mask to perform an AHT[37]: breathing a gas mixture with 12% O_2 for 20 min while monitoring the changes in the subject's cardiovascular parameters and SaO_2. This study analyzed the indices at the 20th min of the test.

From the next morning, after a light breakfast, all participated subjects received the sessions of IHT three times a week for the subsequent three weeks, i.e. each subject received total of nine sessions of IHT. Each session consisted of four cycles of 5-min hypoxia (12% inspired O_2) followed by 5-min normoxia (room air breathing). The normobaric hypoxia was administered to the subjects in sitting position, using a hypoxic apparatus—Hypotron® (Kiev Polytechnic Institute, National Technical University of Ukraine). The subjects' SBP, DBP, HR, and SaO_2 were

continuously monitored and recorded. Next day and one month after the end of three-week IHT, the post-test examinations were conducted in the same manner as the pre-test ones (Table 2).

Determination of gene expression

mRNA expression of HIF-1α, INSR, SLC2A1, and KCNJ8 was determined in circulating blood leukocytes collected in various time points using real-time polymerase chain reaction (RT-PCR) assay. Blood leukocytes were obtained by centrifuging the blood samples at 1500 g for 1.5 min. After centrifugation, supernatant with interphase fraction was collected and transferred in new tube. After a secondary centrifugation (3000 g for 3 min) the supernatant was removed, the precipitate was used for RNA isolation using phenol–chloroform extraction after homogenization with guanidine isothiocyanate (Trizol RNA Prep 100 Kit, Russian Federation). Total RNA concentration was determined with a spectrophotometer ND1000 (NanoDrop Technologies Inc., USA). cDNA was synthesized from 5 μg of total RNA by reverse transcription with 10 mmol/L Tris-HCl (pH 9.0), 5 mmol/L $MgCl_2$; 1 mmol/L dNTPs; 20 U Ribo-Lock, Random hexamer primers (0.5 $\mu g \cdot \mu L^{-1}$) and 200 U RevertAid H Minus M-MuLV Reverse Transcriptase. PCR was performed using an Applied Biosystems 2700 (PerkinElmer, USA).

Gene expression of HIF-1α (Assay ID: Hs00153153_m1), SLC2A1 (Hs00892681_m1), INSR (Hs00961554_m1), and KCNJ8 (Hs00958961_m1) was determined using TaqMan® Gene Expression Assay (Applied Biosystems, USA). The pairs of forward and reverse primers for genes above mentioned and the TaqMan® probes for the target mRNAs were designed by Applied Biosystems based on the human mRNA sequence. Gene expression in each probe was normalized with β-actin, using a TaqMan® human β-actin control reagent. The thermal cycles of PCR amplification consisted of initial denaturation step at 95°C for 20 s, followed by treatment at 95°C for 3 s, and at 60°C for 30 s and for 50 cycles using a 7500 Fast Real-time PCR equipment (Applied Biosystems). The cycle threshold is defined as the number of cycles required for the fluorescence signal to exceed the detection threshold. The expression level of each target gene was calculated relative to the housekeeping gene (β-actin) as the difference between the threshold values of the two genes. Each PCR step was performed in duplicate and the calculations were done using the 7500 Fast System SDS software (Applied Biosystems).

Statistical analysis

All data were analyzed using SPSS software version 21.0 (SPSS Inc., USA). Student's *t*-test was used to test anthropometric differences between healthy and prediabetes groups (Table 1). To evaluate the changes of blood glucose concentration over time in both groups (Table 3), two-way analysis of variance (ANOVA) with repeated measures was used followed by Bonferroni post hoc test to determine both group main effect (healthy vs. prediabetes) and time effect (for 3 time-points: Pre-IHT baseline, 1 day after IHT, 1 month after IHT). To assess the differences between physiological parameters at 20th min of acute hypoxia test before and after IHT (Table 4), three-way ANOVA with repeated measures and Bonferroni post hoc test was used to analyze both the group main effect, time effect, and hypoxia effect for each of dependent variables. Pearson product–moment correlation coefficient (r) was calculated to show the degree of linear relationship between variables. The level of statistical significance was set at $P < 0.05$. Data are expressed as mean ± SD.

Results

All subjects well tolerated the entire process of medical examination and IHT sessions. No subjective discomforts and/or any other adverse effects were reported.

Blood glucose level

Table 3 demonstrates the effects of IHT on blood glucose level in healthy subjects and prediabetic patients. Prior to

Table 3 Glucose blood serum concentration during oral glucose tolerance test (OGTT) before and after the three-week sessions of IHT[a]

	Healthy ($n=7$)	Prediabetes ($n=11$)	Group main effect healthy vs. prediabetes	Time effect 3 time-points	Group + time effect
Fasting glucose (mmol/L)					
Pre-IHT baseline	4.6 ± 0.4	5.6 ± 0.6	$F=25.967$	$F=0.845$	$F=1.084$
1 day after IHT	4.5 ± 0.3	5.4 ± 0.7	$P=0.000$	$P=0.434$	$P=0.348$
1 month after IHT	4.6 ± 0.5	5.2 ± 0.4*			
2 h post-OGTT glucose (mmol/L)					
Pre-IHT baseline	5.3 ± 1.5	7.9 ± 1.5	$F=25.757$	$F=2.590$	$F=9.570$
1 day after IHT	5.0 ± 1.2	7.0 ± 1.9*	$P=0.000$	$P=0.102$	$P=0.007$
1 month after IHT	4.9 ± 1.2	6.4 ± 1.0**			

IHT: intermittent hypoxic training; SaO_2: blood oxygen saturation; HR: heart rate; SBP: systolic blood pressure.
[a]Data are mean ± SD and were analyzed with two-way ANOVA with repeated measures.
*$P < 0.05$ versus pre-IHT baseline; **$P < 0.01$ versus pre-IHT baseline.

Table 4 Arterial blood oxygen saturation and cardiovascular indices at 20th min of acute hypoxia test (12% inspired O_2) before and after the three-week sessions of IHT[a]

	Healthy		Prediabetes						
	Normoxia	Hypoxia	Normoxia	Hypoxia	Group main effect	Time effect	Hypoxia effect	Time + Hypoxia Effect	Group + hypoxia + time effect
SaO_2 (%)									
Pre-IHT baseline	98.8 ± 0.6	80.9 ± 3.1	98.6 ± 0.5	80.3 ± 3.0	$F = 0.030$	$F = 20.247$	$F = 1536.695$	$F = 28.454$	$F = 0.038$
1 day after IHT	98.9 ± 0.5	86.1 ± 3.3*	98.7 ± 0.5	85.0 ± 2.8*	$P = 0.834$	$P = 0.000$	$P = 0.000$	$P = 0.000$	$P = 0.848$
1 month after IHT	98.8 ± 0.6	84.5 ± 3.2*	98.8 ± 0.6	83.8 ± 2.5*					
HR (beat per min)									
Pre-IHT baseline	65.4 ± 3.9	74.1 ± 12.2	69.8 ± 3.4	75.5 ± 4.6	$F = 1.354$	$F = 5.098$	$F = 15.328$	$F = 0.466$	$F = 0.018$
1 day after IHT	63.0 ± 2.9	68.4 ± 8.2*	66.4 ± 3.4	68.1 ± 7.6*	$P = 0.262$	$P = 0.020$	$P = 0.001$	$P = 0.666$	$P = 0.982$
1 month after IHT	64.5 ± 4.2	68.9 ± 7.4*	67.4 ± 8.4	69.4 ± 10.4*					
SBP (mmHg)									
Pre-IHT baseline	128 ± 21	146 ± 18	132 ± 20	154 ± 18	$F = 0.932$	$F = 17.264$	$F = 31.718$	$F = 26.126$	$F = 0.360$
1 day after IHT	124 ± 16	134 ± 19*	123 ± 14*	136 ± 15*	$P = 0.350$	$P = 0.001$	$P = 0.000$	$P = 0.000$	$P = 0.704$
1 month after IHT	125 ± 20	138 ± 21	128 ± 13	140 ± 17					

IHT: intermittent hypoxic training; SaO_2: arterial blood oxygen saturation; HR: heart rate; SBP: systolic blood pressure.
[a]Data are mean ± SD and were analyzed with three-way ANOVA with repeated measures.
*$P < 0.05$ vs. pre-IHT baseline.

IHT, the fasting glucose level was within the normal range in both groups, but in healthy group it was significantly lower (~18%) than prediabetes group. The results of OGTT indicated that 2 h after 75 g glucose ingestion the plasma glucose concentration increased by 15% in the healthy subjects, but by 41% in the prediabetic patients ($P < 0.05$).

Two-way ANOVA results indicated that main group effect (healthy vs. prediabetes) was significant ($P < 0.01$). On the other hand, although the general time effect was not significant due to the absence of IHT influence on healthy subjects, a separate Bonferroni post-hoc analysis in the prediabetic patients showed a significant difference between fasting glucose pre-IHT baseline and one month after IHT ($P < 0.05$), between 2-h post-OGTT glucose pre-IHT baseline and one day after IHT ($P < 0.05$) as well as one month after IHT ($P < 0.01$). Both group main effect and group + time effect were statistically significant for 2-h post-OGTT glucose (Table 3).

Tolerance to acute hypoxia

Table 4 demonstrates the values of blood oxygen saturation and cardiovascular indices during AHT before and after the three-week sessions of IHT. Under acute hypoxia (12% inspired O_2 for 20 min), SaO_2 (a non-invasive indicator of hypoxic tolerance) dropped at the initial stage by 18% in healthy group and 19% in prediabetes group. No statistical difference between the two groups was observed (group main effect $F = 0.030$, $P = 0.834$). At the end of three-week IHT, SaO_2 fell much less (by 13% or 14%, respectively) suggesting the body has gained an increased tolerance to hypoxia. This effect maintained at one month after IHT completion (time effect $F = 20.247$, $P = 0.001$; time + hypoxia effect $F = 28.454$, $P = 0.001$). The indices of cardiovascular response to acute hypoxia also showed an increased tolerance to acute hypoxia. For examples, HR was significantly lower during 20-min hypoxic load in both groups at the end of or one month after the three-week IHT sessions (time effect $F = 5.098$, $P = 0.02$; hypoxia effect $F = 15.328$; $P = 0.001$). Similarly, the hypoxia-triggered increases in SBP were reduced right after the end of IHT (time effect $F = 17.264$, $P = 0.001$; hypoxia effect $F = 31.718$, $P = 0.001$; time + hypoxia effect $F = 26.126$, $P = 0.001$). Three-way ANOVA test did not show significant common effect group + hypoxia + time effect, which demonstrates the absence of differences between groups in adaptive reactions of cardiovascular indices to hypoxia.

HIF-1α mRNA expression

Initial level of HIF-1α mRNA expression was comparable in the healthy group and prediabetes group (Figure 1(a)). IHT resulted in approximately four-fold (Healthy) and five-fold (Prediabetes) increase during the first week of IHT. In the next two weeks HIF-1α expression returned to the baseline level in the healthy subjects. However, in the prediabetic patients HIF-1α expression continued to increase, exceeding the initial level for 6.5 times and remained two-fold higher one month after the end of IHT.

SLC2 mRNA expression

mRNA expression of SLC2—the insulin-independent glucose transporter (Figure 1(b)) was not different significantly between the two groups prior to IHT. At the end of three-week IHT sessions, SLC2 mRNA expression increased significantly in the healthy participants with subsequent 80-fold augmentation in a month after IHT termination, but no changes were observed throughout the test period in prediabetes group, indicating a prediabetes-related defect in this transporter in response to intermittent hypoxia.

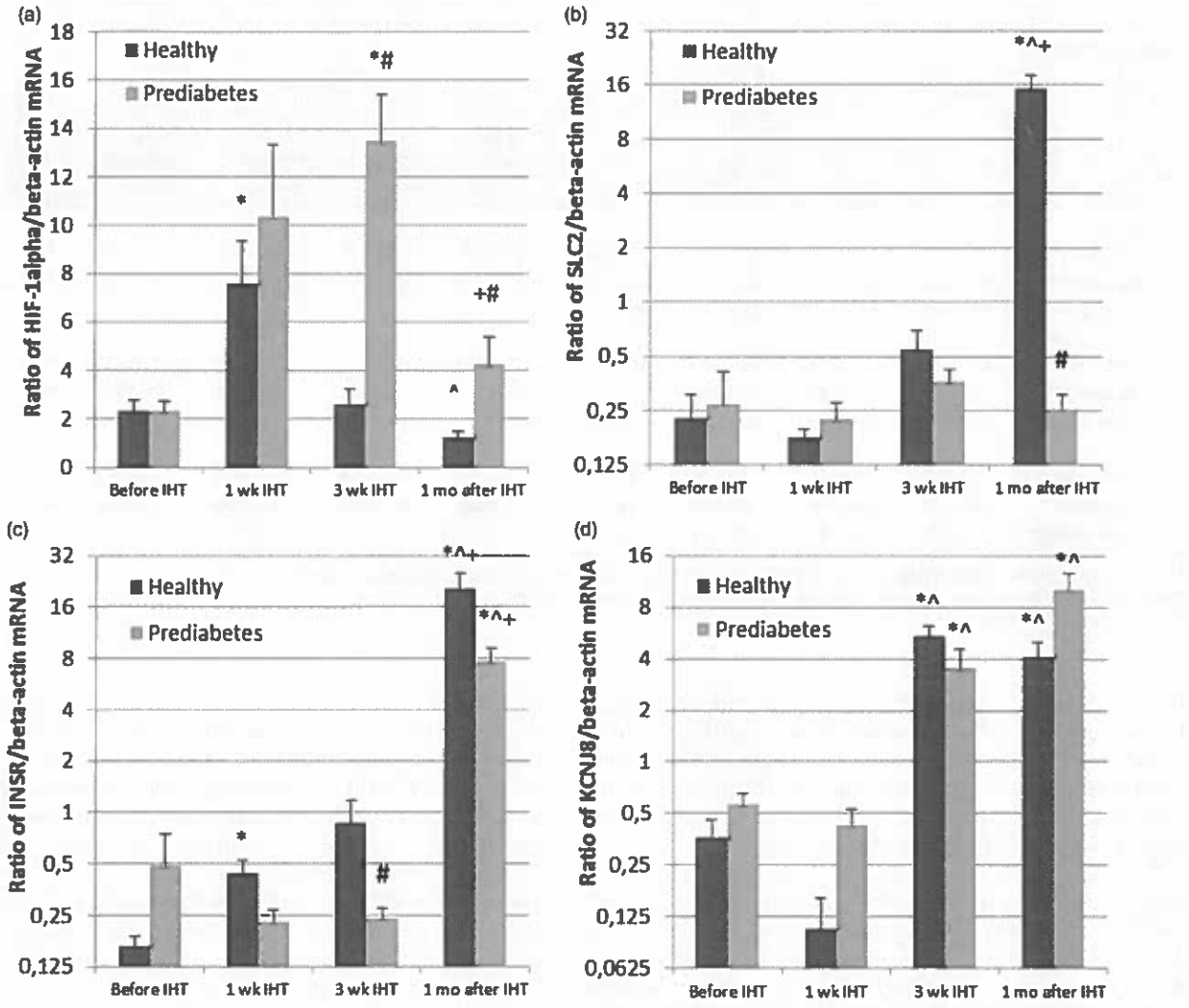

Figure 1 Effect of IHT on mRNA expression of hypoxia inducible factor 1α (HIF-1α) (a), facilitated glucose transporter-solute carrier family-2 (SLC2) (b), insulin receptor (INSR) (c), and potassium voltage-gated channel subfamily J (KCNJ8) (d) in healthy subjects and prediabetic patients. Data are presented as mean ± SD. *$P < 0.05$ vs. pre-IHT baseline; ^$P < 0.05$ vs. 1 wk IHT; +$P < 0.05$ vs. 3 wk IHT; and #$P < 0.05$ vs. healthy group. IHT: Intermittent hypoxia training

INSR mRNA expression

Basal INSR mRNA expression was not statistically different between the two groups, mainly due to large individual variance among the prediabetes patients, from 0.1 to 12 units (Figure 1(c)). During IHT this parameter gradually increased in the healthy subjects reaching a five-fold increase by the end of IHT and continued to increase in the coming month, exceeding more than 100 times above the baseline value. Meanwhile, in the prediabetic patients, IHT caused slight decrease in INSR mRNA expression during training period, and only in a month after IHT termination this parameter increased to a level of 16-folds above the baseline, suggesting a remarkable delay in response to IHT.

KCNJ8 mRNA expression

The pre-IHT basal mRNA expression of KCNJ8 potassium channels was identical in both groups (Figure 1(d)). During the first week of IHT no significant changes was observed, but at the end of three-week IHT sessions KCNJ8 increased about 15-folds in healthy group and six-folds in prediabetes group. Interestingly, one month after IHT termination, the augmented level of KCNJ8 was maintained in healthy group, but showed more pronounced increase in prediabetes group.

Correlation analysis

Figure 2 demonstrates the relationship between SaO_2 at 20th minute of acute hypoxic test (the marker of tolerance to acute hypoxia) and several measured parameters. Although no significant correlation was found between the levels of SaO_2 and baseline fasting blood glucose (Figure 2(a)) or 2-h post-OGTT blood glucose (Figure 2(b)) in healthy group, a strong negative correlation was identified in prediabetes group. In these prediabetes patients, the lower SaO_2 under acute hypoxic test, the higher fasting glucose (Figure 2(a), $r = -0.75$; $P < 0.01$) and 2-h post-OGTT

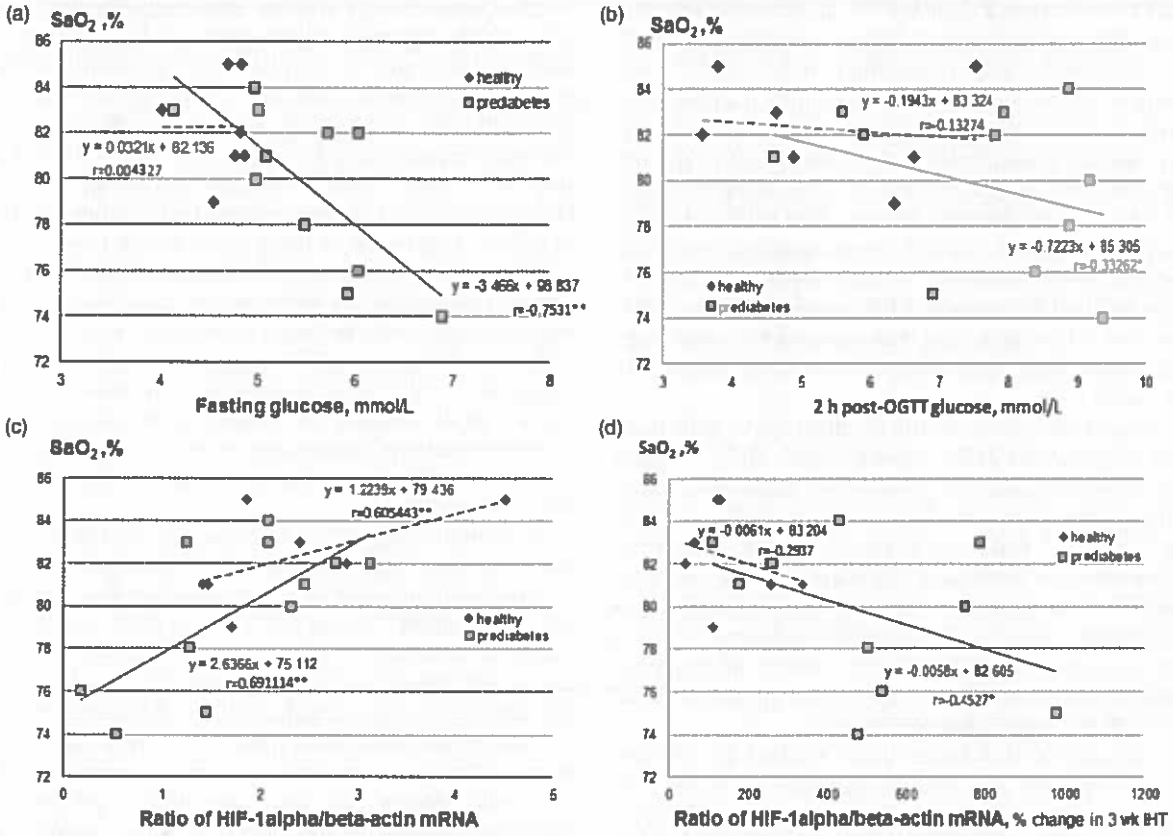

Figure 2 Relationships between baseline SaO_2 at 20th minute of acute hypoxic test (AHT, breathing with 12% of oxygen) and baseline fasting glucose (a), baseline 2 h post-OGTT glucose (b), baseline mRNA expression of HIF-1α (c), and % changes in mRNA expression of HIF-1α between the pre-IHT baseline and post-IHT values (d). HIF: hypoxia inducible factor; OGTT: oral glucose tolerance test. Symbols of correlation coefficient (*$P < 0.05$ or **$P < 0.01$) indicate a significant degree of linear relationship between the variables

glucose level (Figure 2(b), $r = -0.33$, $P < 0.05$). In addition, we also found a significant positive correlation between SaO_2 and baseline HIF-1α mRNA expression in both healthy ($r = 0.61$; $P < 0.01$) and prediabetes groups ($r = 0.69$; $P < 0.01$) (Figure 2(c)). Besides, a negative correlation between SaO_2 and % changes in mRNA expression of HIF-1α between baseline and the post-IHT examination in prediabetes group (Figure 2(d), $r = -0.42$, $P < 0.05$). These data indicate that the subjects with lower tolerance to hypoxia had initially higher blood glucose level and greater increase in HIF-1α mRNA expression under IHT.

Discussion

The present study revealed the following salient findings: (1) three-week sessions of IHT afforded beneficial effects on glucose homeostasis in patients with prediabetes (Table 3), particularly IHT significantly reduced fasting and 2-h post-OGTT blood glucose levels with the most pronounced beneficial effects observed at one month after the end of IHT; (2) IHT significantly increased the body's tolerance to acute hypoxia and improved cardiovascular function under hypoxia in both healthy and prediabetic individuals (Table 4); (3) subjects with higher initial level of blood glucose had lower tolerance to hypoxia (Figure 2(a) and (b)); (4) IHT stimulated HIF-1α mRNA expression in blood leukocytes in a bi-phasic manner, which showed early activation during the first week of IHT and subsequently returned to the pre-IHT basal level in healthy men, but in prediabetic patients the maximum response to IHT was observed in a delayed manner at the end of IHT (Figure 1(a)); (5) HIF-1α regulated genes such as INSR, SLC2, and KCNJ8 were differentially affected by IHT in the healthy and prediabetic individuals; the greatest changes in mRNA expression of the target genes occurred one month after termination of IHT and coincided with the largest decrease in blood glucose levels, both fasting and under hyperglycemia load (Figure 1(b) to (d)); and (6) the higher expression of HIF-1α was positively associated with higher tolerance to hypoxia and better glucose homeostasis in both healthy and prediabetes subjects and interestingly the greater increase in HIF-1α mRNA expression under IHT was observed in the subjects with lower resistance to hypoxia.

The abovementioned findings are conceptually supportive to the notion of anti-diabetes effect of IHT, which was first demonstrated by Kolesnyk *et al.* in rats[9] and subsequently confirmed in other animal and human studies using different IHT models[13,38–42] as well as during the high altitude hypoxia adaptation.[43,44] However, it is notable that severe intermittent hypoxia, such as those found in patients with obstructive sleep apnea (OSA) may cause various negative effects, including the suggested association

between OSA and type 2 diabetes.[45–47] It is increasingly realized that different patterns of intermittent hypoxia could result in divergent effects on metabolic function[21,48–52] and the specific mode of hypoxia, including depth, duration, and cyclic frequency, can be critical for determining the healing or harmful results of intermittent hypoxia.[2,53] Our present investigation provided direct evidence suggesting a moderate and non-invasive protocol of four short cycles of 5-min hypoxic breathing (12% O_2) and 5-min normoxic breathing (room air), three times a week for three weeks is sufficient to reduce fasting and 2-h post-OGTT hyperglycemia in prediabetic patients, while increasing their resistance to acute hypoxia with improved cardiovascular functional parameters under hypoxia.

Hypoxia is well known to initiate an adaptive gene transcription program via HIFs, among which HIF-1 triggers hypoxia-dependent gene expression in regulating many metabolic processes for the improvement of O_2 transport capacity.[11,17,20,21,54] Reduced levels of HIF-1α have been found in the cells or tissues collected from diabetic animals or patients, indicating an inhibitory effect of hyperglycemia on HIF-1α expression.[18,55–59] This diabetes-blunted HIF-1α response to hypoxic conditions may result in impaired angiogenesis and inability to upregulate glycolytic ATP generation in the type 2 diabetic heart.[60]

The selection of leukocytes to be studied on cellular response to hypoxia had several rationales. The primary rationale to study leukocytes is their cellular lifespan (2–10 days), which allows observing the cell phenotypic changes under intermittent hypoxia sessions. In fact, leukocytes are the only nucleated fraction in blood cells, in which the changes in gene expression under hypoxic stimuli can be non-invasively quantified and it has been thought to reflect better the processes of genetic activation in cells than the mRNAs extracted from blood plasma. For example, a previous study by Tissot van Patot *et al.*[61] using leukocytes demonstrated that HIF-1 DNA binding activity was enhanced *in vivo* in response to acute hypoxia in 14 men exposed to hypobaric hypoxia (4300 m or equivalent to 12% O_2) in a hypoxic chamber for 8 h, both HIF-1 DNA binding and HIF-1α protein levels in leukocytes were elevated, in association with plasma and urinary markers of hypoxic stress. To our best knowledge this is the first time to show HIF-1α mRNA increased following IHT sessions. The present study also showed that the maximum increase in HIF-1α expression induced by IHT occurred much earlier in healthy people than those in prediabetic patients, which may indicate the inhibitory effect of higher blood glucose levels on HIF-1α response to IHT in the prediabetic patients. In supporting this notion, recent study by Xiao *et al.*[59] demonstrated that whereas hyperglycemia upregulates HIF-1α signaling in some cell types, high glucose can also inhibit HIF-1α and its target genes. Regarding the mechanisms of HIF-1α impairment under diabetic conditions, the negative effects of various diabetes-associated factors include overproduction of reactive oxygen species, increased sensitivity to Von Hippel-Lindau (VHL) machinery, and altered osmolarity and proteasome activity, which could deactivate HIF-1α.

Our present study further investigated mRNA expression of several main target genes of HIF-1α, which are likely participating in insulin reception (INSR), facilitated glucose transport (SLC2A1), and regulation of insulin secretion in the β-cells (KCNJ8). It is known that as a part of adaptive response to hypoxia, there is upregulated expression of several genes encoding glycolytic enzymes.[27] However, to our best knowledge, an IHT-induced change in mRNA expression of these genes has not been reported prior to our current study. Our present results showed that whereas no significant difference in baseline expression of the examined genes between prediabetes and healthy subjects, the changes in HIF-1α-regulated gene expression in response to IHT was subsequent to those of HIF-1α mRNA in the healthy group and the maximum response was observed one month after the end of IHT (Figure 1(b) to (d)). Notably the prediabetes group exhibited different temporal profiles.

It is well recognized that physiological effects of insulin implemented mainly through INSR by binding to α subunit of INSR and stimulating the intrinsic kinase activity of β subunit of INSR.[28] Increased levels of INSR could alleviate insulin resistance, because overexpression of INSR improved obese and diabetic phenotypes in rodents.[62] KCNJ8 is one of the subunits of K_{ATP} channels and presents in many tissues, including pancreatic islet cells, therefore it is considered as metabolic sensors via coupling cellular metabolic status to cell membrane potential.[32,35,63] Functional and structural defects in K_{ATP} channels impair insulin secretion leading to the onset of diabetes.[64] Our present study demonstrated an increased KCNJ8 mRNA expression at the end of IHT in both healthy and prediabetic subjects and more pronounced effect at one month after IHT in prediabetic patients. Based on the observed similarity in KCNJ8 mRNA expression among healthy and prediabetes subjects under IHT, we postulate that KCNJ8 channels may be less vulnerable during the development of diabetes. Such a hyperactive KCNJ8 following IHT may lead to the increase in insulin secretion by β-cells. Previous laboratory animal studies suggested an organ-protective role of vascular K_{ATP} channel under diabetic condition, via suppressing diabetic oxidative stress.[32] Cardioprotective and antiarrhythmic effect of adaptation to intermittent hypoxia is also mediated via activation of K_{ATP} channels.[34]

GLUT-1 is one of the key components of the HIF-1α-mediated hypoxia response.[24,65] It is responsible for basal glucose uptake and expressed in virtually all tissues under normal conditions.[66] HIF-1α accelerates the expression and activation of GLUT-1 and induces glucose uptake and glycolysis,[26] which in turn induces HIF-1α degradation[67] and GLUT-1 mutations that reduce its function are associated with reduced glucose uptake.[68] HIF-1α and GLUT-1 levels increased significantly in the cells exposed to chronic intermittent hypoxia, suggesting a transcriptional activation and adaptive response to intermittent hypoxia.[24,65] Alterations in glucose transporter genes are also associated with major pathologies, e.g. Alzheimer's disease.[69] GLUT-1 itself is a relatively stable protein as compared with HIF-1α[70] and therefore its changes in response to hypoxia-reoxygenation should have a more sustained profile. In our present study,

IHT elicited no change in SCL2A1 mRNA expression in the prediabetes group. The exact molecular mechanism underlying the loss of ability of IHT to upregulate GLUT-1 in prediabetic patients requires further investigation.

Notably, the most significant changes in mRNA expression of HIF-1α target genes were observed a month after IHT termination and were coincided with the largest decrease in fasting blood glucose and 2-hour post-OGTT glucose levels in the prediabetic patients (Table 3). Such long-term cause-and-effect relationships are not fully understood. Our previous studies in healthy people have also shown that the most pronounced changes in circulating hematopoietic stem and progenitor cell counts were observed not during IHT but two weeks after the completion of IHT.[71] Mechanism underlying the much delayed augmentation mRNA expression of HIF-1α target genes by IHT remains unclear and should be elucidated in future studies.

In addition, it was suggested that glucose sensing in the carotid bodies may play a role in metabolism.[72,73] We previously described in prediabetic patients the relationships between the tolerance to hypoxia and cardiorespiratory response to acute hypoxia as well as the severity of glucose metabolism disorder.[37] Our current study also found that prediabetic patients with impaired glucose homeostasis had lower tolerance to hypoxia (Table 4, Figure 2). The higher expression of HIF-1α was positively associated with higher tolerance to hypoxia and better glucose homeostasis in both healthy and prediabetes subjects. Besides, the greatest increase in HIF-1α mRNA expression under IHT was observed in the subjects with lower tolerance to hypoxia (Figure 2(d)). This observation is in accordance with previous findings from the rats with high or low resistance to hypoxia,[74,75] which reported higher increase in HIF-1 mRNA in the brain tissue of low tolerant animals following adaptation to intermittent hypoxia.

Taken together, the close relationships between the IHT-reduced blood glucose levels and the IHT-enhanced tolerance to hypoxia, which are also associated with the IHT-enhanced HIF-1α expression and its target genes, clearly suggested a better normalization of carbohydrate metabolism during IHT.

Nevertheless, several limitations of the present study include that we only investigated the transcription of HIF-1α as a possible mechanism of potentiation of the "weak links" of the HIF-1α-mediated responses in diabetic patients. At the same time, the obtained results about the induction of target genes indirectly indicate the effectiveness of HIF-1α protein stabilization and its transcriptional activity. Undoubtedly, future studies focusing on posttranslational modifications of HIF-1α protein would be highly warranted and informative.

Conclusion

The present study elucidated the poorly understood molecular mechanism underlying the beneficial effects of IHT under prediabetic conditions. We found that three-week moderate IHT induced higher HIF-1α and its target genes mRNA expressions, which were positively correlated with higher tolerance to acute hypoxia and better glucose homeostasis in both middle-aged healthy and prediabetic subjects. Our results suggest a potential utility of IHT as an effective non-pharmacologic preventive therapy for management of prediabetes patients.

Author contributions: All authors participated in the design and interpretation of the studies, data analysis, review, and final approval of the manuscript. TVS designed the study and wrote the manuscript. TVS, AGP, and VBS elaborated the study protocols. AGP and TID performed and interpreted genetic analyses. VIP, EE, AVG, SN, VC, and VBS conducted physiological investigations and performed statistical analyses of the results. LX critically edited the manuscript. VBS provided the enrollment and clinical examination of the subjects as well as general research management.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Intermittent Hypoxia-Hyperoxia Exposures Improve Cardiometabolic Profile, Exercise Tolerance and Quality of Life: A Preliminary Study in Cardiac Patients

Oleg Glazachev[1], Davide Susta[1,2], Elena Dudnik[1], Elena Zagaynaya[1]

[1] I.M. Sechenov First Moscow State Medical University, Moscow, Russia, [2] School of Health and Human Performance, Dublin City University, Dublin, D9, Ireland

ABSTRACT

Study design: randomized controlled before-and-after and in follow-up trial. Forty-six CAD patients volunteered to take part in the study: a group of 27 patients undertook an Intermittent Hypoxia (O_2 at 10%) - Hyperoxia (O_2 at 30%) Training (IHHT), while a control group (CTRL) of 19 patients was allocated to sham IHHT treatment (breathing via face mask by room air, O_2 at 21%). Exercise performance, blood and metabolic profile, quality of life (MOS SF-36, Seattle Angina Questionnaire, SAQ) were measured before and after IHHT/sham IHHT in both groups; the intervention group was also assessed one month after completing the IHHT.

The IHHT intervention group showed improved exercise capacity (+1,8 ml O_2/min/kg, p=0,02), reduced resting systolic and diastolic blood pressures (151/85 before vs 130/73 after p<0,01), enhanced Left Ventricle Ejection Fraction (62,6±5,5% vs 58±6,2%, p<0,01), glycemia was significantly reduced only at 1-month follow-up (6,18±1,7 after vs 7,10±2,34 mmol/l at baseline, p=0,037). Frequency of angina as reason to stop exercising was significantly reduced after treatment and at 1-month follow-up.

In CAD patients an Intermittent Hypoxia-Hyperoxia Training program is associated with improved exercise tolerance, risks factors profile and quality of life (SF-36, SAQ). IHHT has proved to be safe, well tolerable and easily applicable in cardiac patients.

Keywords: *Intermittent hypoxia-hyperoxia training, exercise tolerance, cardiometabolic profile, coronary artery disease, quality of life, cardiac rehabilitation*

INTRODUCTION

In recent years, the structure of cardiac rehab methods is expanding due to the combined use (along with individually adjusted exercise training) of new high-tech instrumental techniques - enhanced external counterpulsation, extracorporeal shock wave therapy, etc. One of the promising approaches is the use of repeated multiple episodes of adaptation to hypoxia - interval hypoxic training (IHT).

Corresponding author:
Oleg Glazachev
I.M. Sechenov First Moscow State Medical University, Moscow, Russia, E-mail: glazachev@mail.ru. comkseniabadz@gmail.com

Intermittent exposure to normobaric hypoxia (IHT) has been shown to improve exercise capacity without exercising in the elderly and in cardiac patients [2,3]. IHT also positively affects the Autonomic Nervous System and pulmonary functioning in various patients [4]. This technique consists of intermittent exposures to hypoxic and normoxic stimuli through a face mask (one cycle of up to 5 hypoxic exposure lasting at least 5–6 min, and being followed by at least 5–6 min of normoxic air breathing) repeated almost daily (4-5 days a week) over 2–3 weeks [2].

In our study we used normobaric Intermittent Hypoxic-Hyperoxic Training (IHHT) as a new alternative treatment, more effective than adaptation to interval hypoxia-normoxia: replacing normoxia with hyperoxia

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during intermittent exposures to hypoxia produces a faster membrane-stabilizing effect in cells of the heart, liver, and brain compared to IHT in experimental research of T.Sazontova et al [8]. During periods of the induced hyperoxia, the induction of reactive oxygen species (ROS), which is necessary to start the cascade of the redox signaling pathways, takes place, which leads to enhanced synthesis of protective intracellular protein molecules, mainly with antioxidant function (antioxidant defense enzymes, iron-binding proteins, heat shock proteins etc.) [7]. IHHT has been proven to be effective and better tolerable in preliminary studies focused on exercise performance [5]. Very recent studies reported that IHHT improved exercise performance in athletes with overtraining syndrome [10], contributed significantly to improvements in cognitive function and functional exercise capacity in multi-morbid geriatric patients [1].

We aimed to conduct a controlled trial to investigate the effects of an IHHT program on exercise tolerance, cardio-metabolic risk-factors and patient-relevant subjective parameters of life quality assessment in CAD patients.

METHOD

Population

Fifty-four patients with diagnosis of CAD (NYHA functional class II and III) in stable clinical condition for the last six months were invited to participate in the study.

Twenty-seven patients were people waiting to start a usual cardiac rehabilitation program and they were allocated to IHHT group. Twenty-seven patients were allocated to control/sham-IHHT group (CTRL), but eight of them dropped out before initial baseline assessment, so that only 19 patients volunteered as controlled group. Participants' drugs plan was unchanged during the entire study period (drugs used by participants included beta-blockers, calcium channel blockers, ACE-inhibitors, ATR-blockers, anti-aggregants, statins, nitrates and diuretics). All participants were blinded to group allocation. Participants were also advised not to change nutrition and levels of daily physical activity during the study.

Exclusion criteria were: history of exercise induced syncope, NYHA class IV, decompensated heart failure, severe angina, grade 3 hypertension at rest (SBP >180 and/or DBP >110 mmHg).

Intervention

Participants in the intervention group undertook a program of Interval Hypoxia-Hyperoxia Training (IHHT) consisting of personalized repeated exposures to hypoxia (breezing through a face mask by gas mixture with 10–12% O_2) and to hyperoxia (30–35% O_2): 3 sessions a week, 5-7 hypoxic periods lasting 4–6 min, with 3-min hyperoxic recovery intervals for 15 sessions in total (ReOxy-Cardio, AiMediq, Luxembourg). This program was based on a 10-min continuous hypoxia test and tailored on individual responses to hypoxia exposure according to previously published principles and protocols guiding the clinical use of intermittent hypoxia exposure (9, 21). Participants in the control group completed a sham program breathing room air (normobaric normoxic mixture following the same schedule of the IHHT group), so completing at least 15 daily sessions over three weeks, each session including up to seven normoxia-normoxia cycles of up to 6 minutes of sham exposure and 3 minutes sham recovery between periods of sham exposure. During each session of both the IHHT and sham IHHT treatments all participants were continuously monitored (blinded) using fingertip pulseoxymeter (pulse rate and SaO_2) and supervised by physicians and/or nurses. Blood pressure measurements where performed before and after each session.

Study protocol

Baseline assessment included:

- Anthropometrics (height, weight, BMI, Seca stadiometer, Vogel & Halke, Germany),
- Resting blood pressure and heart rate (Omron; Omron Healthcare, Japan);
- Cardiopulmonary stress test (Cardiovit AT-104 PC Ergo-Spiro, Switzerland). A six-lead electrocardiogram was recorded continuously. The selected exertion protocols were Bruce and Modified Bruce depending on patients' clinical conditions. Peak oxygen uptake (VO_2 peak) was defined as the highest 15-s average of oxygen uptake obtained at the end of the test (i.e. at the highest mechanical output achieved). Test was stopped according to internationally agreed criteria (8). Blood pressure, and Ratings of Perceived intensity

of Exertion according to the Borg scale were determined at the end of each workload;

- Echocardiographic study in M-mode (ESAOTE Mylab Alpha) was conducted before starting the program and within one week after completing the program;

- Blood samples (fasting): Red and white blood cell count, haemoglobin concentration, reticulocytes, serum total and high density lipoprotein (HDL) cholesterol, triglycerides and glucose concentrations were analyzed by the central biochemical laboratory of our University (I.M. Sechenov Moscow State Medical University) using standardized analytical methods on fasting blood samples;

- Health related quality of Life (HRQoL) was tested by the Medical Outcomes Study 36-item Short Form Health Survey (MOS SF-36), one of the most often-used generic instruments to measure HRQoL in cardiac populations (23) and is reported to be the most appropriate in its validity, reliability, and sensitivity. Russian version of the SF-36 Health Survey was used in the study and reflects the eight scales: physical functioning, role-physical, bodily pain, general health, vitality, social functioning, role-emotional and mental health (5)

- Additionally HRQoL was monitored by disease-specific survey - Seattle Angina Questionnaire SAQ, (22).

All assessments were repeated 3 days (range 2–5 days) after completion of the IHHT program (or sham program in control group) and at 1-month follow-up (only in IHHT group).

Data analysis

Statistical analyses were performed using SAS statistical software version 9.3 for Windows (SAS Institute Inc, Cary, USA). All data are reported as Mean±SD and statistical significance was at $p<0.05$. After testing for normality by Shapiro-Wilk test, Student's paired t-test or Wilcoxon's signed rank test were used to compare values before and following the IHHT program. Also comparisons were performed between IHHT and Control groups at same time points.

The study was approved by the Ethical Committee of I.M. Sechenov Moscow State Medical University and carried out in conformity with the ethical standards laid down in the Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects (Bulletin of World Health Organization 2001). Written informed consent was obtained from all participants.

RESULTS

Primary outcome was exercise tolerance measured as stress test response and aerobic capacity (Bruce and modified Bruce incremental workload tests protocols and indirect calorimetry). Secondary outcomes were patient-centered (Health related quality of Life (HRQoL) assessed by MOS SF-36 and Seattle Angina Questionnaire, [20,21]) and clinically relevant to better manage CAD (blood pressure, lipid profile, glycemia, Left Ventricular Ejection Fraction, LVEF).

Eight patients in the control group withdrew before baseline assessment. All the patients allocated in the IHHT group completed the program and were tested before and after the IHHT and at 1-month follow-up. Characteristics of the participants are shown in Table 1. In the control group only 19 participants (out of 27 initially recruited) made themselves available to be assessed before starting and after the sham program.

Cardiovascular adaptations

At baseline, control group showed significantly ($p<0.05$) lower Blood Pressure values (SBP 131±18 vs 151±19 mmHg, DBP 78±10 vs 85±11 mmHg), higher aerobic capacity (16.8 ± 3.9 vs 14.25 ± 4.2 $mlO_2/min/kg$ measured as VO_{2peak} using modified Bruce and Bruce protocols depending on patients' clinical conditions). These findings were somewhat unexpected and were explained by less pronounced CAD and Heart Failure symptoms and better medication in the control group, allocated later to sham treatment.

Table 2 summarizes cardiovascular responses after IHHT (stress test according to Bruce and modified Bruce protocols, VO_{2peak} measurement using indirect calorimetry and gas analysis, Resting Systolic and Diastolic Blood Pressure, Resting and maximal effort Heart Rates, Left Ventricle Ejection Fraction): The IHHT group showed after treatments and at follow-up lower frequency of 'angina as reason to stop exercising', significant increase in aerobic capacity, lowering in resting systolic and diastolic blood pressure, resting heart rate and improved myocardial performance. No significant changes have been observed in CTRL.

Blood biochemistry

Table 3 shows relevant blood biochemistry and hematological parameters (Hemoglobin and Reticulocytes, Total Cholesterol and Low-Density Lipoproteins, Glycemia). Lipid profile improved as well as the Atherogenic Index, mainly because of a significantly lowered Total Cholesterol. In IHHT group hemoglobin and glycemia were unchanged after IHHT, but glycemia was significantly lower at 1-month follow-up. No significant hematological changes have been revealed in CTRL.

DISCUSSION

Our results show that, after 15 daily session of IHHT, cardiopulmonary fitness was significantly improved as the values of VO_{2peak} were higher than those measured at baseline. These values are not likely to be clinically meaningful as their magnitude is around 0.5 METS but they show that improving cardiopulmonary fitness without exercising is feasible in patients with very low baseline values and co-morbidities. Linked to this it is worth putting emphasis on the significant reduction of the number of patients reporting angina as a reason to stop exercising while undertaking a stress test. Our results are aligned with previous studies on Intermittent Hypoxia-Normoxia exposure in different forms: Intermittent Hypoxia Training (breathing hypoxic mixtures via a facial mask while resting, usually comfortably sitting) and Training in Hypoxia (continuous exposure to hypobaric or normobaric hypoxia while exercising). Both these "strategies" have been shown to be effective in improving exercise tolerance and performance in athletes by triggering hematological and non-hematological adaptations [6].

Table 1. Descriptive statistics at baseline of the IHHT and Control groups.

	IHHT group (n=27)	Control group (n=19)
Males n (%)	9 (33%)	9 (47%)
Average age, years (range)	63,9 (52-77)	63,2 (43-83)
Body mass (kg)	81,6 ± 13, 9	79,1 ± 12,5
Heart Rate (bpm)	71,5 ± 11,4	68,9 ± 9,6
Systolic Blood Pressure (SBP, mmHg)	151 ± 19	131 ± 18
Diastolic Blood Pressure (DBP, mmHg)	85 ± 11	78 ± 10 (p=0,05)
Current smokers, n (%)	5 (18,5%)	4 (18,5%)
Hypertension, n (%)	22 (81,5%)	17 (89,5%)
Diabetes, n (%)	8 (29,6%)	3 (15,8%) (p=0,04)
Exertional Angina, II FC	20 (74,1%)	17 (89,5%)
Exertional Angina, III FC	7 (25,9%)	2 (10,5%) (p=0,04)
Previous MI, n (%)	8 (29,6%)	8 (42,1%)
Paroxismal AF, n (%)	5 (18,5%)	2 (10,5%)
COPD, n (%)	2 (7,4%)	2 (10,5%)
VO_2 peak (ml/min/kg)	14,3 ± 4,2	16,5 ± 4,2 (p=0,05)
LV Ejection fraction (%)	58,0 ± 6,2	62,2 ± 7,2

Table 2. Cardiovascular responses and haemodynamic parameters of the IHHT and the control groups before and after the program and at 1-month follow-up (in IHHT group only)

	Group	Before (HT1)	After (HT2)	1 Month follow up (HT3)
Angina as a reason to stop test, n (%)	IHHT	12 (44,44%)	6 (22,22%)*	3 (11,11%)**
	Control	4 (21,05%)	6 (31,57%)	---
Exercise time, s (M-BRUCE)	IHHT (n=13)	354 ± 194	383 ± 141	395 ± 130** p=0,01
	Control (n=5)	280 ± 92	323 ± 64 # p=0,02	---

Cont... **Table 2. Cardiovascular responses and haemodynamic parameters of the IHHT and the control groups before and after the program and at 1-month follow-up (in IHHT group only)**

Exercise time, s (BRUCE)	IHHT (n=14)	280 ± 126	295 ± 79	332 ± 113** p=0,01
	Control (n=14)	335 ± 121	355 ± 96	---
VO_{2peak} ($mlO_2/min/kg$)	IHHT	14,3 ± 4,2	16,1 ± 4,2* p=0,02	15,4 ± 4,5** p=0,03
	Control	16,8 ± 3,9 #	17,8 ± 4,9	---
SBP, mmHg	IHHT	151 ± 19	130 ± 13* p=0,0001	129 ± 11** p=0,005
	Controls	131 ± 18 #	131 ± 17	---
DBP, mmHg	IHHT	85 ± 11	73 ± 7* p=0,0002	75 ± 9** p=0,002
	Controls	78 ± 10 #	79 ± 10	---
Heart Rate at rest (bpm)	IHHT	71,5 ± 11,4	67,7 ± 8,3* p=0,03	66,6 ± 10,0** p=0,02
	Controls	68,9 ± 9,6	66,8 ± 10,2	---
Heart Rate Max (bpm)	IHHT	122 ± 19	120 ± 14* p=0,03	116 ± 14** p=0,01
	Control	124 ± 13	119 ± 17	---
Left Ventricle Ejection Fraction %	IHHT	58,0 ± 6,2	62,6 ± 5,5* p=0,0008	61,6 ± 6,3*** p=0,007
	Controls	62,2 ± 7,2 #	61,3 ± 6,0	---

p-values for differences between:

* HT1 and HT2, ** HT1 and HT3 *** HT2 and HT3; # - IHHT and Control group at same time point.

Table 3. Hematological and metabolic variables of the IHHT and the control groups before and after the program and at 1-month follow-up (in IHHT group only)

	Group	Before (HT1)	After (HT2)	1 Months Follow-up (HT3)
Hemoglobin, g/L Reticulocytes, ‰ Total Cholesterol, (TCh), Mmol/L	IHHT	134 ± 12	136 ± 13	136 ± 12
	Controls	145 ± 10	145 ± 10	---
	IHHT	9,0 ± 5,5	11,3 ± 6,2* p=0,02	9,2 ± 4,8
	Controls	6,4 ± 3,6	5,11 ± 3,13	---
	IHHT	5,6 ± 1,4	5,1 ± 1,2* p=0,041	5,5 ± 1,4
	Controls	5,5 ± 0,9	5,6 ± 1,0#	---

Cont... **Table 3. Hematological and metabolic variables of the IHHT and the control groups before and after the program and at 1-month follow-up (in IHHT group only)**

Low-density lipoproteins (LDL), Mmol/L	IHHT	3,5 ± 1,2	3,2 ± 0,9* p=0,006	2,6 ± 1,3*** p=0,007
	Controls	3,6 ± 0,8	3,5 ± 0,8	---
Atherogenic Index (TCh - HDL) / HDL	IHHT	4,7 ± 1,8	3,4 ± 1,3* p=0,0018	3,5 ± 1,5** p=0,002
	Controls	3,6 ± 1,1	3,4 ± 1,0	---
Glucose, mmol/L	IHHT	7,10 ± 2,3	6,45 ± 1,7	6,18 ± 1,7** p=0,037
	Controls	5,83 ± 0,65	5,97 ± 0,68	---

p-values for differences between:

* HT1 and HT2, ** HT1 and HT3 *** HT2 and HT3;
# - IHHT and Control group at same time point.

CONCLUSION

A novel modality of intermitted hypoxic repeated preconditioning - interval hypoxic-hyperoxic training (IHHT) has been tested and found to be safe, deliverable to cardiac patients and associated with improved exercise tolerance, a more protective cardio-metabolic profile and superior quality of life self-assessment.

Positive multiple effects of IHHT in correction of cardio-metabolic risk factors and quality of life subjective assessments in patients with stable angina raise the prospect of potential using IHHT as an additional method of treatment and rehab of CAD patients with metabolic syndrome, as well as with orthopedic comorbidity and low adherence to exercise training.

These very encouraging results should be confirmed by randomized, well controlled trials. Further research is also needed to explain the mechanisms behind adaptations to IHHT and to better tailor individual exposures to Hypoxia – Hyperoxia cycles.

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DS, ED and EZ none to declare"

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