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Intermittent hypoxic exposure with or without exercise improved cardiopulmonary functions in people with cardiovascular risk factors

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Abstract

This research aimed to explore the effects of intermittent hypoxic exposure (IH) with or without exercise on lung function, lipid profile, and a 6-min walk in people with three cardiovascular risk factors (hypertension stage I, hyperlipidemia, and obesity). Thirty participants were randomly allocated into 3 groups: a control group (CON, n=11) received no training, an intermittent hypoxic exposure during rest group (IHR, n=9), and an intermittent hypoxic training group (IHT, n=10) who combined IH with walking. Both IHR and IHT performed 8 sessions of 3-min of hypoxic breathing (14% O2) alternated with 3-min of normoxic breathing (21% O2) for 48-min per day, twice a week, for 6 consecutive weeks. All participants were measured before and after 6 weeks of theexperimental period. After training, IHR group significantly increased vital capacity (*p*=0.038) and forced vital capacity (*p*=0.025) compared to baseline. Similarly, compared to baseline, participants in the IHT group revealed significantly increased vital capacity (*p*=0.030), forced vital capacity (*p*=0.031), and forced expiratory volume in 1 second (*p*=0.042). Compared to CON, only IHT showed a significant increase in forced vital capacity of 8.6 ±4.5% (*p*=0.034) and forced expiratory volume in 1 second of 7.0 ± 3.9%, (*p*=0.033) after 6 weeks. Both the IHR and IHT participants demonstrated a significantly increased 6-min walk distance (*p*=0.048 and *p*=0.004, respectively) compared to CON. The study demonstrated that IH programs can improve lung function and cardio-pulmonary fitness which indicates that IH with or without exercise improves some cardiopulmonary functions in at risk patients.

Keywords: Hypoxic training, Lung function, Vital capacity, Body mass index, Six-min walk distance

1. Introduction

Cardiovascular disease (CVD) is the most common non-communicable disease globally and is the leading reason of death worldwide [1]. This disease has been proven to be the major cause of death in both developed and developing countries [2]. There are a number of risk factors associated with CVD [3] including, high blood pressure, high serum cholesterol, and obesity as well as other unhealthy behaviors such as excessive alcohol use, smoking, and sedentariness [3]. Current advice suggests modification of major cardiovascular risk factors through lifestyle changes and medication. It is also well-established that regular exercise is associated with reduced overall mortality as well as reduced risk of developing cardiovascular disease [4].

Improved mental health and reduced body mass are both associated with regular exercise which improves bone mass, muscle strength, and cardiovascular and metabolic health [5]. Aerobic performance indicates the capability of the cardiopulmonary system and microcirculatory competency to transport oxygen to skeletal muscle during exercise and is an essential indicator of metabolic and cardiovascular health [6]. Promoting regular exercise and physical activity are cost-effective procedures to enhance public health [5]. Unfortunately, the recommended regular exercise and physical activity goals are not met by the general population [7], who cite a lack of time and motivation as the major barriers.

A relatively new intervention to improve health is hypoxic exposure either at rest (passive hypoxic exposure) or with exercise [8]. In recent years, increased attention has been given to the notion of using a hypoxic intervention as a therapeutic agent to sustain the treatment and prevention of non-communicable diseases and to improve the quality of life of patients and older adults [9]. There is substantial evidence that exercise under hypoxic conditions, triggers specific responses, not found following similar exercise in normoxia [10]. Exercise under hypoxic conditions is an independent and highly influential metabolic stressor [11]. Generally, acute hypoxic exposure diminishes arterial O_2 saturation levels. Undertaking hypoxic training therefore significantly reduces the O_2 partial pressure within the mitochondria of the working organs by concurrently increasing O_2 demand (via exercise) and decreasing O_2 supply (via hypoxia) [12]. Inhaling and exhaling hypoxic air engages similar metabolic pathways as endurance exercise such as upregulation of the hypoxia-inducible factor 1 hormonal system [13]. Upregulation of hypoxia-inducible factor 1α (HIF-1α) protein expression during exercise could further enhance other endothelial pathways including the activation of vascular endothelial growth factor (VEGF) [13] and nitric oxide. Muangritdech et al. (2020) found that after 6 weeks of intermittent normobaric hypoxic (FiO2; 0.14) exposure at rest (IHR) or during exercise (IHT) in hypertensive patients, both groups showed reduced blood pressure along with enhanced nitric oxide levels [14].

Hypoxic breathing also affects the pulmonary system with a previous study indicating an improvement in pulmonary function (increased vital capacity (VC) by 9.2 ± 2.5%, and forced vital capacity (FVC) by 6.1 ± 2.9%) after two weeks of intermittent normobaric hypoxic training in patients with high-risk COPD [15]. Furthermore, a three-week, 15 session IHT intervention (12-15% O_2) resulted in significant benefits for mild COPD patients, with increased inspiratory muscle function and thickness in the diaphragm, improved lung volumes (forced expiratory volume in 1 sec (FEV_1) and ratio of forced expiratory volume in 1 sec and forced vital capacity (FEV_1/FVC)), increased aerobic capacity and level of physical activity [16]. Reports on the benefits of exercising in hypoxia on lipid profiles are less common. However, Lu et al. (2014), found levels of serum total cholesterol (TC) were significantly lower, and levels of low-density lipoprotein cholesterol (LDL-C) tended to diminish in obese rats in the hypoxic group compared to rats in the normoxic group after four weeks of hypoxic training, suggesting that exercise training under a hypoxic environment may lower hypercholesterolemia [17]. In humans, 5 weeks of high-intensity interval training (HIIT) under normobaric hypoxic conditions reduced blood lipids in young overweight Chinese females. This study showed significant improvements in the TC/HDL-C and TG/HDL-C ratios [18]. Currently, there is a lack of information on the effects of IH (at rest or during exercise) on lung function and lipid profiles of people at risk of CVD (i.e., have risk factors for CVD).

Breathing hypoxic gas has been suggested as one strategy for health promotion through increasing cardiovascular and respiratory function in some patients and older people [8, 19]. The theoretical justification for hypoxic training is that oxygen transport and oxygen utilization are enhanced by reduced O_2 availability over time. For a given O_2 demand the ventilation volume must be increased correspondingly. Moreover, the acclimatization to hypoxia can cause various physiological changes such as respiratory muscle strength improvements, and increases in alveolar PO_2 [20]. While lung volumes are an important indicator of lung health very few studies have investigated the effect of light-to-moderate exercise under hypoxic conditions on lung function. Moreover, the beneficial effect of IH on common risk factors for CVD including lipid metabolism, body composition and cardiorespiratory fitness are yet to be fully ascertained. Therefore, the present study aims to investigate the benefits of 6 weeks of intermittent hypoxic exposure at rest or combined with light to moderate exercise on these variables in people with three cardiovascular risk factors: hypertension stage 1, hyperlipidemia and obesity.

2. Materials and methods

2.1 Participants

Thirty participants with cardiovascular risk aged between 30 to 59 years old participated in this study. The sample size was calculated by the G^*power program using the mean subject numbers (n) from a previous study (Muangritdech et al., 2020) [21]. The participants were randomized into three groups including the control group (CON, n=11) who received no intervention, the IHR, n=9, and the IHT, n=10 who combined hypoxic breathing with walking on a motorized treadmill at a light to moderate intensity, based on individual heart rate reserves (35-50% of HRR). All participants were recruited from the Outpatient Department and the Hypertension Clinics, the Faculty of Medicine (Srinagarind Hospital, Khon Kaen University). Inclusion criteria included stage I hypertension (systolic blood pressure ≥ 140 mmHg and diastolic blood pressure (DBP) ≥ 90 mmHg) diagnosed by medical specialists, LDL-C ≥ 130 mg/dL, body mass index more than 23 kg/m^2 in overweight and more than 25 kg/m^2 in obese, under optimal medication treatment for at least six months, and clinically stable (i.e., no crises or changes in medication) for at least three months. Exclusion criteria include serious respiratory disorder (e.g., asthma, COPD), history of cardiovascular diseases (e.g., acute myocardial infarction, and/or unstable angina), history of heart failure and stroke, history of neuromuscular and musculoskeletal diseases for more than 1-month, chronic kidney disease (stage I or more), type II diabetes mellitus and pregnancy.

2.2 Study design

2.2.1 Intermittent hypoxia protocol

The participants in the IHR group received hypoxic exposure via a mask during rest in a seated position, and the participants in the IHT group received hypoxic exposure via a mask during light to moderate-intensity walking exercise on a motorized treadmill (Commercial Treadmill M90T, Motus Co., Ltd, Korea). The participants in the the IHR and the IHT groups were given hypoxia from a Hypoxicator (ATS-HP-Hyperoxic, from Altitude Technology Solutions Co., Ltd. (ATS), Australia), but only the IHT group were asked to perform light to moderate exercise which was based on their maximum heart rate (HRmax). All participants in both groups were given 3-min hypoxic air (14% O_2) alternated with 3-min of normoxic air (21% O_2) for 48-min per day, twice a week, for consecutive 6 weeks. All participants were measured with on all parameters before and after 6 weeks of the experimental period. During the IH intervention, real-time monitors of heart rate (HR) and pulse oxygen saturation (%SpO_2) were recorded using a pulse oximeter (Beurer model PO30, USA). During the test, if the participants had any adverse symptoms for example, hypertensive crisis, uncontrolled blood pressure or dizziness, the researchers informed the participants to breathe normoxic air.

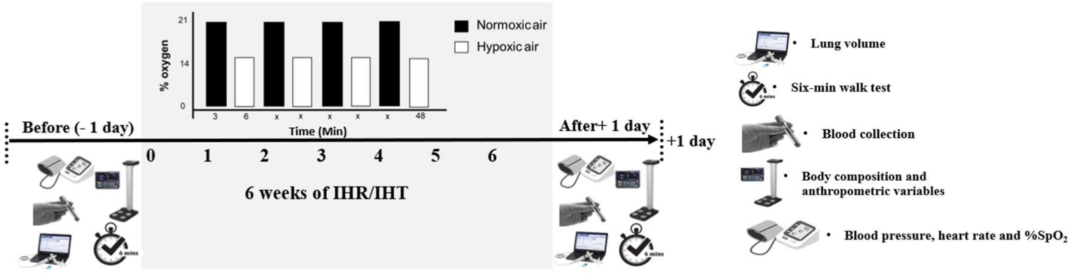

Figure 1 Timeline of intermittent hypoxic (IH) programs.

2.3 Variables

2.3.1 Hemodynamic variables

Systolic blood pressure (SBP) and DBP were measured by an autonomic blood pressure monitor (Bliss BL-B920., Shenzhen, China). All participants were measured at the same time of day after at least 15 min of rest.

2.3.2 Lung function

Lung function was assessed using a Vitalograph pneumotrac (Vitalograph, Ireland). Before testing, the spirometer was calibrated with a pneumotachograph. This system was calibrated before each test using a 3-liter syringe. All participants were familiarized with the equipment and procedures before trials. The measurements were taken when the participants were relaxed and in a seated position with a nose clip on.

The FVC and the FEV_1 were measured in at least 3 trials (3 min rest between trials). The values are expressed in liters (L) and % predicted. Each participant was instructed to inhale as deeply as possible and exhale with as much effort as possible without any pause for at least 6 sec. All participants were asked to put maximum effort into their test which aims to reach the highest values. Each spirometric parameter was accepted when the variation between trials fell to less than ± 5%.

The highest values of the three best trials were taken as the value for each measurement according to the standard methods of the American Thoracic Society and European Respiratory Society [22].

2.3.3 Lipid profile and hematological variables

Participants fasted for over 8 hours and after stabilization, 6 mL of a venous blood from an antecubital vein was collected in EDTA tubes between 8:00 and 9:00 am by the specialist nurse Three mL was used for the complete blood count (CBC) and three mL blood sample for serum TC, serum triglycerides (TG), serum LDL-C, serum high-density lipoprotein cholesterol (HDL-C) measures. The biochemical assays were completed at the Clinical Laboratory Unit of Queen Sirikit Heart Center of Northeastern Thailand. The equation for estimating LDL-C levels from the standard lipid profile was calculated by the formula. The fasting blood sugar (FBS) levels were measured using a glucometer (Accutrend® Plus System AU).

$$\text{LDL-C (mg/dL)} = \left(\text{Total cholesterol} - \text{HDL-C} - \frac{\text{Triglyceride}}{5}\right) \quad (1)$$

2.3.4 Body composition and anthropometric variables

Body composition measurement included body weight, body mass index, body fat mass, percentage of body fat, and fat-free mass using a body composition analyzer (BC-418 Segmental Body Composition Analyzer, USA). The body composition was taken twice; once at the start and again at the end of the 6-week intervention period. Waist circumference (WC) was measured at the mid-level between the lower rib margin and the iliac crest by using a flexible inch tape. Hip circumference was measured at the level of the greater trochanter by using a flexible inch tape. These measurements were used to calculate the waist-to-hip ratio (WHR) using the formula.

$$\text{Waist-to-hip ratio (WHR)} = \frac{\text{WC (cm)}}{\text{HC (cm)}} \quad (2)$$

2.3.5 Six-min walk test (6MWT)

According to a standard method of the American Thoracic Society, the 6MWT was measured as the distance walked over a span of 6 min. A standard distance in a straight line (30 m) was marked with two cones. A chair was set at the halfway distance so that the participants could stop to sit if necessary. Participants were asked to walk as far as possible in the 6-min period. At the end of the six-min, the participants are told to stop and estimate their level of effort during the six-min walk using the Rating of Perceived Exertion (RPE) scale. Recording the distance and recording the subject's RPE were taken.

2.4 Statistical analysis

The data are expressed as mean ± standard deviation (SD). Statistical analyses were made using STATA version 13.0 (StataCorp College Station, TX). The Shapiro-Wilks test was used to assess the normal distribution and homogeneity variance of data. A Student's paired t-test was used to compare differences in lung function, body composition, lipid profile, hematological variables, and the 6-min walk test before and after 6 weeks of the experimental period. Analysis of covariance was used to test for differences between the groups. An alpha level of $p<0.05$ was considered to be statistically significant.

3. Results

Thirty participants completed the experiment (Figure 2). Participant baseline characteristics were shown in Table 1. All parameters were not significantly different between the CON, the IHR, and the IHT groups at baseline.

3.1 Lung function

Compared to their baseline, both the IHR and the IHT groups showed significant improvement in %predicted VC (80.6 ± 4.5 vs 83.7 ± 4.0, p=0.038, 80.6 ± 5.5 vs 86.0 ± 4.5, p=0.030, respectively) and %predicted FVC (76.7 ± 7.0 vs. 81.8 ± 4.9, p=0.025, 78.4 ± 5.4 vs. 83.5 ± 4.7, p=0.031). Moreover, only the IHT showed significantly improved %predicted FEV_1 (76.6 ± 6.2 vs. 81.7 ± 5.6, p=0.042). Interestingly, the VC and FVC (% predicted) was improved only in the IHT group (81.7 ± 6.5 vs. 86.0 ± 4.5, p=0.027, 80.1 ± 8.7 vs. 83.5 ± 4.7, p=0.034, respectively), when compared the CON. Additionally, compared to the CON, only the IHT group's, FVC, and FEV_1 showed a significant increase (2.4 ± 0.8 vs. 2.9 ± 0.7, p=0.034, 2.1 ± 0.6 vs. 2.5 ± 0.7, p=0.033, respectively) (Table 2).

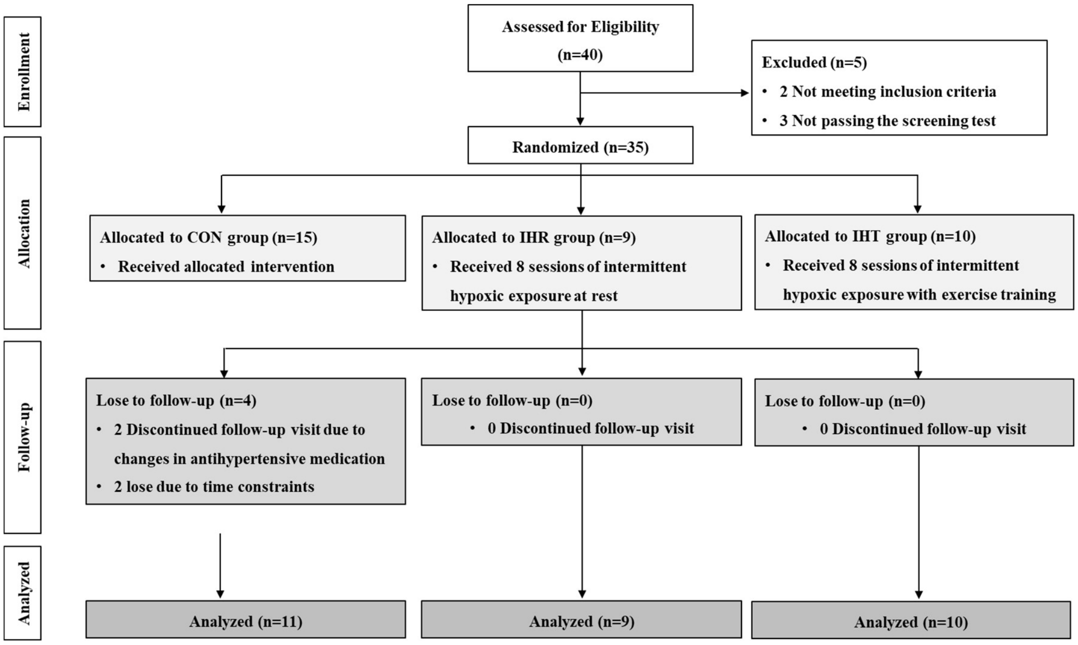

Figure 2 CONSORT flow diagram.

Table 1 Baseline characteristics of participants in the CON, IHR, and IHT groups.

Variables	CON (n=11)	IHR (n=9)	IHT (n=10)
General characteristics			
Age (years)	50.6±4.9	44.4±7.1	45.3±6.7
Gender (male/female)	2/9	2/7	3/7
Bodyweight (kg)	72.0±9.2	66.5±7.6	74.5±14.1
Height (cm)	156.0±7.7	154.4±6.5	160.0±8.7
Body mass index (kg/m^2)	29.7±3.3	27.3±2.4	28.9±4.6
Waist circumference (cm)	94.8±9.9	91.4±7.1	93.4±9.4
Hip circumference (cm)	104.1±5.9	101.2±6.3	104.9±8.1
Waist-to-hip ratio	0.9±0.1	0.9±0.1	0.9±0.1
Hemodynamic parameters			
Resting HR (beat per min)	74.0±7.6	70.0±8.7	71.6±6.2
Resting SBP (mmHg)	140.3±8.7	137.1±3.8	142.6±9.7
Resting DBP (mmHg)	84.3±7.2	83.6±7.6	92.0±9.2
Rating pulse oxygen saturation (%SpO$_2$)	97.8±0.8	97.6±1.0	98.2±0.9
Fasting blood sugar (mg/dL)	88.2±15.2	87.6±12.4	97.6±11.3
Clinical blood lipid profile			
LDL-C (mg/dL)	144.4±23.4	137.1±14.6	142.9±22.9
HDL-C (mg/dL)	46.8.4±5.0	51.3±8.2	52.0±10.0

Values are presented as mean ± SD.

Table 2 Lung function of participants in the CON, IHR, and IHT groups.

Variables	CON (n=11)		IHR (n=9)		IHT (n=10)	
	Before	After	Before	After	Before	After
Liter						
VC	2.3±0.6	2.3±0.6	2.4±0.7	2.5±0.6	2.5±0.6	2.7±0.6
FVC	2.4±0.7	2.4±0.8	2.5±0.7	2.6±0.9	2.7±0.6	2.9±0.7*
FEV$_1$	2.1±0.6	2.1±0.6	2.2±0.6	2.2±0.5	2.3±0.7	2.5±0.7*
FEV$_1$/FVC	0.9±0.1	0.9±0.1	0.9±0.1	0.9±0.1	0.9±0.1	0.9±0.2
%predicted						
VC	81.6±6.6	81.7±6.5	80.6±4.5	83.7±4.0$^\#$	80.6±5.5	86.0±4.5$^{\#*}$
FVC	81.5±7.8	80.1±8.7	76.7±7.0	81.8±4.9$^\#$	78.4±5.4	83.5±4.7$^{\#*}$
FEV$_1$	86.3±8.3	84.9±8.5	79.2±4.5	82.9±5.1	76.6±6.2	81.7±5.6$^\#$

Values are presented as mean ± SD. % predicted is a predicted value when compared to the normal values for the same aged and sex. $^\# p<0.05$, before vs. after, $^* p<0.05$, IHT vs. CON.

3.2 Lipid profile

After 6 weeks of the experimental period, FBS and all lipid profile variables in the three groups showed no significant differences ($p>0.05$) when compared to their baseline levels.

Table 3 Lipid profile of participants in the CON, IHR, and IHT groups.

Variables	CON (n=11)		IHR (n=9)		IHT (n=10)	
	Before	After	Before	After	Before	After
FBS (mg/dL)	88.2±15.2	90.4±10.1	87.6±12.4	86.4±10.5	97.6±12.4	94.6±11.3
Lipid profile						
TC (mg/dL)	233.7±36.0	235.7±38.7	210.7±25.0	199.3±21.3	226.9±33.5	206.1±45.7
TG (mg/dL)	144.2±34.3	140.6±33.5	150.9±48.9	145.3±41.3	168.1±66.0	148.2±53.5
HDL-C (mg/dL)	46.8±5.0	46.0±3.3	51.3±8.2	52.7±13.0	56.0±16.9	56.3±17.0
LDL-C (mg/dL)	144.4±23.4	147.5±27.0	137.1±14.6	130.1±14.8	142.9±22.9	131.1±28.3
VLDL-C (mg/dL)	28.8±6.9	28.1±6.7	30.2±9.8	29.1±14.3	33.6±13.2	29.6±10.7
TC/HDL	5.0±0.8	5.2±1.0	4.2±0.7	3.9±0.8	4.3±1.2	3.9±1.4
None-HDL	186.9±34.8	189.7±38.9	159.3±24.4	146.7±17.1	170.9±31.3	149.8±44.3
LDL/HDL	3.1±0.7	3.2±0.8	2.7±0.4	2.6±0.6	2.7±0.8	2.5±0.9

Values are presented as mean ± SD. VLDL-C = very low-density lipoprotein cholesterol.

3.3 Haematological variables

No significant differences were found in any of the groups haematological variables except for WBC which increased significantly in the IHT compared to the CON group over the 6 weeks of the study (6.8 ± 1.5 vs. 7.2±1.8, p=0.046) (Table 4).

Table 4 Hematological variables of participants in the CON, IHR, and IHT groups.

Variables	CON (n=11)		IHR (n=9)		IHT (n=10)	
	Before	After	Before	After	Before	After
Oxygen-transporting capacity						
RBC (10^6/µl)	4.9±0.4	4.9±0.4	4.8±0.3	4.9±0.3	5.1±0.4	5.1±0.4
Hb (g/dL)	13.4±1.7	13.2±1.5	13.3±1.0	13.5±1.1	13.9±1.4	13.8±1.3
Hct (%)	41.1±3.5	41.0±3.8	41.2±2.6	41.2±2.7	42.4±3.0	42.2±3.2
MCV (pg)	83.9±3.1	84.0±2.7	85.2±5.3	84.5±4.4	84.7±7.0	84.8±6.6
MCH (pg)	27.4±1.6	27.3±1.5	27.7±2.1	27.8±2.1	27.8±2.3	27.6±2.6
MCHC (g/dL)	32.6±1.2	32.4±0.8	32.5±1.2	32.7±1.3	32.9±1.1	32.5±0.8
RDW	14.0±0.7	13.8±0.8	14.2±1.4	14.2±1.3	13.9±0.8	14.0±1.1
Immune function						
WBC (10^3/µl)	7.4±1.5	6.8±1.5	6.5±1.6	6.7±2.0	6.4±2.1	7.2±1.8*
Neutrophil (%)	54.6±7.8	53.6±6.9	50.4±14.0	50.6±9.8	52.4±13.6	56.3±8.3
Lymphocyte (%)	33.5±7.6	34.7±6.6	40.0±12.3	37.3±7.2	33.6±9.7	30.9±7.0
Monocyte (%)	7.7±1.8	8.1±1.4	6.5±2.5	6.2±1.4	7.5±1.3	7.4±1.4
Eosinophil (%)	3.3±1.5	3.4±1.9	3.0±2.1	3.2±2.4	5.3±5.4	4.8±5.1
Basophil (%)	0.8±0.6	0.7±0.2	0.8±0.5	0.7±0.2	1.2±1.4	0.7±0.5

Values are presented as mean ± SD. MCV = mean cell volume, MCH = mean cell hemoglobin, MCHC = mean cell hemoglobin concentration, RDW = RBC distribution width. $^*p<0.05$, IHT vs. CON

3.4 Body composition and anthropometric variables

As shown in Table 5, there were no significant differences ($p>0.05$) in body composition including body weight, BMI, body fat, %body fat, and fat-free mass. Moreover, anthropometric variables such as waist circumference, hip circumference, and waist-to-hip ratio showed no significant change ($p>0.05$) after 6 weeks of both the IHR and the IHT. However, after 6 weeks compared to the baseline IHT group showed a significantly decreased hip circumference by 1.1 cm (104.9 ± 8.1 vs. 103.8 ± 8.0 cm, p=0.030).

Table 5 Body composition and anthropometric variables of participants in the CON, IHR, and IHT groups.

Variables	CON (n=11)		IHR (n=9)		IHT (n=10)	
	Before	After	Before	After	Before	After
Body composition						
BW (kg)	72.2±9.2	72.2±9.0	66.5±7.6	66.5±7.6	74.5±14.1	74.9±15.3
BMI (kg/m^2)	29.7±3.3	29.8±3.2	27.3±2.4	27.4±2.4	28.9±4.6	29.1±4.8
BF (kg)	26.4±5.4	26.6±5.4	25.1±5.7	24.0±5.1	28.2±9.9	27.3±10.8
% BF	36.8±5.7	36.9±5.9	37.7±7.2	35.9±5.3	37.5±8.9	36.1±10.0
FFM (kg)	45.5±7.7	45.6±7.8	41.4±6.7	42.5±5.2	46.3±10.8	47.6±12.0
Anthropometrics						
WC (cm)	94.8±9.9	95.1±9.2	91.4±7.1	91.1±7.7	93.4±9.4	92.9±10.5
HC (cm)	104.1±5.9	104.5±5.1	101.2±6.3	100.1±5.8	104.9±8.1	103.8±8.0$^\#$
WHR	0.9±0.1	0.9±0.1	0.9±0.1	0.9±0.1	0.9±0.1	0.9±0.1

Values are presented as mean ± SD. BW = body weight, BMI = body mass index, BF = body fat, % BF = % body fat, FFM = fat-free mass, HC = hip circumference. $^\#p<0.05$, before vs. after.

3.5 6MWT

Compared to baseline the IHT group showed a significant increase in the 6MWD by 42.9 m (536.1 ± 45.6 to 579.2 ± 28.7 m, *p*=0.025), along with a similar increase in the IHR group (517.2 ± 28.7 to 549.0 ± 32.7 m, *p*=0.008). Moreover, after 6 weeks, the 6MWD increased in the IHT (501.5 ± 40.3 m vs. 579.0 ± 56.5 m, *p*=0.004) and the IHR (501.5 ± 40.3 m vs. 549.0 ± 32.7 m, *p*=0.048) groups compared to the CON group (Figure 3).

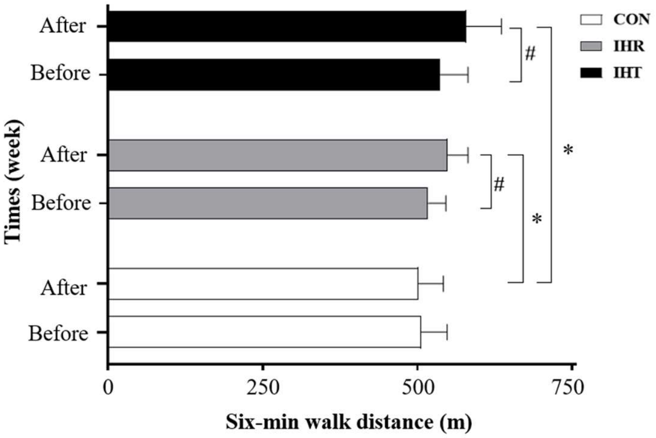

Figure 3 6MWT in all three groups before and after experimental periods. Values are presented as mean ± SD for the CON, IHR, and IHT groups. $^{\#}p<0.05$, before vs after, $^{*}p<0.05$, the IHR and IHT vs CON.

4. Discussion

Our study has demonstrated that after 6 weeks of IH (either at rest or during exercise) pulmonary function in the form of % predicted VC, FVC was significantly improved. In addition, IH during exercise also improved FEV_1 compared to the control who did not receive any hypoxia. In addition, both the IHR and IHT demonstrated a significantly increased 6MWD compared to the CON after the 6 weeks intervention. These results indicate that after these exercise interventions, lung function and cardiovascular fitness (6MWT) improved substantially. Moreover. Compared to the CON, only the IHT presented increased in the WBC. However, the WBC increase found in this study is in normal limit.

4.1 Lung function

Rapid lung function decline has been related to raised risk of cardiovascular disease (CVD) in the general population [23]. Over the past several years it has been proposed that intermittent hypoxia also had a small advantageous effect on FEV_1 and FEV_1/FVC. We know that FEV_1 and FEV_1/FVC are significant diagnostic measures for the degree of airway obstruction, but their association with exercise capacity is controversial. Previous work has shown VC and FVC improvements after intermittent normobaric hypoxic training in patients with high-risk COPD [15]. A previous study reported a three-week with 15 sessions of the IHT (12-15% O_2) resulted in significant benefits for mild COPD patients, with increased inspiratory muscle function and thickness in the diaphragm, improved lung volumes (FEV_1 and FEV_1/FVC), increased aerobic capacity and level of activity [16]. Our study found the IHT improved %predicted VC and FVC when compared to the CON. However, the IHR presented a nonsignificant increase when compared to control. These results related with 6MWD increased seen in this study which indicated the cardiovascular fitness improvement. Consistent with previous studies, they demonstrated that after the 6 weeks of the IHE was substantially higher in the 6MWD than the CON group. This result indicated that the training program helped improve cardiovascular fitness in patients with excess weight and hypertension [21]. Other studies conducted on COPD patients or overweight people presented improvement in exercise performance following the IHR [8,24]. Similarly, this research found a beneficial outcome resulting in hypoxic programs both at rest and with exercise on the 6MWT. Taken together, exposure to hypoxic air either at rest or during moderate-intensity exercise results in hematological and non-hematological adaptations [25,26] which may consider for fitness improvements. The lung volume improvement found in this study may result from respiratory muscle strength increased which was enhanced by hypoxic training, and cardiovascular fitness (6MWD) increased correspondingly. This study focused on the effects of intermittent hypoxic exposure at rest or combined with light to moderate exercise on cardiopulmonary function. However, further research is required to elucidate the effect of lung volumes improvement on cardiovascular fitness.

4.2 Lipid profile and hematological variables

Participants lipid profile is an essential indicator of cardiovascular disease (CVD) risk. Commonly, the high risk of CVD is related to elevations in triglycerides (TG), LDL-C, and decreases in HDL-C levels [27]. The mechanisms responsible for the improvement of the lipid profile levels induced by hypoxia have not yet been completely described. The effects of hypoxic training in a previous study show significantly decreased total cholesterol (−4.2% to −30%) and LDL-C (−2.6% to −14.3%) but no significant change in HDL-C and TG levels [28]. However, the current study did not observe any improvements in blood lipid profiles after 6 weeks of intermittent hypoxic exposure at rest or combined with light to moderate exercise. While trending in the right direction we postulate a longer period of training (more than 6 weeks) may be required for lipid changes in cardiovascular-risk participants. Thus, future studies may need to lengthen the intervention period or alter the hypoxic dose to get lipid changes in people at risk of cardiovascular disease.

4.3 Body composition and anthropometric variables

Hobbins et al. [29] conducted a systematic review to investigate the effects of exercise training in a hypoxic environment on cardiometabolic health and body composition, who reported that exercise training in a hypoxic environment causes increased energy expenditure, improved cardiometabolic health, and decreased body weight and body fat mass. It has been proposed that the hypoxic condition stimulates the hypoxia-inducible factor (HIF) which plays a crucial role in effective metabolism regulation such as preserving body mass, glucose homeostasis, and liver metabolism, and thereby in the prevention of obesity [30]. Our results are in contrast to previous studies that have shown that hypoxic training is successful in body weight and body fat loss in overweight or obese subjects [29,31]. Similar results were found with overweight and obese patients with T2DM where exercise combined with hypoxia caused a reduction in body weight and fat mass [9,32]. A previous study also reported that combined endurance and strength training under hypoxic conditions (simulated altitude: 2,100-3,200 m, 14.5-16.5% O_2) for 4 weeks (11 times per week) reduced body weight more than under normoxic environments in obese adults [31]. Similarly, Wiesner et al. [33] indicated that after 4 weeks of hypoxic exercise training body composition was improved in both obese women and men with insulin-resistance. In the current study, most of the body composition parameters have shown no changes except hip circumference. One possible reason is that we conducted this research on an unhealthy subject group (i.e., they had at least 3 risk factors for cardiovascular disease) who performed only light-to-moderate exercise under a hypoxic environment. Because of the higher risk patients in this study, we used an exercise intensity that may not be conducive to fat loss. In addition, the length of the intervention was only 6 weeks which may not be long enough to get significant body weight reduction. Finally, the degree of hypoxia (14%) or the number of hypoxic sessions (only 2 per week) may not have been adequate to stimulate lipolysis and further work should investigate what hypoxic level and intervention length is appropriate for fat loss in individuals with cardiovascular disease risk factors.

4.4 6MWT

6MWT is a simple cardiopulmonary functional test that gives an indication of the individual’s ability to complete aerobic exercise. An important indicator of metabolic and cardiovascular fitness is aerobic exercise performance. This reflects the ability of the cardiopulmonary system and microcirculation to transport oxygen to muscle tissue while exercising [34].Previous studies demonstrated that endurance training (at 70-80% maximal oxygen uptake, 5-6 sessions/week, 3-6 weeks) performed under hypoxic conditions produces improvement in muscle oxidative capacity when compared to endurance exercise under normoxia conditions [35]. The present study showed 6 weeks of the IHR and the IHT (moderate hypoxia, FiO_2=0.14) substantially improved 6-min walk distance in people with cardiovascular risk factors. Therefore, both IH programs, particularly the IHT, have the possibility to be a valuable non-pharmacological therapy which aims to improve aerobic capacity and endurance.

Breathing normobaric hypoxic air with or without exercise has significant benefits compared with other forms of hypoxia including high-mountain therapy (living in the mountains or at high altitude) or hypobaric hypoxic exposure via a hypobaric chamber, particularly cost-effectiveness and accessibility of use in the clinical setting. As no irritation or adverse effects occurred during our intermittent hypoxic intervention with or without light to moderate-intensity exercise, this type of intervention appears to be feasible to use in people with current cardiovascular risk factors. Overall, the findings of this study revealed that light to moderate-intensity aerobic exercise with hypoxic stress may play a role in lung function and cardiopulmonary function improvement. However, little is known in regard to the optimal combination between the exercise training (e.g., the intensity of exercise, type of exercise) and degree of hypoxic (e.g., level of altitude, optimal dose of hypoxia, normobaric hypoxia vs. hypobaric hypoxia etc.) which should be the focus in further research studies.

4.5 Limitations of study

In considering the results of this study we must also state the obvious limitations which may have affected the results. Firstly, the study sample size is relatively small and although we used a statistical procedure to find the appropriate subject numbers, with such small numbers in each group the risk of bias increases. Secondly, the generalizability of our findings is limited to people with several cardiovascular risk factors and cannot be extended to otherwise healthy cohorts. Finally, the study did not require participants to control other lifestyle variables which have been shown to have an effect on subsequent health and fitness (e.g., diet, supplement intake, smoking etc.). We therefore consider these results to be speculative until substantiated with further research.

5. Conclusion

We demonstrated for the first time that 6 weeks of intermittent hypoxic training improved lung function and cardiopulmonary performance in people with cardiovascular risk factors. The consecutive 6 weeks of the IHT showed beneficial effects on lung volumes (FVC and FEV_1) and cardiovascular fitness (6MWD). This study indicated that IH (whether at rest or during exercise) can improve cardiopulmonary function in people with cardiovascular disease risk factors.

6. Ethical approval

All participants signed an informed consent, and the study was approved by the Human Ethical Committee of the Khon Kaen University (reference HE621492) in accordance with the 1964 Declaration of Helsinki.

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