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Helpful to Live Healthier? Intermittent Hypoxic/Ischemic Training Benefits Vascular Homeostasis and Lipid Metabolism with Activating SIRT1 Pathways in Overweight/Obese Individuals

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Keywords
Intermittent hypoxic training · Remote ischemic preconditioning · Obesity management · Vascular homeostasis · Lipid metabolism

Abstract

Introduction: The present study aimed to investigate whether and how normobaric intermittent hypoxic training (IHT) or remote ischemic preconditioning (RIPC) plus normoxic training (RNT) has a synergistic protective effect on lipid metabolism and vascular function compared with normoxic training (NT) in overweight or obese adults. ***Methods:*** A total of 37 overweight or obese adults (36.03 ± 10.48 years) were randomly assigned to 3 groups: NT group (exercise intervention in normoxia), IHT group (exercise intervention in normobaric hypoxic chamber), and RNT group (exercise intervention in normoxia + RIPC twice daily). All participants carried out the same 1-h exercise intervention for a total of 4 weeks, 5 days per week. Physical fitness parameters were evaluated at pre- and postexercise intervention. ***Results:*** After training, all three groups had a significantly decreased body mass index ($p < 0.05$). The IHT group had reduced body fat percentage, visceral fat mass ($p < 0.05$), blood pressure ($p < 0.01$), left ankle-brachial index (ABI), maximal heart rate (HRmax) ($p < 0.05$), expression of peroxisome proliferator-activated receptor-γ (PPARγ) ($p < 0.01$) and increased expression of SIRT1 ($p < 0.05$), VEGF ($p < 0.01$). The RNT group had lowered waist-to-hip ratio, visceral fat mass, blood pressure ($p < 0.05$), and HRmax ($p < 0.01$). ***Conclusion:*** IHT could effectively reduce visceral fat mass and improve vascular elasticity in overweight or obese individuals than pure NT with the activation of SIRT1-related pathways. And RNT also produced similar benefits on body composition and vascular function, which were weaker than those of IHT but stronger than NT. Given the convenience and economy of RNT, both intermittent hypoxic and ischemic training have the potential to be successful health promotion strategies for the overweight/obese population.

Introduction

Obesity, typically defined as excess adiposity storage, rapidly develops into a public health concern. The World Health Organization (WHO) determines a body mass index (BMI) ≥ 25 as overweight and a BMI ≥30 as obese

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[1]. Obesity is a major public life-threatening health issue since it magnifies the risk of developing various chronic diseases, deteriorating life quality and life expectancy [2–5]. As traditional approaches to losing weight frequently generate disappointing short- or long-term consequences, innovative solutions are being investigated [6, 7]. Consequently, how to lose weight effectively has emerged as an urgent clinical challenge.

In recent years, numerous studies have revealed that participants lost significant weight after they reach a plateau under hypoxic conditions [8, 9]. Other investigations have also confirmed altitude is inversely associated with obesity prevalence [10, 11]. Hence, studies mentioned above imply that hypoxia plus exercise may help with weight loss. With technological advancement, people could exercise in an artificial hypoxic environment (inspired oxygen fraction: 16.5–14.5%, simulated altitude: 2,000–3,000 m) while living in normoxia, widely known as "intermittent hypoxic training (IHT)" [12–14]. IHT benefits from hypoxic stimulation without adverse health complications [15]. In comparison to normoxic exercise, IHT could increase basal metabolic rate and energy expenditure [16, 17], enabling exercisers to attain the same amount of metabolic stimulation even at lower mechanical loads during physical exercise, thus minimizing the risk of orthopedic injury [16]. Although the exercise conditions are different, IHT does generate greater benefits than a pure normoxic exercise on the cardiovascular system [18–20], metabolic function [21–24], and body composition [25]. IHT has the potential as a promising type of physical exercise for managing obesity and obesity-associated diseases.

Remote ischemic preconditioning (RIPC) is a nonpharmacological therapeutic strategy to protect vascular function through intermittent ischemia. RIPC initiates alternate and intermittent episodes of ischemia by cutting off blood flow to nontarget tissue (often the arms or legs) [26]. Moreover, numerous clinical trials have already supported RIPC's anti-ischemic protective effects on the heart, brain, and other important organs [27–29]. Besides reducing infarct size and blood pressure (BP), RIPC improves vascular endothelial function in both the cardio- and cerebrovascular systems [30–32]. Plenty of studies and investigations generally concentrated on the protective effect of RIPC against ischemia-reperfusion injury, emphasizing RIPC may provide a powerful, universal stimulus for vascular adaptation and protection.

In brief, both RIPC-induced intermittent localized ischemia compensation and IHT-induced systemic hypoxia compensation are vasoprotective, but it is still equivocal whether the mechanisms and pathways involved are the same and whether vascular protection is approximately equivalent. Our study integrated diet management and exercise training with normobaric hypoxia or RIPC to explore whether IHT or RIPC plus normoxic training (RNT) can increase the efficacy of weight loss and improve the expression of metabolic-related biomarkers, thereby slowing down vascular ageing and the related pathways and mechanisms. According to our hypothesis, after exposure to hypoxia, IHT could effectively enhance vascular function and elasticity as well as modulate lipid metabolism. However, RNT, a combination of normoxic training (NT) and intermittent local ischemia and hypoxia, may also evoke similar vascular protective effects by mimicking mechanisms of IHT. Therefore, our findings would cast new insights and provide new perspectives for vascular protection and weight control in the overweight/obese population.

Materials and Methods

Subjects

Thirty-seven overweight or obese volunteers (aged: 20–58 [36.03 ± 10.48] years) were recruited for the study. Inclusion criteria were aged from 18 to 60 years; 25≤ BMI ≤35; have not been to the plateau or participated in a hypoxic program in the last 6 months; if the participant has hypertension or diabetes, he/she should be on regular medication for at least 6 months and stable for at least 3 months. Subjects were excluded if they met any one of the following items: acute and chronic physical or psychological illnesses, including muscle and joint injuries, cardiac diseases, cerebrovascular diseases, peripheral vascular diseases, pulmonary disease, migraine, moderate or higher anxiety and/or depression, insomnia, etc.; alcohol abuse or smoking; women who are pregnant or in preparation for pregnancy or breastfeeding; a history of upper limb thrombosis; serious local soft tissue injury or fracture of the upper limb; adjustment or change in the medication taken within the last 4 weeks; physical activity twice a week or more. The sample size was estimated by G*Power software (version 3.1.9.6, University of Kiel, Kiel, Germany). The sample size was calculated with an alpha of 0.05, a desired power of 0.80, and the effect size (ES = 0.69) published in a previous study of the effect of exercise on vascular endothelial function [20]. The total sample size required to satisfy statistical significance was calculated to be 30 individuals.

Prior to the start of the study, all volunteers were informed about the purpose and process of the study and they provided informed consent after receiving an adequate explanation about the experiment and potential adverse effects. Afterward, they were randomized to one of three groups: IHT (n = 13), NT (n = 12), and RNT (n = 12) by using a computerized random number generator. Four participants (NT: 3 and RNT: 1) were dropped from the trial, owing to withdrawal (n = 2) and healthy problems (n = 2). The Consolidated Standards of Reporting Trial (CONSORT) flow diagram is shown in Figure 1.

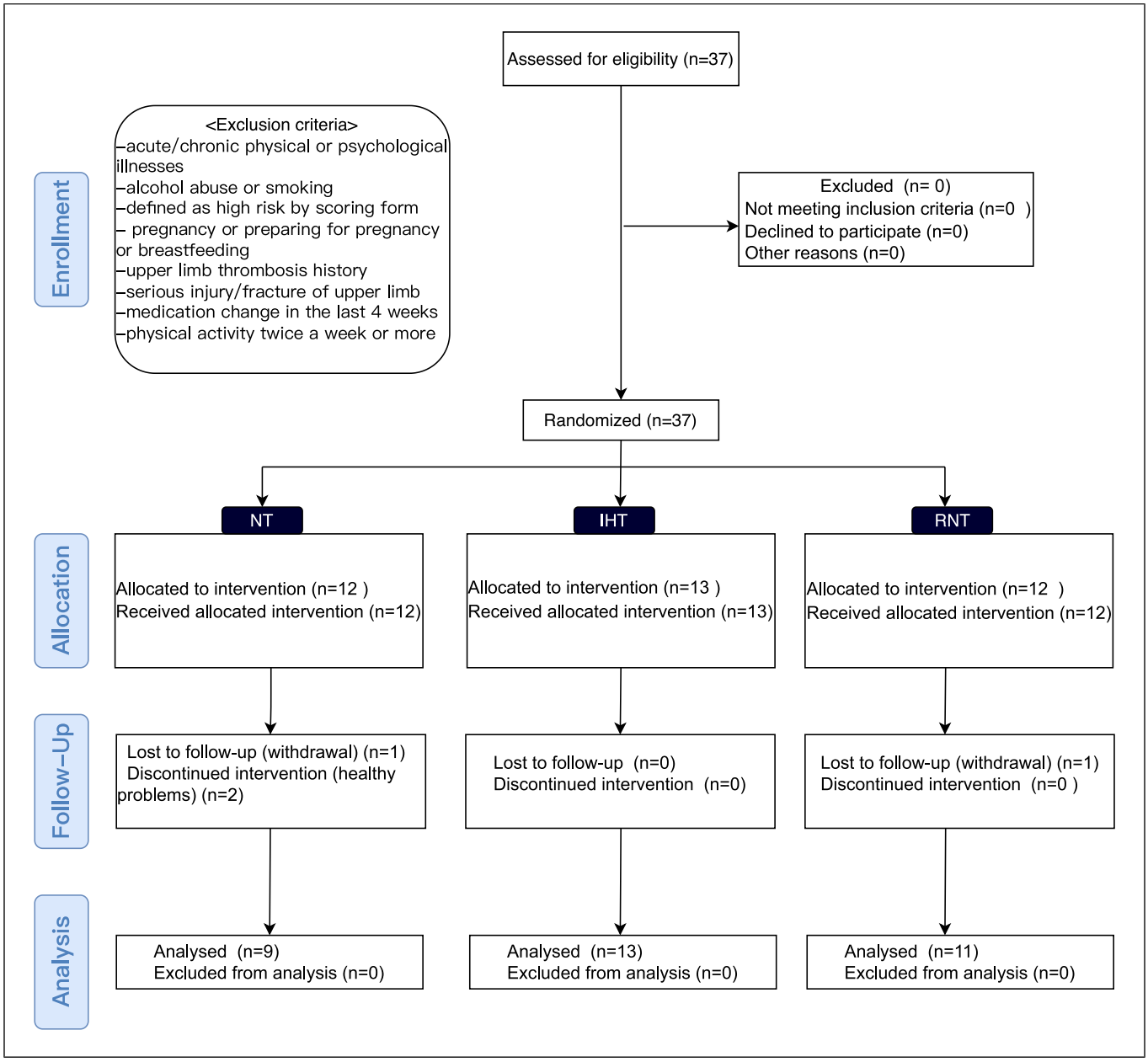

Fig. 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram. NT, normoxic training group; IHT, intermittent hypoxic training group; RNT, remote ischemic preconditioning plus normoxic training group.

Study Design

The study design consisted of a day of pretesting session, a 4-week intervention, and a day of post-testing session (Fig. 2). Before and after the exercise intervention, all participants fasted for at least 8 h, and a venous blood sample was collected in the morning, for the analysis of hemorheological parameters and metabolic biomarkers. Then, their body composition and arterial stiffness were assessed. Following a light meal, participants completed a cardiopulmonary exercise test, and their BP and heart rate were measured in the afternoon.

During the 4-week exercise intervention, subjects exercised for 60 min after eating a fat-reducing nutritional lunch (approximately 600 kcal, 25% adipose, 35% protein, 40% carbohydrates) on weekdays, and all of them finished the same moderate-to-high-intensity (metabolic equivalent task ≥3 mL/kg/min; ≥70% maximal oxygen consumption) workout program designed by professional exercise specialists (online suppl. Table 1; for all online suppl. material, see https://doi.org/10.1159/000536093). The IHT group exercised in a normobaric hypoxic room (inspired oxygen fraction: 15%, a 2,700-m stimulated altitude), while others worked

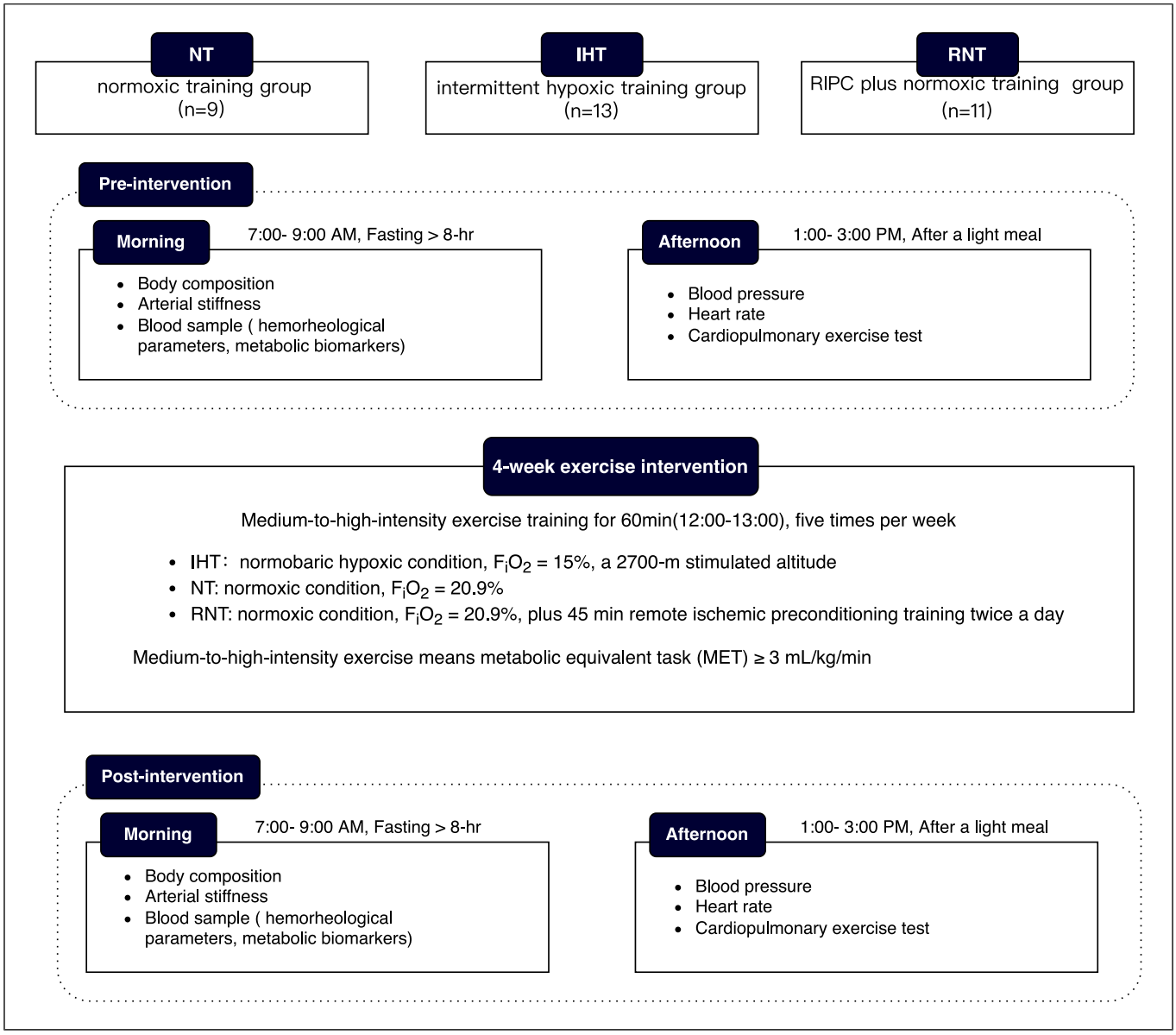

Fig. 2. Study design. IHT, intermittent hypoxic training group; NT, normoxic training group; RNT, remote ischemic preconditioning plus normoxic training group; FiO_2, inspired oxygen fraction.

out in a normoxic condition. Besides, participants of the RNT group also achieved 45 min RIPC twice daily for 4 weeks. All subjects were required to adhere to a weight loss diet constructed by a nutritionist. Additionally, they also need to walk briskly for at least 2.5 km per day on weekends or finish other moderate-to-high-intensity exercises depending on personal circumstances.

Body Composition

The body composition parameters (height, weight, BMI, lean body mass, fat mass, muscle mass, protein, body fat percentage, waist-hip ratio [WHR], basal metabolism, total energy metabolism, visceral fat area, visceral fat content, subcutaneous fat content) of all the subjects were analyzed by a body composition analyzer (MC-980MA, BAI-LIDA, Dongguan, China), and participants wore light clothing and stood barefoot on the analyzer, aligning the palms of his or her feet with the electrodes to ensure intimate contact between the whole sole of the foot and the foot electrodes. Participants grasped the handle with both hands, with both upper limbs naturally dangling at an angle of 15° to the trunk and both lower limbs standing.

Arterial Stiffness

After at least 20 min of rest, the participants' resting brachial-ankle pulse wave velocity and ankle-brachial index (ABI) were measured using an automatic oscillometric device (BP-203RPEIII,

Omron, Osaka, Japan). This device captures the brachial and ankle BP and the brachial-ankle pulse wave velocity on both the left and right sides. Electrocardiography cuffs and wrist electrodes were put bilaterally, along with the brachium and ankles. Both a plethysmographic sensor, which calculated the volume pulse shape, and an oscillometric pressure sensor, which measured BP, were attached to the cuffs. Using a semiconductor pressure sensor, the waveforms of the brachial and ankle pulse volumes were captured.

Blood Pressure

After participants had rested for at least 20 min, their BP in their right brachial artery was monitored twice with an ECG monitor (MX700, Philips, Shanghai, China), and the average value was utilized as the result value. If the first and second measurements yielded mixed conclusions, the measurement was repeated after a 10-min pause. Mean arterial BP was calculated as 1/3 × systolic BP (SBP) + 2/3 × diastolic BP (DBP). Pulse pressure difference was calculated as SBP minus DBP.

Cardiopulmonary Exercise Test

With the use of a continuous incremental exercise load procedure, cardiopulmonary exercise testing was carried out using a cardiopulmonary exercise tester (quarkb2, COSMED, Italy). The subjects warm up by treading for 2 min at 50 revolutions per minute while unloaded, followed by 5 min of rest. The treadmill was used for 2 min with an initial load of 50 W and a speed of 50 r/min. Thereafter, a load increase of 25 W was made every 2 min until exhaustion. The entire test procedure took roughly 12 min. During the recovery phase, the exercise was performed in the unloaded state for 2–5 min.

Blood Sample Collection and Analysis

All participants fasted for at least 8 h prior to and following the exercise intervention, and venous blood samples were obtained for the analysis of hemorheological parameters and metabolic biomarkers. During each blood collection, venous samples were collected and directly sent to the blood laboratory (Xiaotangshan Hospital, Beijing, China) for hematological analysis; 5 mL was collected by EDTA-coated vacutainers and centrifuged at 3,000 rpm for 15 min. Then, those samples were separated into plasma, white blood cells, and red blood cells and stored in 1.5 mL microtubes at −80°C until subsequently analyzed for plasma levels of metabolic biomarkers through ELISA.

ELISA

Serum insulin, HIF-1α, platelet-derived growth factor-BB (PDGF-BB), platelet-derived growth factor-AB (PDGF-AB), vascular endothelial growth factor-A (VEGF-A), interleukin 6 (IL-6), tumor necrosis factor α (TNFα), erythropoietin (EPO), von Willebrand factor (vWF), SIRT1, and endothelial nitric oxide synthase (eNOS) were measured by commercial enzyme-linked immunosorbent assay kits (RayBiotech, Atlanta, USA) following the manufacturer's specifications. Serum nitrotyrosine, endothelin-1 (ET-1), oxidized low-density lipoprotein (ox-LDL), peroxisome proliferator-activated receptor-γ (PPARγ), PPARγ coactivator 1α (PGC-1α), and platelet endothelial cell adhesion molecule-1 (PECAM-1) were measured by commercial enzyme-linked immunosorbent assay kits (Sabbiotech, MD, USA) following the manufacturer's specifications. All samples were measured in triplicate, and the mean was scored.

Statistical Analysis

Data are presented as means ± standard deviations. Prior to performing the parametric tests, the Shapiro-Wilks W test was used to confirm the assumptions of normality and homoscedasticity. A two-way ANOVA with repeated measures for time-dependent pretest value was carried out to assess the effects of the 4-week intervention on each dependent parameter. The Bonferroni test was applied as a post hoc analysis to determine the differences when an ANOVA revealed a significant interaction. GraphPad Prism 9.0 was used for all analyses (GraphPad Software, CA, USA).

Results

Demographic and Anthropometric Characteristics of Participants

Thirty-three participants (17 females and 15 males) aged 20–58 years have completed the 4-week exercise intervention. At baseline, three groups had similar demographic and anthropometric characteristics, and no significant differences among groups were observed for any variable (Table 1, $p > 0.05$).

Body Composition

A significant decrease in BMI ($p < 0.05$ in three groups) of all groups was found after the 4-week exercise intervention. Compared to the baseline, the body weight percentage ($p = 0.026$) and fat mass ($p = 0.011$) of the IHT group significantly reduced, while the RNT group had considerably lowered WHR ($p = 0.042$) and visceral fat area ($p = 0.002$). Moreover, the two experimental groups IHT and RNT had markedly less visceral fat mass (IHT: $p = 0.044$, RNT: $p = 0.014$) following the intervention (Table 2).

Vascular Function

SBP (IHT: $p = 0.0002$, RNT: $p = 0.006$) and mean arterial BP (IHT: $p = 0.005$, RNT: $p = 0.005$) of the IHT and RNT groups significantly decreased. DBP ($p = 0.019$) of the RNT group remarkably lowered as well. Regarding the IHT group, the pulse pressure difference ($p = 0.008$) and left ABI ($p = 0.013$) significantly decreased (Table 3).

Hematologic Parameters

There were no statistically significant differences in hematological parameters among the three groups during the 4-week exercise intervention (Table 4).

Cardiopulmonary Fitness

The maximal heart rate (HRmax) (IHT: $p = 0.025$, RIPC: $p = 0.0009$) of the IHT and RNT groups significantly decreased (Table 5).

Table 1. Anthropometric characteristics of participants

Variables	Groups			*F*-value	*p* value
	NT (*n* = 9)	IHT (*n* = 13)	RNT (*n* = 11)		
Age, years	31.33±8.75	36.62±9.54	39.18±12.68	1.414	0.259
Female, %	4 (44.44)	8 (61.54)	5 (45.45)	0.404	0.671
Height, cm	170.2±11.57	169.3±7.87	170.8±11.66	0.064	0.938
Weight, kg	84.98±19.39	80.70±9.95	84.90±16.76	0.303	0.741
BMI	29.28±5.81	28.08±2.10	28.87±3.50	0.278	0.760
WHR	0.93±0.08	0.93±0.04	0.93±0.04	0.106	0.890
Body fat percentage, %	33.50±6.91	36.40±6.41	33.69±8.80	0.561	0.576
Fat mass, kg	28.66±10.07	29.38±5.62	28.28±8.05	0.061	0.941

Data were presented as means ± standard deviation. NT, normoxic training group; IHT, intermittent hypoxic training group; RNT, remote ischemic preconditioning plus normoxic training group; BMI, body mass index; WHR, waist-to-hip ratio.

Table 2. Pre- and postexercise intervention results for body composition

Variables	Baseline versus postexercise					
	groups	before	after	changes of variables (after − before)	mean diff. (95% CI)	*p* value
BMI	NT	29.28±5.81	28.66±5.39	−0.62±0.61	0.62 (0.04, 1.20)	0.033
	IHT	28.08±2.10	27.38±2.23	−0.7±0.65	0.70 (0.22, 1.18)	0.003
	RNT	28.87±3.50	28.33±3.16	−0.55±0.79	0.55 (0.02, 1.07)	0.040
Body fat percentage, %	NT	33.50±6.91	32.57±7.55	−0.93±1.32	0.93 (−0.21, 2.07)	0.134
	IHT	36.40±6.41	35.35±6.44	−1.05±1.44	1.05 (0.11, 2.00)	0.026
	RNT	33.69±8.80	32.68±8.72	−1.01±1.27	1.01 (−0.02, 2.04)	0.056
WHR	NT	0.93±0.08	0.93±0.08	−0.01±0.01	0.01 (−0.005, 0.02)	0.410
	IHT	0.93±0.04	0.92±0.04	−0.01±0.02	0.01 (−0.004, 0.02)	0.435
	RNT	0.93±0.04	0.92±0.05	−0.01±0.01	0.01 (0.0003, 0.02)	0.042
Fat mass, kg	NT	28.66±10.07	27.19±9.43	−1.47±1.72	1.47 (−0.16, 3.10)	0.088
	IHT	29.38±5.62	27.68±5.50	−1.70±2.10	1.70 (0.34, 3.06)	0.011
	RNT	28.28±8.05	26.89±7.71	−1.39±1.89	1.39 (−0.08, 2.87)	0.069
Visceral fat area, cm^2	NT	106.1±40.69	101.1±41.17	−4.94±4.56	4.94 (−0.44, 10.33)	0.080
	IHT	113.7±33.44	110.5±32.07	−3.15±7.08	3.15 (−1.34, 7.63)	0.237
	RNT	117.4±33.17	110.1±35.48	−7.38±6.76	7.38 (2.51, 12.25)	0.002
Visceral fat mass, kg	NT	5.12±3.31	4.76±3.05	−0.37±0.41	0.37 (−0.02, 0.76)	0.070
	IHT	5.19±1.23	4.85±1.25	−0.33±0.45	0.33 (0.007, 0.66)	0.044
	RNT	5.16±2.19	4.74±2.06	−0.43±0.52	0.43 (0.08, 0.78)	0.014
Muscle mass, kg	NT	53.22±12.38	53.01±12.79	−0.21±0.88	0.21 (−0.48, 0.90)	0.829
	IHT	48.59±9.37	48.13±9.14	−0.46±0.67	0.46 (−0.11, 1.04)	0.144
	RNT	53.55±14.20	53.18±13.66	−0.37±0.92	0.37 (−0.25, 0.10)	0.366

Data were presented as means ± standard deviation. NT, normoxic training group; IHT, intermittent hypoxic training group; RNT, remote ischemic preconditioning plus normoxic training group; BMI, body mass index; WHR, waist-to-hip ratio; mean diff., mean difference; CI, confidence interval.

Table 3. Pre- and postexercise intervention results for vascular function

Variables	Baseline versus postexercise					
	groups	before	after	changes of variables (after − before)	mean diff. (95% CI)	*p* value
SBP, mm Hg	NT	125.5±10.86	117.0±15.22	−8.50±9.87	8.50 (−1.98, 18.98)	0.140
	IHT	126.2±9.89	110.2±8.96	−16.00±9.82	16.00 (7.44, 24.56)	0.0002
	RNT	128.2±11.17	116.3±16.52	−11.91±14.40	11.91 (2.97, 20.85)	0.006
DBP, mm Hg	NT	82.63±7.75	74.25±9.07	−8.38±9.91	8.38 (−0.73, 17.47)	0.078
	IHT	79.67±9.90	73.17±6.81	−6.50±9.70	6.50 (0.93, 13.93)	0.100
	RNT	83.27±7.93	74.27±8.84	−9.00±10.74	9.00 (1.24, 16.76)	0.019
MBP, mm Hg	NT	96.92±8.43	88.50±9.10	−8.42±8.18	8.42 (−0.19, 17.03)	0.057
	IHT	95.17±9.23	85.50±7.23	−9.67±9.21	9.67 (2.64, 16.70)	0.005
	RNT	98.24±8.61	88.27±10.02	−9.97±10.84	9.97 (2.63, 17.31)	0.005
Pulse pressure difference, mm Hg	NT	42.88±6.11	42.75±12.02	−0.13±11.81	0.13 (−8.79, 9.04)	>0.9999
	IHT	46.50±7.59	37.00±4.94	−9.50±6.72	9.50 (2.22, 16.78)	0.008
	RNT	44.91±6.49	42.00±13.86	−2.91±11.34	2.91 (−4.69, 10.51)	0.712
Heart rate, beats/min	NT	74.13±3.27	83.38±9.44	9.25±10.18	−9.25 (−19.03, 0.53)	0.068
	IHT	77.67±9.78	80.50±12.46	2.83±10.23	−2.83 (−10.82, 5.15)	0.756
	RNT	81.27±13.53	78.64±13.37	−2.64±7.74	2.64 (−5.70, 10.97)	0.814
Left ABI	NT	1.04±0.09	1.08±0.07	0.04±0.08	−0.04 (−0.10, 0.024)	0.321
	IHT	1.08±0.08	1.02±0.07	−0.06±0.07	0.06 (0.01, 0.12)	0.013
	RNT	1.09±0.12	1.12±0.09	0.02±0.08	−0.02 (−0.07, 0.04)	0.855
Right ABI	NT	1.07±0.08	1.09±0.10	0.02±0.06	−0.02 (−0.09, 0.04)	0.722
	IHT	1.10±0.12	1.08±0.09	−0.02±0.08	0.02 (−0.04, 0.07)	0.803
	RNT	1.11±0.08	1.09±0.10	−0.02±0.06	0.02 (−0.03, 0.07)	0.730
Left baPWV, cm/s	NT	1,112±102.5	1,093±73.33	−19.25±104.06	19.25 (−68.69, 107.2)	0.927
	IHT	1,173±173.0	1,126±223.2	−46.83±108.81	46.83 (−24.97, 118.6)	0.292
	RNT	1,295±273.5	1,254±252.3	−41.00±79.18	41.00 (−34.00, 116.0)	0.441
Right baPWV, cm/s	NT	1,094±103.1	1,139±58.62	45.63±129.89	−45.63 (131.5, 40.29)	0.465
	IHT	1,167±176.3	1,158±196.7	−9.25±93.29	9.25 (−60.90, 79.40)	0.983
	RNT	1,274±273.1	1,266±240.3	−7.45±65.31	7.45 (−65.82, 80.73)	0.992

Data were presented as means ± standard deviation. NT, normoxic training group; IHT, intermittent hypoxic training group; RNT, remote ischemic preconditioning plus normoxic training group; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean arterial blood pressure; ABI, ankle-brachial index; baPWV, brachial-ankle pulse wave; mean diff., mean difference; CI, confidence interval.

The Expression of Metabolic Biomarkers

After exercise intervention, as shown in Figure 3, the expression of SIRT1 of two experimental groups grew, which of the IHT group elevated significantly (baseline vs. postexercise: 0.82 ± 2.14 vs. 6.02 ± 10.45, $p = 0.041$). Moreover, compared to the NT group, the expression of SIRT1 of the IHT group was significantly higher (NT vs. IHT: 0.06 ± 0.18 vs. 6.02 ± 10.45, $p = 0.022$). The expression of VEGF of both NT and IHT groups significantly increased (NT baseline vs. postexercise: 0 ± 0 vs. 267.0 ± 248.2, $p = 0.011$; IHT baseline vs. postexercise: 11.39 ± 33.11 vs. 268.8 ± 316.3, $p = 0.003$). All groups' PPARγ expression showed a decreasing trend, among which, PPARγ of the IHT group significantly reduced (baseline vs. postexercise: 9.87 ± 2.79 vs. 8.12 ± 1.37, $p = 0.008$). Statistical analysis of all metabolic biomarkers is shown in online supplementary Table 2.

Discussion

The main finding of our study showed that a 4-week moderate-to-high-intensity exercise intervention with calorie restriction under normobaric intermittent hypoxia or combined with RIPC significantly improved body composition and lipid metabolism, ameliorated

Table 4. Pre- and postexercise intervention results for hematologic parameter

Variables	Baseline versus postexercise					
	groups	before	after	changes of variables (after − before)	mean diff. (95% CI)	*p* value
LDL-C, mmol/L	NT	2.48±0.59	2.83±0.55	0.35±0.55	−0.35 (−0.86, 0.15)	0.235
	IHT	2.52±0.87	2.82±0.57	0.30±0.73	−0.30 (−0.72, 0.12)	0.214
	RNT	3.29±0.76	3.38±0.64	0.10±0.43	−0.10 (−0.55, 0.36)	0.931
TG, mmol/L	NT	2.33±2.93	1.28±0.74	−1.05±2.26	1.05 (−0.06, 2.16)	0.069
	IHT	1.53±0.60	1.20±0.47	−0.33±0.51	0.33 (−0.60, 1.26)	0.755
	RNT	2.06±1.11	1.52±0.84	−0.54±0.89	0.54 (−0.47, 1.55)	0.458
HDL-C, mmol/L	NT	1.19±0.29	1.28±0.26	0.09±0.15	−0.09 (−0.22, 0.05)	0.299
	IHT	1.35±0.44	1.34±0.32	−0.02±0.21	0.02 (−0.10, 0.13)	0.971
	RNT	1.12±0.21	1.18±0.25	0.06±0.08	−0.06 (−0.18, 0.06)	0.560
TC, mmol/L	NT	4.30±0.35	4.34±0.49	0.04±0.34	−0.04 (−0.41, 0.34)	0.993
	IHT	4.43±0.73	4.34±0.59	−0.09±0.43	0.09 (−0.23, 0.40)	0.870
	RNT	5.03±0.99	4.87±0.78	−0.16±0.53	0.16 (−0.18, 0.50)	0.576
hs-CRP, mg/L	NT	1.53±1.69	1.54±2.17	0.01±0.78	−0.01 (−1.36, 1.34)	>0.9999
	IHT	2.76±1.70	2.85±2.78	0.09±2.36	−0.09 (−1.21, 1.03)	0.996
	RNT	1.42±1.76	1.60±1.50	0.19±0.69	−0.19 (−1.40, 1.03)	0.973
Glucose, mmol/L	NT	4.75±0.28	4.71±0.31	−0.05±0.40	0.05 (−0.41, 0.50)	0.992
	IHT	4.98±0.10	5.07±0.78	0.09±0.60	−0.09 (−0.47, 0.29)	0.920
	RNT	4.97±0.96	5.23±0.90	0.26±0.57	−0.26 (−0.67, 0.16)	0.334
WBC, $\times 10^9$/L	NT	4.75±0.28	4.71±0.31	−0.18±1.54	0.18 (−0.69, 1.05)	0.939
	IHT	4.98±0.10	5.07±0.78	−0.52±0.81	0.18 (−0.69, 1.05)	0.212
	RNT	4.97±0.96	5.23±0.90	−0.66±0.72	0.66 (−0.13, 1.44)	0.124

Data were presented as means ± standard deviation. NT, normoxic training group; IHT, intermittent hypoxic training group; RNT, remote ischemic preconditioning plus normoxic training group; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; hs-CRP, high-sensitive C-reactive protein; WBC, white blood cell; mean diff., mean difference; CI, confidence interval.

BP, regulated vascular elasticity, as well as activating SIRT1 pathways in overweight or obese individuals. To the best of our knowledge, under the condition of well-controlled calorie intake per meal, this is the first study to investigate the impacts and mechanisms of weight loss and vascular homeostasis following exercise training with intermittent hypoxia or RIPC in overweight or obese subjects.

The current investigations have made determined efforts to understand and uncover the relationship between hypoxia and diseases, due to the fact that poor tissue oxygenation is positively related to excessive adiposity which causes sleep disorders [33]. However, in addition to the cause of obesity, hypoxia, e.g., IHT, is a potential obesity therapy. Our results were in agreement with previous studies that IHT helped obese individuals to significantly optimize body composition such as reduction of BMI, body fat percentage, and fat mass [19, 25, 34]. Moreover, we unexpectedly found that visceral fat mass was significantly lowered, indicating that IHT may be helpful to alleviate visceral obesity and further reduce visceral-obesity-related mortality and morbidity [3, 35]. Some clinical trials also found that IHT has a positive effect on lipid profiles, and subjects of the IHT group tend to have increased high-density lipoprotein cholesterol levels and decreased total cholesterol, low-density lipoprotein cholesterol, and triglycerides [36]. However, our results did not show significant changes in lipid profiles. Wood et al. [37] have discovered that high-intensity interval training improves high-density lipoprotein cholesterol more than moderate-intensity continuous training. Given the fact that most of the subjects were nontrained, we adopted a combination of medium-to-high-intensity exercise training programs instead of high-intensity interval training, which may explain the lack of significant improvement in the lipid profiles of our

Table 5. Pre- and postexercise intervention results for cardiopulmonary fitness

Variables	Baseline versus postexercise					
	groups	before	after	changes of variables (after − before)	mean diff. (95% CI)	*p* value
VO_2max, mL/min/kg	NT	22.48±6.71	23.31±5.28	0.84±2.34	−0.84 (−4.06, 2.38)	0.885
	IHT	21.01±3.52	21.68±4.15	0.67±2.89	−0.67 (−3.23, 1.96)	0.893
	RNT	20.33±7.59	22.95±6.04	2.62±4.79	−2.62 (−5.36, 0.13)	0.065
HRmax, beats/min	NT	161.3±16.11	155.9±13.58	−5.38±6.89	5.38 (−2.49, 13.24)	0.256
	IHT	155.3±9.35	148.1±9.70	−7.17±8.68	7.17 (0.74, 13.59)	0.025
	RNT	158.3±22.07	147.4±18.65	−10.91±9.94	10.91 (4.20, 17.62)	0.0009
Peak MET, mL/kg/min	NT	6.44±1.92	6.64±1.52	0.20±0.68	−0.20 (−0.95, 0.55)	0.880
	IHT	6.02±1.00	6.18±1.18	0.17±0.84	−0.17 (−0.78, 0.45)	0.873
	RNT	6.22±1.32	6.55±1.77	0.33±0.94	−0.33 (−0.97, 0.32)	0.501
Peak cardiac output, L/min	NT	6.44±1.92	6.64±1.52	−0.58±2.08	0.58 (−0.99, 2.15)	0.880
	IHT	6.01±1.00	6.18±1.18	−0.23±1.54	0.23 (−1.06, 1.51)	0.873
	RNT	6.22±1.32	6.55±1.73	0.01±1.71	−0.01 (−1.35, 1.33)	0.501

Data were presented as means ± standard deviation. NT, normoxic training group; IHT, intermittent hypoxic training group; RNT, remote ischemic preconditioning plus normoxic training group; VO_2max, maximal oxygen consumption; HRmax, maximal heart rate; MET, metabolic equivalent; mean diff., mean difference; CI, confidence interval.

subjects. Furthermore, it has been demonstrated that IHT could effectively augment aerobic exercise performance [38, 39], and results of our study showed significantly decreased HRmax, which is associated with lower cardiac output, thereby reducing myocardial oxygen demand and preserving cardiac integrity [40]. In terms of vascular homeostasis, the current investigations indicate that intermittent normobaric hypoxia and exercise training have synergistic impacts on ameliorating BP and reducing arterial stiffness [41]. Similar findings were observed in our trial, where subjects in the IHT group not only had lower BP but also had decreased pulse pressure difference as well as left ABI that was dramatically reduced to about 1, indicating enhanced vascular flexibility and lower risk for arterial disease [42].

In the last decade, few studies on IHT have concentrated on its mechanisms or have simply discovered the elevated expression of HIF-1α and its downstream targets VEGF and NO [41, 43] but not the interlocking relationship between lipid metabolism and vascular protection, where various biomarker factors and pathways may be involved. Considering SIRT1 works as a hypoxia-responsive energy sensor and plays a key epigenetic role in lipid homeostasis, metabolic regulation, and vascular endothelial function [44–47], we have noticed the multiple protective roles of SIRT1 in lipid metabolism and vascular homeostasis after analyzing the expression of relative metabolic biomarker proteins. SIRT1 is a class III nicotinamide-adenine-dinucleotide (NAD+)-dependent histone deacetylase (HDAC) that plays a crucial role in the maintenance of genome stability, apoptosis, autophagy, senescence, proliferation, and lifespan extension [48, 49]. As a survival factor, SIRT1 expression is inversely correlated with the presence of energy substrates, with levels rising during fasting and falling during overeating. In adipose tissue, SIRT1 represses the master regulator of adipogenesis and lipid metabolism PPARγ at the transcriptional level, inhibiting adipogenesis and activating lipolysis [50]. Generally speaking, SIRT1 overexpression reduces body weight and fat mass and circulating levels of free fatty acids, cholesterol, leptin, and adiponectin and increases glucose tolerance [51–53]. In terms of vascular homeostasis, SIRT1 protects vascular functionality, promotes vasorelaxation and angiogenesis, and ameliorates arterial stiffness by upregulating the expression of vascular protective factors such as eNOS and VEGF and reducing oxidative stress [54].

Our results were in line with the above investigations. On one hand, after exercise intervention, the expression of SIRT1 and VEGF in the IHT group was significantly elevated, which was consistent with previous research. SIRT1 deacetylates and stabilizes HIF-1α, then upregulates the expression of downstream VEGF protein [55], increasing endothelium-dependent vasorelaxation and promoting angiogenesis [56, 57]. On the other hand, the

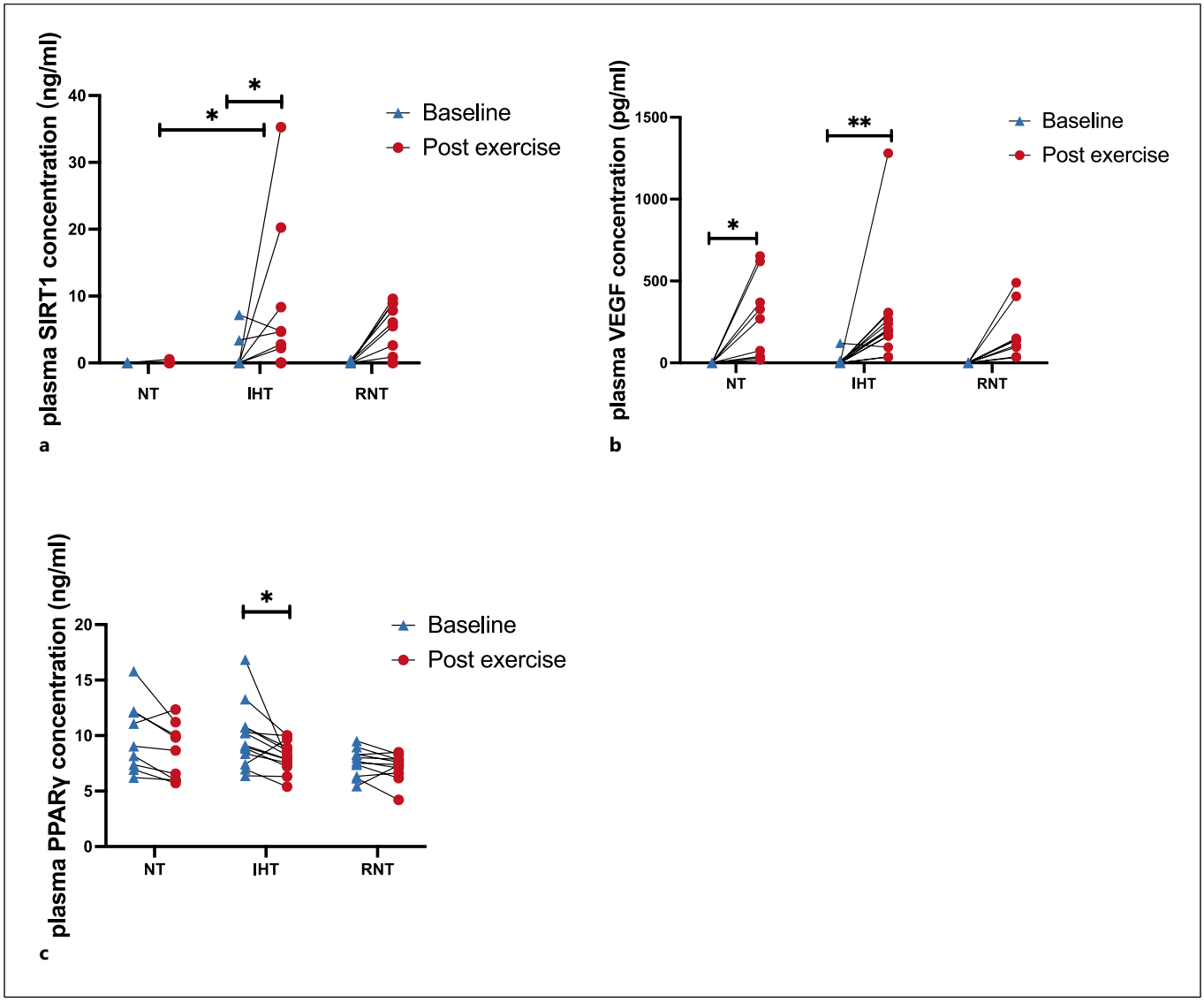

Fig. 3. Changes of plasma metabolic biomarkers concentration in three groups at baseline and postexercise. **a** Plasma SIRT1 concentration. **b** Plasma VEGF concentration. **c** Plasma PPARγ concentration. *p value <0.05; **p value <0.01. NT, normoxic training group; IHT, intermittent hypoxic training group; RNT, remote ischemic preconditioning plus normoxic training group.

activation of SIRT1 suppressed PPARγ [58]. Therefore, the activation of the SIRT1/PPARγ pathway may explain why subjects of the IHT group lose more fat mass than those of the NT group. The observed relationship between IHT-mediated impacts on body composition and vascular homeostasis may validate such underlying SIRT1 mechanisms.

In addition, RIPC is a non-pharmacological strategy and provides multi-organ protection [59]. The protection of ischemia tolerance induced by RIPC comprises two phases: an early phase appears within minutes from the initial ischemic stimulus and lasts for a few hours, and a late phase emerges 12–24 h later and lasts 3–4 days. Early-phase protection is provided by immediate post-translational modification of preexisting proteins, while late-phase protection is most probably triggered by the synthesis of new protective proteins, explaining why early-phase protection is more effective than late-phase protection [60]. However, it has never been studied whether RIPC, a form of localized intermittent ischemia,

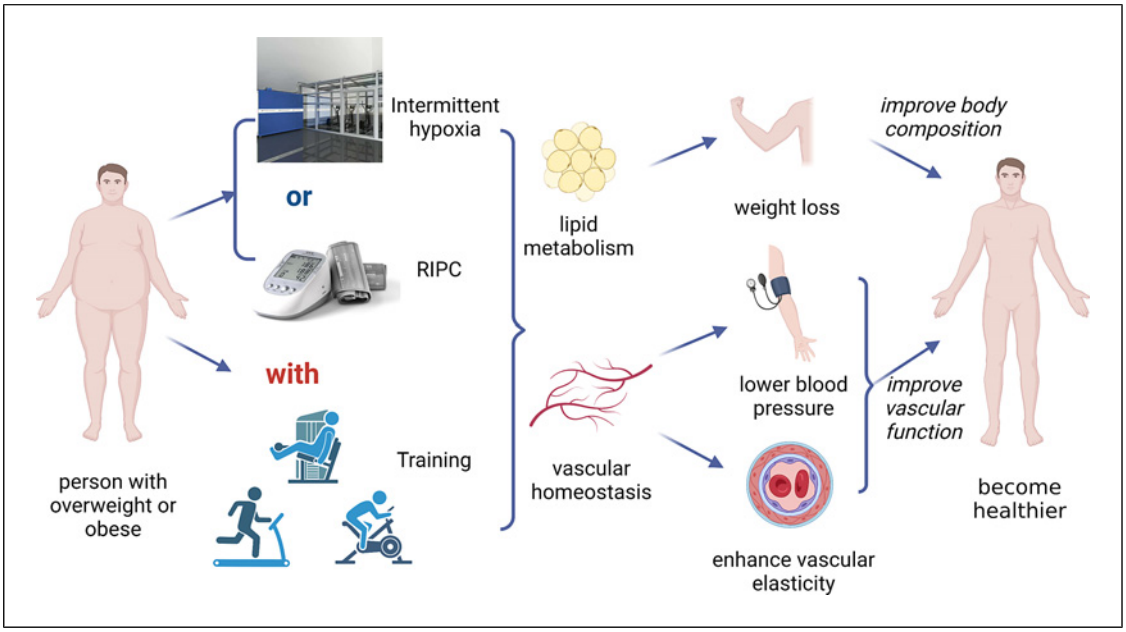

Fig. 4. Both IHT and RNT benefit lipid metabolism and vascular homeostasis in overweight or obese people, such as lowering body weight and BP and improving vascular elasticity, thus enabling these people to live healthier.

can work cooperatively with normobaric exercise to enhance vascular elasticity or metabolic parameters. To our astonishment, similar to the results of IHT, RNT significantly lowered BMI, WHR, and visceral fat mass and decreased BP as well as HRmax after exercise intervention (Fig. 4). Meanwhile, after the intervention, albeit not significant, RNT showed the tendency of increased expression of SIRT1 and VEGF as well as reduced expression of PPARγ, suggesting that RNT may improve body composition and enhance vascular function through mimicking mechanisms of IHT. However, RNT was marginally less effective than IHT in terms of losing weight, improving vascular stiffness, or activating SIRT1-related pathways.

Furthermore, subjects of the control group, named as the NT group, also presented significantly lower BMI and higher expression of VEGF after the 4-week of NT. And plenty of research has already demonstrated regardless of hypoxia that pure physical activity has overwhelming advantages in improving lipid profiles, aerobic exercise performance, ameliorating BP, and enhancing vascular homeostasis [61]. In other words, both intermittent hypoxia and intermittent ischemia (RIPC) work as "the icing on the cake," which means intermittent hypoxia or ischemia has the potential to maximize the health benefits of normoxic exercise on the body.

IHT was initially proposed as an innovative training for athletes, climbers, and pilots to improve their aerobic performance and cardiorespiratory fitness [62]. But during the past few years, IHT's potential for losing weight, reducing insulin resistance, and elevating vascular function has gained a great deal of attention [19, 43, 63]. Based on those previous trials and studies, we have lately discovered that 4-week IHT successfully reduced visceral fat mass and improved ABI, an indicator of lower limb vascular elasticity, in overweight or obese individuals. This finding serves as a reminder that IHT has been vastly underestimated for its potential to improve visceral obesity and vascular elasticity. We also conducted a preliminary exploration of the mechanisms and pathways involved in IHT. According to our results, after the 4-week intervention, the expression of SIRT1 and VEGF were significantly upregulated, while PPARγ was significantly suppressed in the IHT group, due to the fact that SIRT1 has diverse protective effects in a broad range of cellular physiologic processes and SIRT1 may facilitate lipid metabolism and protect vascular homeostasis through complicated mechanisms including SIRT1/PPARγ and SIRT1/HIF-1α/VEGF pathways. That is to say that the activation of SIRT1 and related pathways, those novel factors and mechanisms that have never been noticed before, may assist IHT to positively impact vascular function as well as lipid metabolism.

When it comes to RIPC, it is mostly applied to provide multi-organ protection against ischemia-reperfusion-induced injury [64]; nonetheless, we realized that, in addition to alleviating sustained ischemia-reperfusion injury, there might be other mechanisms to protect visceral organs. Apart from weight loss, the RNT group exhibited a significant reduction in both visceral fat area and visceral fat mass, highlighting that RNT has meaningful effects on visceral obesity. Visceral obesity, a complicated phenotype typified by dysfunctional adipose tissue and ectopic triglyceride storage, is a strong independent predictor of cardiovascular and metabolic disorders [65]. Hence, we hypothesized that RIPC may have an entirely new mechanism for managing visceral obesity, besides those recognized mechanisms to improve BP and cardiopulmonary fitness [32, 64, 66]. The precise pathways and factors involved in this mechanism require further investigation and exploration, which is our succeeding destination.

Our study has several limitations. First, our trial was designed as a pilot study with a limited sample size. Although our results were in agreement with previous larger, multicenter studies, the sample size should be expanded in future studies to track and analyze the influence of IHT and RNT on lipid metabolism and vascular elasticity to further validate the applicability and significance of this study. Second, we were unable to compare the gender difference when analyzing body composition changes due to the limited sample size. Third, our study lacked relevant histological examination such as biopsy and vascular slides, to intuitively verify changes of vascular and body composition after intervention. We will incorporate histology examination into our investigation of the SIRT1-related pathway in animal experiments.

Conclusion

Compared with NT, intermittent normobaric hypoxic training elicited better and stronger responses in improving body composition, regulating vascular homeostasis, elevating cardiopulmonary fitness, and activating SIRT1/HIF-1α/VEGF and SIRT1/PPARγ pathways in overweight or obese individuals. Moreover, although its potency was a little bit weaker than that of the IHT group, intermittent ischemic (RIPC) training could achieve multi-visceral-organ protection through the novel mechanism of reducing visceral fat area and content, in addition to the known effect of lowering BP. Thus, our findings demonstrated that intermittent hypoxic and ischemic training not only resulted in significant weight loss but also reduced visceral obesity, protected visceral organs, lowered obesity-related risks, and improved vascular homeostasis in overweight or obese individuals.

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Statement of Ethics

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Xuanwu Hospital, Capital Medical University (protocol code: KS2022157-1 and date of approval: September 13th, 2022). Informed consent was obtained from all subjects involved in the study. And written informed consent has been obtained from the subjects to participate in the study and to publish this paper.

Conflict of Interest Statement

The authors declare no conflicts of interest.

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Author Contributions

Methodology: G.W.N; software: M.Q.L and R.L.; validation: M.Q.L. and X.Q.J.; data curation: J.L.L. and L.W.; writing – original draft preparation: X.Q.J.; writing – review and editing: X.H.G.; supervision: L.M.W. and C.M.L.; project administration: X.M.J. All the authors have read and agreed to the published version of the manuscript.

Data Availability Statement

Data are not publicly available due to ethical reasons. Further inquiries can be directed to the corresponding author.

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