Article

Not peer-reviewed version

Evaluating the Therapeutic Potential of Exercise in Hypoxia and Low-Carbohydrate High-Fat Diet in Managing Hypertension in Elderly Type 2 Diabetes Patients: A Novel Intervention Approach

Raquel Kindlovits *, Ana Catarina Sousa, João Luís Viana, Jaime Milheiro, Bruno M. P. M. Oliveira, Franklim Marques, Alejandro Santos, Vitor Hugo Teixeira

Posted Date: 4 January 2025

doi: 10.20944/preprints202501.0270.v1

Keywords: Hemoglobin; Blood Pressure; Normobaric Hypoxia; Carbohydrates; Exercise; Diabetes; Elderly

 doi:10.20944/preprints202501.0270.v1

Article

Evaluating the Therapeutic Potential of Exercise in Hypoxia and Low-Carbohydrate High-Fat Diet in Managing Hypertension in Elderly Type 2 Diabetes Patients: A Novel Intervention Approach

Raquel Kindlovits [1,*], Ana Catarina Sousa [2], João Luís Viana [3], Jaime Milheiro [3] Bruno M. P. M. Oliveira [4], Franklim Marques [5], Alejandro Santos [6] and Vitor Hugo Teixeira [7]

[1] Faculty of Nutrition and Food Sciences, University of Porto, FCNAUP, Porto, Portugal, 4200-465

[2] Research Center in Sports Sciences, Health Sciences and Human Development, CIDESD, University of Maia, Maia, Portugal, 4475-690

[3] CMEP, Exercise Medical Centre Laboratory, Porto, Portugal, 4150-044 and Centre of Research, Education, Innovation and Intervention in Sport, CIFI2D, Faculty of Sport, University of Porto, Porto, Portugal, 4200-540

[4] Faculty of Nutrition and Food Sciences, University of Porto, FCNAUP, Porto, Portugal, 4200-465 and Laboratory of Artificial Intelligence and Decision Support, Institute for Systems and Computer Engineering, Technology and Science (LIAAD, INESC-TEC), Porto, Portugal

[5] Laboratory of Biochemistry, Department of Biological Sciences, UCIBIO, REQUIMTE, Faculty of Pharmacy, University of Porto, Porto, Portugal, 4050-313

[6] Faculty of Nutrition and Food Sciences, University of Porto, FCNAUP, Porto, Portugal, 4200-465 and Institute for Research and Innovation in Health, i3S, Porto, Portugal, 4200-135

[7] Faculty of Nutrition and Food Sciences, University of Porto, FCNAUP, Porto, Portugal, 4200-465 and Research Center in Physical Activity, Health and Leisure, CIAFEL, - Faculty of Sports - University of Porto, FADEUP Portugal, 4200-540 and Laboratory for Integrative and Translational Research in Population Health, ITR, Porto, Portugal, 4050-600

* Correspondence: nutri@raquelkin.com

Abstract. Type 2 diabetes mellitus (T2DM) is a chronic disease characterized by hyperglycemia, which could alter metabolic, vascular and hematological parameters. A low-carbohydrate, high-fat diet (LCHF) diet benefits glycemic and blood pressure control. In turn, exercise in hypoxia (EH) is known to improve insulin sensitivity, erythropoiesis and angiogenesis. LCHF combined with EH seem to be a potential therapeutic strategy for T2DM and hypertension (HTN), but evidence is still scarce. The aim of this study was to evaluate the effects of eight weeks of EH combined with a LCHF on hematological and lipid profiles, inflammation and blood pressure in older patients with T2DM and coexistent HTN. Diabetic patients with HTN (n=42) were randomly assigned to a (1) control group: control diet (high-carbohydrate and low-fat diet) + exercise in normoxia; (2) EH group: control diet + EH; (3) intervention group: LCHF + EH. Baseline and eight-week measurements included systolic, diastolic, and mean blood pressure (SBP, DBP, MAP, respectively), and hematological and lipid profiles and inflammation biomarkers. Blood pressure decreased after interventions ($p<0.001$), with no differences among groups (SBP: $p=0.151$; DBP: $p=0.124$ and MAP: $p=0.18$). There were no differences in lipid profile and C-reactive protein ($p>0.05$). Mean corpuscular hemoglobin (MCH) increased in the EH group ($p=0.027$), while MCH concentration decreased in the EH+LCHF group ($p=0.046$). In conclusion, there is no additional benefit in adding hypoxia to exercise or restricting carbohydrates, on blood pressure in patients with T2DM and coexisting HTN. Further elucidation of the mechanisms underlying hematological adaptations is imperative. Trial registration number: NCT05094505.

 doi:10.20944/preprints202501.0270.v1

Keywords: Hemoglobin; Blood Pressure; Normobaric Hypoxia; Carbohydrates; Exercise; Diabetes; Elderly

1. Introduction

Diabetes is a metabolic disease that shows itself clinically as chronic hyperglycemia. Poorly or uncontrolled diabetes could be associated with many physiological processes that may affect lipid metabolism, regulation of inflammation, vasodilatation, vascular, immunological, and hematological parameters [1]. In light of this, hypertension (HTN), defined by systolic blood pressure ≥ 140mmHg or diastolic blood pressure ≥90mmHg [2], is prevalent in over 50% of individuals with type 2 diabetes (T2DM) and significantly escalates the risk of cardiovascular diseases (CVD) by fourfold compared to normotensive non-diabetic individuals [3]. Notably, individuals with T2DM who also have HTN at the time of diagnosis exhibit elevated rates of mortality and cardiovascular events, particularly pronounced in older populations [4], suggesting that much of this excess risk is attributable to coexistent HTN [5], underscoring the critical need for effective therapeutic strategies [6].

Most T2DM patients are not active [7] and a sedentary lifestyle, together with poor nutrition, are considered as the major risk factors for T2DM and its complications [8]. Current international guidelines recommend aerobic and resistance exercise training for T2DM patients for improving blood lipids, inflammation, glycemic and blood pressure control [9]. Exercise and Sports Science Australia guidelines [10] recommends supervised exercise as an effective modality for improving weight loss and both blood pressure and glycemic in T2DM patients.

Hyperglycemia in T2DM was negatively correlated with some hematological indices, as red blood cells (RBC), hemoglobin (Hb) and mean corpuscular hemoglobin (MCH), due the augmented oxidative stress [11]. Improvements in these biomarkers, mainly by the production of erythropoietin (EPO) and its consequent erythropoiesis, can be a helpful physiological mechanism, but do not occur as a result of an exercise training in normoxia [12]. Conversely, adding hypoxia to exercise promotes relevant hematological adaptations [12,13]. Recent investigations have shown promising outcomes in elderly patients with CVD [14] and in individuals with T2DM [15] performing exercise in hypoxia (EH) at simulated altitudes, suggesting EH may offer additional benefits compared to exercise in normoxia.

Physiological adaptations occur in hypoxic environments [16], mainly driven by hypoxia inducible factor 1α (HIF-1α) activation [17] and increased expression of hypoxia-responsive genes [18]. EH-induced erythropoiesis and angiogenesis may contribute to improved blood pressure control and enhanced tissue oxygenation. Not least, HIF-1α promotes lowering of blood glucose levels through the stimulating on glycolysis and as a result of the increased expression of GLUT4 glucose transporters in muscle tissue independently of muscle work [19]. Collectively, these adaptations could potentially mitigate vascular, hematological and metabolic complications associated with T2DM and HTN.

The optimal dietary approach remains a subject of debate among experts [20,21]. The low-carbohydrate, high-fat (LCHF) diet has emerged as a promising therapeutic option for individuals with T2DM and HTN, endorsed by the American Diabetes Association for glycemic control and weight management [22]. Notably, LCHF diets have demonstrated efficacy in reducing blood pressure [23], even surpassing the Dietary Approaches to Stop Hypertension (DASH) diet [24], the standard recommendation for blood pressure management by the American Heart Association [21].

Furthermore, LCHF diets have also been associated with hematological adaptations, including reductions in MCH and in mean corpuscular hemoglobin concentration (MCHC)[25]. Decreases in MCH and MCHC were inversely linked with insulin resistance and high blood pressure [26]. It was previously shown that hematological changes could occur as an effect of oxidative stress promoted by T2DM [11], and that these biomarkers may also serve as predictors of the disease's evolution [27], due to their role as determinants of blood viscosity [28]. Increased blood viscosity could contribute to the development of T2DM [29] and HTN [26,30].

 doi:10.20944/preprints202501.0270.v1

Thus, this study aimed to evaluate the effects of an eight-week normobaric EH intervention at 3000m of simulated altitude combined with an LCHF diet on the hematological and lipid profiles, inflammation and blood pressure in older patients with T2DM and coexistent HTN. We hypothesize that the combination of chronic EH and LCHF dietary modifications will lead to improvements in blood pressure, with associated changes in hemoglobin levels, offering novel insights into therapeutic interventions for this high-risk population.

2. Materials and Methods

2.1. Ethical Considerations

The study was approved by the Ethics Committee of the Faculty of Nutrition and Food Sciences, University of Porto (Approval Number 45/2021/CEFCNAUP/2021 in July 2021. It was conducted following the declaration of Helsinki for studies in humans [31]. It has been registered in the Clinical Trial database (NCT05094505).

2.2. Study Design

This is a controlled, single-blind, three-arm, parallel, randomized controlled trial (RCT). Participants were randomly assigned into three independent groups (n=14, in each): control group (control diet + exercise in normoxia), EH group (control diet + EH) and EH+LCHF group [32]. The experimental design consisted of (1) pre-intervention tests, (2) a familiarization period, (3) an experimental intervention, and (4) post-intervention tests [33].

2.3. Participants

To be included, participants met the following criteria: (1) old individuals (>65 years) of either sex diagnosed with T2DM and HTN by their medical doctor for at least one year; (2) hemoglobin A1c (HbA1c) between 6.5% and 10%; (3) systolic blood pressure ≥ 140mmHg or diastolic blood pressure ≥ 90mmHg (4) pharmacological regimen stabilized for at least three months; (5) previous participation in supervised exercise programs in the last six months, and (6) absence of smoking in the last six months. Participants were excluded if: (a) they were insulin-dependent; (b) had uncontrolled microvascular or macrovascular complications related to diabetes, such as retinopathy, nephropathy, diabetic foot and atherosclerosis, diabetic cardiomyopathy or acute myocardial infarction; (c) presented other uncontrolled metabolic or vascular comorbidities; (d) were sedentary or (e) had a physical limitation that prevented them from exercising.

2.4. Dietary Plan

To each participant an individualized dietary plan was prescribed using the Dietbox® software, version 7.0. The energy content of the dietary plan met 100% of the estimated energy requirement (EER) for each participant. EER was calculated by multiplying the resting metabolic rate obtained by the Harris-Benedict equation, the most accurate at the individual level for older adults [34], by a physical activity level, assessed using the International Physical Activity Questionnaire (IPAQ short form, last seven days, elderly, self-administered format). Participants were categorically rated into one of three levels of physical activity: low, moderate or high [35].

The energy distribution by macronutrients was 60% from carbohydrates, 20% from protein and 20% from fat for the control diet, and 40% of energy from carbohydrates, 20% from protein, and 40% from fat for the LCHF diet. Both diets emphasized low–glycemic index foods to convey conventional dietary guidelines [36]. According to the classification of diets as (1) very low-carbohydrate: less than 26% of the energy intake; (2) low-carbohydrate: 26-45% of the energy intake; and (3) high carbohydrate ≥ 45% of the energy intake [37], we compared a low-carbohydrate diet (EH+LCHF group) to a high-carbohydrate diet (EH group). Compliance with the dietary plan was assessed using

 doi:10.20944/preprints202501.0270.v1

weekly 24-hour recalls. Participants met individually in appointments with a nutritionist twice over the eight weeks to encourage adherence to the dietary plan.

2.5. Exercise Protocol

Exercise sessions, either in normoxia or hypoxia (at 3000m of simulated altitude, via nitrogen dilution), took place in a hypoxic chamber at CMEP – Exercise Medical Center, 3 times per week during a period intervention of eight weeks. The chamber allows the control of O_2 (11% to 20.97%), temperature (up to 50ºC), relative humidity (up to 80%), and altitude above sea level (up to 8000m). Altitude rating can be defined as (1) high altitude: 1500 to 3500m; (2) very-high altitude: 3500 to 5500m; and (3) extreme altitude: above 5500m [38].

Before starting the intervention period, six familiarization sessions were held for two weeks so the participants could learn the exercise techniques and acclimate to the simulated altitude, with an increment of 500m at each visit until reaching 3000m of altitude. Exercise intensity was set at 75% of the heart rate reserve, quantified in the pre-intervention cardiopulmonary test (CPET). Heart rate and oxygen saturation were constantly monitored using a finger pulse oximeter (Globus YM201, Milan, Italy), and the Borg Rating of Perceived Exertion (RPE) was recorded after each exercise session [39]. All exercise sessions occurred at the same time of day (± 1 hour), and visits were separated by at least 48 hours of recovery.

The total duration of each exercise session was approximately 60min, which included a 5min warm-up with body mobilization and dynamic stretching, followed by 40min of moderate aerobic exercise, alternated every 9min on a cycle ergometer (Life Fitness, Illinois, United States) and a treadmill (Life Fitness, Illinois, United States), with a 1min rest between them. At the end of each session, and alternately between weeks, three strength exercises were performed (pectoral, shoulders, back, arms, thighs, legs and abdominals) using a structure of 3 series of 12 to 15 repetitions per exercise, with a 1min rest between sets, totaling ~ 15min of strength exercises.

2.6. Measurements

In all groups, hematological parameters, blood lipids, inflammation marker, and blood pressure were evaluated at baseline and 48h after the last exercise session (eighth week), after fasting for 12 hours and without any strenuous exercise in the last 24 hours and no alcohol consumption in the previous 72 hours.

2.7. Blood Samples Analyses

Hematological markers were determined, including erythrocytes (L), hemoglobin (g/dL), globular volume, (MCV, %), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCH, g/dL), red cell distribution width (RDW, %), leukocytes (L), neutrophils (%), eosinophils (%), basophils (%), lymphocytes (%), monocytes (%) and platelets (L). Lipid parameters included total cholesterol (mmol/L), high-density lipoprotein cholesterol (HDL-c, mmol/L), low-density lipoprotein cholesterol (LDL-c, mmol/L), triglycerides (TAG, mmol/L); and inflammatory marker, as CRP (mg/dL) were collected and analyzed.

2.8. Blood Pressure

Systolic, diastolic, and mean blood pressure (SBP, DBP and MAP) were measured using an automated sphygmomanometer (Dinamap Pro; Florida, United States) in the left arm, in a sitting position and after ten minutes of rest in the same day of blood sample collection. The measurements were performed in triplicate, and the average was expressed in mmHg.

2.9. Statistical Analysis

For sample and power calculations, this study was powered based on changes in hemoglobin A1c in the RCTs included in the meta-analysis by Zuuren et al. [40]. To detect an effect size of Cohen's

 doi:10.20944/preprints202501.0270.v1

d=1.14 with 80% power (alpha=0.05, two-tailed), G*Power software [41] suggested we would need 28 participants in a paired samples t-test, totaling 42 individuals to the three experimental groups. Assuming a 20% dropout rate, it was recruited 48 participants. All data was reported as mean (standard deviation SD). Normality was assessed using Shapiro-Wilk's test. Continuous variables that did not follow a normal distribution were transformed using the logarithm function: y=log(x-L) with L < minimum of x, if skewness was positive; or y=log(H-x) with H > maximum of x, if the skewness was negative; except for the diastolic blood pressures, that were transformed using the Box-Cox transformation with parameter λ=0.75. A two-way repeated measures ANOVA was used to examine changes in hematological markers, CRP, lipid profile and blood pressure over the chronic exercise period (zero vs. eighth weeks) and whether the magnitude of the chronic exercise-mediated adaptations differ in time and differ among groups. A Tukey post hoc test for multiple pairwise comparisons was performed to identify differences between groups when a significant main- or interaction effect was found. Statistical analysis was performed using SPSS Statistics software version 28.0, 2021 (IBM Company, Chicago, United States), and statistical significance was assumed at $p \leq 0.05$.

3. Results

3.1. Baseline Characteristics

The baseline characteristics of the participants are shown in Table 1. None of the participants got injured or had adverse responses to the EH or LCHF diet. The groups did not differ significantly at this moment, neither in terms of gender or age.

Table 1. Baseline characteristics of the participants.

Variables	CTRL group	EH group	EH+LCHF group	p-Value
Gender (male:female)	7:7	8:6	9:5	0.747
Age (years)	74.4 (3.6)	71.6 (3.8)	70.7 (4.0)	0.110
Body mass index (kg/m^2)	29.4 (4.1)	28.3 (4.0)	29.3 (3.4)	0.707
Hemoglobin A1c (%)	6.9 (0.8)	7.1 (0.7)	6.8 (0.5)	0.647
Fasting glucose (mg/dL)	118.7 (27.8)	117.9 (22.3)	108.2 (19.7)	0.435
Systolic blood pressure (mmHg)	154.7 (20.9)	142.3 (18.2)	148.0 (18.9)	0.254
Diastolic blood pressure(mmHg)	77.5 (8.1)	76.3 (11.7)	82.9 (16.3)	0.347
Mean arterial blood pressure (mmHg)	107.9 (16.9)	98.7 (13.6)	107.1 (15.8)	0.234

42 subjects participated in this study. All values are presented as the mean (SD).

3.2. Dietary and Exercise Interventions

The detailed data regarding exercise and diet interventions were previously published [33]. Briefly, the EH+LCHF group had a lower carbohydrate intake ($p<0.001$), a higher total fat intake ($p<0.001$), while no significant differences were found between groups about energy ($p=0.69$) and fiber ($p=0.49$) intakes. Physiological parameters obtained during eight weeks of exercise sessions, showed that the groups that exercised in hypoxia (EH and EH+LCHF) presented lower mean values of oxygen saturation when compared to the CTRL group ($p<0.001$), but the average heart rate was similar among groups ($p=0.63$). Also, subjective effort perception was collected and the EH and EH+LCHF groups achieved the highest score of RPE ($p=<0.001$) used to measure the level of subjective intensity of physical exercise.

3.3. Cardiovascular Risk Factors

 doi:10.20944/preprints202501.0270.v1

Cardiovascular risk factors were evaluated by lipid profile and CRP. No effect from baseline to post-interventions was observed on total cholesterol (p=0.08), HDL-c (p=0.987), LDL-c (p=0.501) and TAG (p=0.435), and among the three groups (p=0.135; p00.511; p=0.119; p=0.518), respectively. CRP did not show differences from baseline to the eighth week of intervention (p=0.090), and did not differ among groups (p=0.66, Table 2).

Table 2. Lipid profile and inflammation marker pre- and post- eight weeks of interventions.

Variables	CTRL group			EH group			EH+LCHF group			p-Value
	Pre	Post	Δ	Pre	Post	Δ	Pre	Post	Δ	Moments Groups
Cholesterol (mmol/L)	181.2 (49.5)	188.1 (51.8)	2.4 (17.7)	178.9 (30.1)	161.9 (23.2)	7.1 (6.7)	158.5 (46.4)	143.2 (42.7)	7.3 (16.4)	0.082 0.135
HDL-c (mmol/L)	53.8 (11.7)	52.5 (10.6)	0.5 (2.2)	52.3 (13.1)	52.0 (9.4)	0.1 (2.8)	56.2 (14.0)	57.2 (13.9)	0.4 (1.4)	0.987 0.511
LDL-c (mmol/L)	100.4 (43.2)	112.5 (48.4)	3.6 (14.3)	104.5 (29.2)	93.8 (19.1)	3.3 (6.4)	87.7 (36.6)	77.7 (28.4)	3.5 (8.3)	0.501 0.119
TAG (mmol/L)	134.6 (61.5)	133.8 (46.4)	1.2 (9.4)	136.5 (59.6)	126.8 (47.1)	2.1 (9.0)	96.2 (29.8)	91.2 (29.6)	2.5 (9.9)	0.435 0.518
CRP (mg/dL)	1.8 (2.4)	3.3 (6.4)	0.1 (0.3)	1.9 (2.1)	1.6 (1.7)	0.1 (0.1)	3.4 (4.7)	1.7 (1.3)	0.2 (0.3)	0.155 0.19

HDL-c: High-density lipoprotein cholesterol; LDL-c: Low-density lipoprotein cholesterol; TAG: Triglycerides; CRP: C-reactive protein.

3.4. Hematological Parameters

The hemogram of the study participants is shown in Table 3. There were no significative differences from pre to post interventions regarding erythrocytes (p=0.585), Hb (p=0.355), globular volume (MCV, p=0.460), RDW (p=0.059), leukocytes (p=0.999), neutrophils (p=0.192), eosinophils (p=0.863), basophils (p=0.691), lymphocytes (p=0.279%), monocytes (p=0.303) and platelets (p=0.105). There were no differences between the moments for MCH (p=0.733) and MCHC (p=0.669), but MCH increased only in the EH group (p=0.027), and MCHC was reduced only in the EH+LCHF group (p=0.046).

Table 3. Hemogram with platelets pre- and post- eight weeks of interventions.

Variables	CTRL group			EH group			EH+LCHF group			p-Value
	Pre	Post	Δ	Pre	Post	Δ	Pre	Post	Δ	Moments Groups
Erythrocytes (L)	4.7 (0.4)	4.7 (0.5)	0.1 (0.1)	4.5 (0.4)	4.5 (0.5)	0.1 (0.2)	4.6 (0.51)	4.7 (0.5)	0.1 (0.2)	0.585 0.122
Hemoglobin (g/dL)	14.2 (1.0)	14.2 (1.2)	0.1 (0.5)	13.9 (1.3)	13.7 (1.4)	0.1 (0.6)	13.8 (1.6)	13.7 (1.7)	0.1 (0.5)	0.355 0.668
MCV (%)	42.7 (3.1)	42.7 (3.5)	0.1 (1.3)	41.7 (3.6)	41.1 (4.3)	0.5 (1.9)	41.9 (4.1)	41.9 (5.0)	0.1 (1.6)	0.460 0.611
MCH (pg)	29.9 (2.2)	29.2 (2.2)	0.7 (2.7)	30.2 (1.2)	31.3 (1.7)	1.1 (1.3)	29.7 (1.8)	29.6 (1.8)	0.1 (0.3)	0.733 0.027#
MCHC (g/dL)	33.3 (1.3)	33.4 (1.3)	0.1 (0.3)	33.3 (1.1)	33.4 (1.3)	0.1 (0.3)	32.9 (0.9)	32.6 (0.8)	0.2 (0.5)	0.669 0.046#
RDW (%)	12.5 (0.5)	12.6 (0.7)	0.1 (0.3)	12.8 (0.5)	12.8 (0.5)	0.1 (0.1)	13.4 (0.6)	13.5 (0.6)	0.1 (0.4)	0.059 0.911
Leukocytes (L)	7.4 (2.1)	7.1 (1.9)	0.2 (1.0)	6.1 (1.3)	6.3 (1.1)	0.2 (1.0)	6.1 (1.4)	5.8 (1.1)	0.3 (0.9)	0.999 0.156
Neutrophils (%)	62.2 (8.0)	60.3 (10.1)	1.8 (5.2)	63.1 (7.2)	63.9 (7.7)	0.9 (5.4)	61.5 (7.5)	59.3 (7.4)	2.1 (4.6)	0.192 0.241
Eosinophils (%)	2.6 (1.4)	2.5 (1.4)	0.1 (0.4)	2.2 (1.1)	1.9 (1.1)	0.2 (0.6)	2.4 (1.4)	2.8 (1.1)	0.37 (1.1)	0.863 0.118
Basophils (%)	0.5 (0.1)	0.4 (0.1)	0.1 (0.1)	0.6 (0.2)	0.7 (0.2)	0.1 (0.2)	0.6 (0.2)	0.6 (0.3)	0.1 (0.2)	0.691 0.138

 doi:10.20944/preprints202501.0270.v1

Lymphocytes (%)	28.6 (6.3)	30.4 (9.1)	1.7 (4.7)	28.1 (6.9)	27.1 (7.1)	0.9 (5.1)	28.7 (7.2)	30.3 (8.1)	1.6 (4.4)	0.279	0.260
Monocytes (%)	5.9 (1.4)	6.2 (1.6)	0.2 (1.3)	5.9 (1.1)	6.1 (1.1)	0.2 (0.6)	6.7 (1.2)	6.90 (1.6)	0.1 (1.1)	0.303	0.967
Platelets (L)	218.2 (38.0)	212.8 (35.4)	5.4 (13.8)	213.5 (43.3)	227.4 (31.2)	13.8 (27.1)	219.1 (35.2)	223.2 (35.9)	4.1 (32.0)	0.105	0.066

= MCH (pg): EH group increased from CTRL and EH+LCHF groups; MCHC (g/dL): EH+LCHF group decreased from EH and CTRL groups. MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration.

3.5. Blood Pressure

SBP, DBP and MAP significantly reduced ($p<0.001$) from pre- to post-intervention (Table 4). While SBP, DBP, and MAP values decreased after interventions, post-hoc analysis revealed that these changes did not reach statistical significance between groups ($p=0.151$; $p=0.124$; $p=0.18$, respectively). No differences were found in resting heart rate regarding moments ($p=0.090$) and groups ($p=0.660$).

Table 4. Blood pressure measures pre- and post- eight weeks of interventions.

Variables	CTRL group			EH group			EH+LCHF group			p-Value	
	Pre	**Post**	**Δ**	**Pre**	**Post**	**Δ**	**Pre**	**Post**	**Δ**	**Moments**	**Groups**
SBP (mmHg)	154.7 (20.9)	142.5 (16.6)	12.2 (10.2)	142.3 (18.2)	124.5 (14.6)	17.7 (15.6)	148.0 (18.9)	126.5 (18.6)	21.5 (10.6)	<0.001*	0.151
DBP (mmHg)	77.5 (8.1)	72.2 (7.7)	5.4 (5.2)	76.3 (11.7)	71.8 (12.0)	4.1 (6.6)	82.9 (16.3)	71.4 (8.4)	9.0 (8.9)	<0.001*	0.124
MAP (mmHg)	107.9 (16.9)	102.9 (10.2)	5.0 (13.3)	98.7 (13.6)	87.0 (17.8)	11.7 (15.6)	107.1 (15.8)	92.0 (12.1)	15.1 (12.3)	<0.001*	0.158
RHR (bpm)	68.5 (9.5)	69.0 (9.7)	0.5 (5.2)	64.7 (10.5)	67.3 (10.1)	2.4 (8.1)	62.1 (8.1)	65.0 (9.5)	2.8 (8.3)	0.090	0.660

SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial blood pressure; RHR: resting heart rate.

4. Discussion

This is the first RCT examining the responses of chronic EH with and without a LCHF diet on hematological and lipid profile, inflammation and blood pressure in patients with T2DM and coexistent HTN. The study's main findings revealed an increased in MCH in patients who exercised in hypoxia, while a reduction in MCH concentration was observed in patients following an LCHF diet. Additionally, the study identified a significant reduction in HTN following eight weeks of exercise in normoxia and hypoxia, with no substantial disparity in the efficacy of EH compared to normoxia, irrespective of dietary carbohydrate content, which contradicts our hypothesis. These findings align with prior research indicating comparable blood pressure outcomes between EH and normoxia-based exercise interventions in older individuals over an eight-week period with same oxygen levels (~15% of FIO_2) [42].

However, contrasting results from existing studies suggest superior effects of regular EH on blood pressure regulation. For instance, moderate exercise at a natural altitude of 1700m exhibited notable reductions in SBP and DBP in individuals with metabolic syndrome over a three-week period [43]. A reduction of 10 mmHg and 7 mmHg in SBP was observed after four weeks of chronic EH at 16.4 and 14.5% of FIO_2, respectively [44,45]. In contrast, other researchers observed a reduction only in DBP after a 13-week of aerobic and strength exercise in normobaric hypoxia simulating 2000–3350m altitude [46]. It is noteworthy that most of these studies reported an improved in body composition after EH intervention [43,44,46], a crucial factor in decreasing blood pressure by exercise [47].

 doi:10.20944/preprints202501.0270.v1

Moreover, there is evidence suggesting that older individuals may exhibit resistance to the reduction of exercise-induced blood pressure [47], and this resistance appears to be closely tied to changes in body composition [44,47]. Published data from the current RCT showed reductions in weight loss, body mass index (BMI) and body fat after eight weeks of interventions [33], and there were no significant additional benefits observed in the groups exercising in hypoxia compared to those exercising in normoxia. Similarly, our investigation into blood pressure failed to reveal any notable advantages of exercising in hypoxia over normoxia, irrespective of dietary carbohydrate content. In agreement, it was demonstrated that an eight-month training program led to improvements in weight, BMI, waist and hip circumference over time, with no discernible differences between the normoxia and hypoxia exercise groups [48].

Despite observing similar improvements in blood pressure with both normoxic and hypoxic exercise interventions, along with comparable levels of Hb mass, MCH, and MCHC before and after chronic EH and LCHF diet interventions, our study revealed higher MCH levels in the EH group. It is widely acknowledged that achieving a substantial increase in hemoglobin content requires an adequate hypoxic dose of >12 hours per day at a sufficient altitude for >21 days (approximately 300 hours) [49,50]. This suggests that the similarity in hemogram biomarkers from pre- to post-eight weeks in the current study may be attributed to the short exposure to simulated altitude, specifically 3 hours per week and 24 hours during the total intervention period.

Compensatory elevation in MCH within a specific range is recognized as a fundamental physiological response to high-altitude hypoxia [51], enhancing blood´s oxygen-carrying capacity and improving tissue oxygenation without increasing cardiac output [52]. This adjustment translates to an increase of approximately 0.30–0.47 g/dL per 1000 meters of altitude [50]. However, when Hb production significantly exceeds the reference range, an increase in cardiac output is required to sustain oxygen transport [52], potentially contributing to blood pressure elevation. Given that Hb production remains within the normal range proposed for sea-level [53], and when corrected by high-altitudes [54], it appears that the increase in MCH production within the minimum cut-off values in patients who exercised in hypoxia was not sufficient to induce significant changes in blood pressure compared to exercise in normoxia, at least with 24h of hypoxic exposure at 3000m simulated-altitude.

Contrary to our findings, previous studies have demonstrated improvements in SPB and DBP following long-term exposure (approximately 6 and 12 months) to natural high-altitude, accompanied by increased Hb levels [55]. Other authors found similar improvements after 15 sessions of hypoxia exposure (14-10% FiO_2) compared to normoxia [56]. This discrepancy may be attributed to altered hydration status in the EH group, increasing Hb content when dehydrated [53]. However, the MCV values in the current study do not support this hypothesis.

Additionally, existing evidence has demonstrated that incorporating hypoxia into exercise, even during short periods and at moderate high altitudes, yields greater benefits in blood pressure, independent of hematological parameters. This suggests the involvement of alternative mechanisms beyond MCH in this response [44–46]. Chronic EH has been linked to reduced arterial stiffness [57], and improvements in metabolic risk factors such as body fat and insulin resistance [58], all of which play a role in regulating blood pressure. EH also induces vasodilation and lowers blood pressure in patients with T2DM [59]. These effects are, in part, mediated by increased HIF-1α protein expression, which is inversely associated with systemic blood pressure [16]. HIF-1α also triggers VEGF activation [60], impacting blood pressure regulation via nitric oxide synthase expression and nitric oxide activity [59]. Unfortunately, we did not evaluate HIF-1α and VEGF levels in our study.

It is well known that elevated levels of Hb, MCH and MCHC are associated with increased blood viscosity [61], which induces decreased blood flow to skeletal muscles and fat tissues, contributing to peripheral vascular resistance and elevating the risk for T2DM development, interfering with insulin-mediated glucose uptake [29], and potentially elevating blood pressure [62] contributing to the development of HTN [26,30]. While the EH group exhibited increased MCH levels within normal ranges, restricting carbohydrate intake appeared to decrease MCH concentration, albeit within appropriate levels [53,54]. Consistent with this, prior research reported that a LCHF diet over twelve

 doi:10.20944/preprints202501.0270.v1

weeks reduced iron intake and lowered both MCH and MCHC in male endurance athletes [25]. This reduction could be attributed to increased inflammation associated with the LCHF diet, affecting the iron regulatory hormone hepcidin [63]. Hepcidin production is stimulated by iron and inflammation but inhibited by hypoxia [64]. Considering that Hb comprises nearly 70% of iron in the body, the LCHF diet's reduction in iron consumption, coupled with hypoxia-induced decrease in hepcidin production, could explain the reduced MCHC values observed in our study.

Moreover, MCHC has been linked to metabolic disorders, with levels increased in obesity and decreased by medications improving cellular insulin sensitivity [64]. Prediabetic patients with higher Hb content have been shown to exhibit impaired blood pressure, HDL-c levels, and waist circumference [65], contributing to a proinflammatory state and worsening metabolic dysfunction and CVD development [66]. Although our study found no differences in lipid profile and CRP levels among time and groups, evidence suggests a positive association between reduced MCHC and carbohydrate-restricted diets, with consequent improvement on insulin resistance and blood pressure, highlighting a crucial mechanism for further exploration in patients with T2DM and coexistent HTN.

In summary, while EH interventions did not demonstrate superior benefits in reducing blood pressure compared to normoxia-based exercise, our findings underscore the intricate interplay between exercise, diet, and hematological parameters in managing metabolic disorders and hypertension in patients with T2DM. Further research is needed to elucidate underlying mechanisms and optimize therapeutic strategies for this population.

Strengths and Limitations

The study had a 100% adherence rate, with no dropouts throughout the testing and intervention phases, surpassing the general cutoff point for sufficient adherence in older adults by 30pp [67]. To uphold participation levels, the research team maintained constant contact with participants throughout the eight-week intervention period, motivating them to complete their participation regularly during the eight weeks of intervention. This proactive approach helped motivate participants, ensuring their consistent involvement in the study. Recognizing potential barriers to attendance, such as transportation challenges, the study provided chauffeured transportation for participants to attend exercise sessions at the clinic, removing logistical hurdles. Regular meetings were convened to assess food consumption and address any queries, fostering correct adherence to the prescribed dietary plan among all participants. This personalized support contributed to the overall adherence success of the intervention.

Some limitations should also be mentioned. Due to logistical and budgetary constraints, the study was limited to an eight-week duration, resulting in a total of 24h of hypoxia exposure. While sufficient for certain outcomes, this timeframe may have been insufficient to induce substantial changes in hematological parameters [50], such as Hb, MCH and MCHC, and their influence on blood pressure regulation. The reasons mentioned above precluded the determination of key biomarkers, including HIF-1α, VEGF, iron deficiency markers, and hepcidin levels. These markers are vital for understanding the mechanistic underpinnings of the intervention and its effects on blood pressure regulation. Future research should delve into these biomarkers, as well as increase the time of exposure to hypoxia, to elucidate their role in the treatment of HTN in type 2 diabetic patients undergoing EH and LCHF dietary interventions.

5. Conclusions

In conclusion, diets and exercise lowered HTN, with no additional benefits from added hypoxia and restrict carbohydrate. Future research is needed to provide a deeper understanding of the precise mechanisms underlying hematological adaptations and their subsequent impact on blood pressure regulation. Additional elucidation of these mechanisms is imperative for a comprehensive understanding of the therapeutic potential and optimization of EH and LCHF dietary interventions in managing related HTN complications in individuals with T2DM.

 doi:10.20944/preprints202501.0270.v1

Author Contributions: Conceptualization, R.K., A.C.S., J.L.V. and V.H.T.; methodology, R.K., A.C.S.; J.L.V., B.M.P.M.O. and V.H.T.; software, R.K. and B.M.P.M.O.; validation, all authors; formal analysis, R.K and B.M.P.M.O.; investigation, R.K.; resources, J.L.V., J.M. and F.M.; data curation, R.K. and B.M.P.M.O.; writing—original draft preparation, R.K.; writing—review and editing, R.K., A.C.S., J.L.V., J.M., B.M.P.M.O., A.S. and V.H.T.; visualization, all authors; supervision, A.C.S., J.L.V., A.S. and V.H.T.; project administration, R.K., A.C.S.; J.L.V. and V.H.T.; funding acquisition, R.K., A.C.S., J.L.V. and V.H.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by FCT (Foundation for Science and Technology), grant number 2020.06520.BD, and FCT/UIDB/00617/2020 and LA/P/0064/2020.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of the Faculty of Nutrition and Food Sciences, University of Porto (Approval Number 45/2021/CEFCNAUP/2021).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author due ethical reasons.

Acknowledgments: The authors would like to thank Sérgio Nunes, Mariana Fonseca and Victoria Paes for their collaboration in the study stages; to João Regufe for his collaboration in recruiting participants at Matosinhos Sport; to Ana Rocha and Daniela Dinis, for their availability and logistical support at CMEP – Exercise Medical Centre and to pharmacist Bárbara Duarte for their contribution to collecting blood samples.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Sobczak AIS, Stewart AJ (2019) Coagulatory Defects in Type-1 and Type-2 Diabetes. *Int J Mol Sci* **20**. 10.3390/ijms20246345
2. Unger T, Borghi C, Charchar F *et al.* (2020) 2020 International Society of Hypertension Global Hypertension Practice Guidelines. *Hypertension* **75**, 1334-1357. 10.1161/HYPERTENSIONAHA.120.15026
3. Yen F-S, Wei JC-C, Chiu L-T *et al.* (2022) Diabetes, hypertension, and cardiovascular disease development. *Journal of Translational Medicine* **20**, 9. 10.1186/s12967-021-03217-2
4. Rapsomaniki E, Timmis A, George J *et al.* (2014) Blood pressure and incidence of twelve cardiovascular diseases: lifetime risks, healthy life-years lost, and age-specific associations in 1.25 million people. *Lancet* **383**, 1899-1911. 10.1016/S0140-6736(14)60685-1
5. Chen G, McAlister FA, Walker RL *et al.* (2011) Cardiovascular outcomes in framingham participants with diabetes: the importance of blood pressure. *Hypertension* **57**, 891-897. 10.1161/HYPERTENSIONAHA.110.162446
6. Dongway AC, Faggad AS, Zaki HY *et al.* (2015) C-reactive protein is associated with low-density lipoprotein cholesterol and obesity in type 2 diabetic Sudanese. *Diabetes Metab Syndr Obes* **8**, 427-435. 10.2147/DMSO.S85451
7. Morrato EH, Hill JO, Wyatt HR *et al.* (2007) Physical activity in U.S. adults with diabetes and at risk for developing diabetes, 2003. *Diabetes Care* **30**, 203-209. 10.2337/dc06-1128
8. Pan B, Ge L, Xun YQ *et al.* (2018) Exercise training modalities in patients with type 2 diabetes mellitus: a systematic review and network meta-analysis. *Int J Behav Nutr Phys Act* **15**, 72. 10.1186/s12966-018-0703-3
9. Cosentino F, Grant PJ, Aboyans V *et al.* (2020) 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. *Eur Heart J* **41**, 255-323. 10.1093/eurheartj/ehz486
10. Hordern MD, Dunstan DW, Prins JB *et al.* (2012) Exercise prescription for patients with type 2 diabetes and pre-diabetes: a position statement from Exercise and Sport Science Australia. *J Sci Med Sport* **15**, 25-31. 10.1016/j.jsams.2011.04.005
11. Ebrahim H, Fiseha T, Ebrahim Y *et al.* (2022) Comparison of hematological parameters between type 2 diabetes mellitus patients and healthy controls at Dessie comprehensive specialized hospital, Northeast Ethiopia: Comparative cross-sectional study. *PLoS One* **17**, e0272145. 10.1371/journal.pone.0272145
12. Yatsutani H, Mori H, Ito H *et al.* (2020) Endocrine and Metabolic Responses to Endurance Exercise Under Hot and Hypoxic Conditions. *Front Physiol* **11**, 932. 10.3389/fphys.2020.00932
13. Baker JM, Parise G (2016) Skeletal Muscle Erythropoietin Expression Is Responsive to Hypoxia and Exercise. *Med Sci Sports Exerc* **48**, 1294-1301. 10.1249/MSS.0000000000000899
14. Glazachev OS, Kryzhanovskaya SY, Zapara MA *et al.* (2021) Safety and Efficacy of Intermittent Hypoxia Conditioning as a New Rehabilitation/ Secondary Prevention Strategy for Patients with Cardiovascular

 doi:10.20944/preprints202501.0270.v1

Diseases: A Systematic Review and Meta-analysis. *Curr Cardiol Rev* **17**, e051121193317. 10.2174/1573403X17666210514005235
15. Kindlovits R, Pereira A, Sousa AC *et al.* (2022) Effects of Acute and Chronic Exercise in Hypoxia on Cardiovascular and Glycemic Parameters in Patients with Type 2 Diabetes: A Systematic Review. *High Alt Med Biol* **23**, 301-312. 10.1089/ham.2022.0029
16. Sato T, Takeda N (2023) The roles of HIF-1alpha signaling in cardiovascular diseases. *J Cardiol* **81**, 202-208. 10.1016/j.jjcc.2022.09.002
17. Lindholm ME, Rundqvist H (2016) Skeletal muscle hypoxia-inducible factor-1 and exercise. *Exp Physiol* **101**, 28-32. 10.1113/EP085318
18. Weidemann A, Johnson RS (2008) Biology of HIF-1alpha. *Cell Death Differ* **15**, 621-627. 10.1038/cdd.2008.12
19. Sakagami H, Makino Y, Mizumoto K *et al.* (2014) Loss of HIF-1alpha impairs GLUT4 translocation and glucose uptake by the skeletal muscle cells. *Am J Physiol Endocrinol Metab* **306**, E1065-1076. 10.1152/ajpendo.00597.2012
20. Arauz-Pacheco C, Parrott MA, Raskin P *et al.* (2004) Hypertension management in adults with diabetes. *Diabetes Care* **27 Suppl 1**, S65-67. 10.2337/diacare.27.2007.s65
21. Eckel RH, Jakicic JM, Ard JD *et al.* (2014) 2013 AHA/ACC guideline on lifestyle management to reduce cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation* **129**, S76-99. 10.1161/01.cir.0000437740.48606.d1
22. Evert AB, Dennison M, Gardner CD *et al.* (2019) Nutrition Therapy for Adults With Diabetes or Prediabetes: A Consensus Report. *Diabetes Care* **42**, 731-754. 10.2337/dci19-0014
23. Bueno NB, de Melo IS, de Oliveira SL *et al.* (2013) Very-low-carbohydrate ketogenic diet v. low-fat diet for long-term weight loss: a meta-analysis of randomised controlled trials. *Br J Nutr* **110**, 1178-1187. 10.1017/S0007114513000548
24. Saslow LR, Jones LM, Sen A *et al.* (2023) Comparing Very Low-Carbohydrate vs DASH Diets for Overweight or Obese Adults With Hypertension and Prediabetes or Type 2 Diabetes: A Randomized Trial. *Ann Fam Med* **21**, 256-263. 10.1370/afm.2968
25. McSwiney FT, Doyle L (2019) Low-Carbohydrate Ketogenic Diets in Male Endurance Athletes Demonstrate Different Micronutrient Contents and Changes in Corpuscular Haemoglobin over 12 Weeks. *Sports (Basel)* **7**. 10.3390/sports7090201
26. Kawamoto R, Tabara Y, Kohara K *et al.* (2013) Hematological parameters are associated with metabolic syndrome in Japanese community-dwelling persons. *Endocrine* **43**, 334-341. 10.1007/s12020-012-9662-7
27. Mansoori A, Sahranavard T, Hosseini ZS *et al.* (2023) Prediction of type 2 diabetes mellitus using hematological factors based on machine learning approaches: a cohort study analysis. *Sci Rep* **13**, 663. 10.1038/s41598-022-27340-2
28. Crawford JH, White CR, Patel RP (2003) Vasoactivity of S-nitrosohemoglobin: role of oxygen, heme, and NO oxidation states. *Blood* **101**, 4408-4415. 10.1182/blood-2002-12-3825
29. Floras JS, Meneilly G (2001) Insulin-mediated blood flow and glucose uptake. *Can J Cardiol* **17 Suppl A**, 7A-10A. https://www.ncbi.nlm.nih.gov/pubmed/11381287
30. Crawford JH, Chacko BK, Kevil CG *et al.* (2004) The red blood cell and vascular function in health and disease. *Antioxid Redox Signal* **6**, 992-999. 10.1089/ars.2004.6.992
31. Goodyear MD, Krleza-Jeric K, Lemmens T (2007) The Declaration of Helsinki. *BMJ* **335**, 624-625. 10.1136/bmj.39339.610000.BE
32. Kindlovits R, Sousa AC, Viana JL *et al.* (2024) Combined low-carbohydrate diet and long-term exercise in hypoxia in type 2 diabetes: A randomized controlled trial protocol to assess glycemic control, cardiovascular risk factors and body composition. *Nutr Health* **30**, 5-13. 10.1177/02601060231190663
33. Kindlovits R, Sousa AC, Viana JL *et al.* (2024) Eight Weeks of Intermittent Exercise in Hypoxia, with or without a Low-Carbohydrate Diet, Improves Bone Mass and Functional and Physiological Capacity in Older Adults with Type 2 Diabetes. *Nutrients* **16**. 10.3390/nu16111624
34. Cioffi I, Marra M, Pasanisi F *et al.* (2021) Prediction of resting energy expenditure in healthy older adults: A systematic review. *Clin Nutr* **40**, 3094-3103. 10.1016/j.clnu.2020.11.027
35. Sjostrom M, Ainsworth BE, Bauman A *et al.* (2005) Guidelines for data processing analysis of the International Physical Activity Questionnaire (IPAQ) - Short and long forms.
36. Dyson PA, Twenefour D, Breen C *et al.* (2018) Diabetes UK evidence-based nutrition guidelines for the prevention and management of diabetes. *Diabet Med* **35**, 541-547. 10.1111/dme.13603
37. Silverii GA, Botarelli L, Dicembrini I *et al.* (2020) Low-carbohydrate diets and type 2 diabetes treatment: a meta-analysis of randomized controlled trials. *Acta Diabetol* **57**, 1375-1382. 10.1007/s00592-020-01568-8
38. Idrose AM, Juliana N, Azmani S *et al.* (2022) Singing Improves Oxygen Saturation in Simulated High-Altitude Environment. *J Voice* **36**, 316-321. 10.1016/j.jvoice.2020.06.031
39. Scherr J, Wolfarth B, Christle JW *et al.* (2013) Associations between Borg's rating of perceived exertion and physiological measures of exercise intensity. *Eur J Appl Physiol* **113**, 147-155. 10.1007/s00421-012-2421-x

 doi:10.20944/preprints202501.0270.v1

40. van Zuuren EJ, Fedorowicz Z, Kuijpers T *et al.* (2018) Effects of low-carbohydrate- compared with low-fat-diet interventions on metabolic control in people with type 2 diabetes: a systematic review including GRADE assessments. *Am J Clin Nutr* **108**, 300-331. 10.1093/ajcn/nqy096
41. Faul F, Erdfelder E, Lang AG *et al.* (2007) G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods* **39**, 175-191. 10.3758/bf03193146
42. Hein M, Chobanyan-Jurgens K, Tegtbur U *et al.* (2021) Effect of normobaric hypoxic exercise on blood pressure in old individuals. *Eur J Appl Physiol* **121**, 817-825. 10.1007/s00421-020-04572-6
43. Schobersberger W, Schmid P, Lechleitner M *et al.* (2003) Austrian Moderate Altitude Study 2000 (AMAS 2000). The effects of moderate altitude (1,700 m) on cardiovascular and metabolic variables in patients with metabolic syndrome. *Eur J Appl Physiol* **88**, 506-514. 10.1007/s00421-002-0736-8
44. Kong Z, Zang Y, Hu Y (2014) Normobaric hypoxia training causes more weight loss than normoxia training after a 4-week residential camp for obese young adults. *Sleep Breath* **18**, 591-597. 10.1007/s11325-013-0922-4
45. Morishima T, Hasegawa Y, Sasaki H *et al.* (2015) Effects of different periods of hypoxic training on glucose metabolism and insulin sensitivity. *Clin Physiol Funct Imaging* **35**, 104-109. 10.1111/cpf.12133
46. Gonzalez-Muniesa P, Lopez-Pascual A, de Andres J *et al.* (2015) Impact of intermittent hypoxia and exercise on blood pressure and metabolic features from obese subjects suffering sleep apnea-hypopnea syndrome. *J Physiol Biochem* **71**, 589-599. 10.1007/s13105-015-0410-3
47. Stewart KJ, Bacher AC, Turner KL *et al.* (2005) Effect of exercise on blood pressure in older persons: a randomized controlled trial. *Arch Intern Med* **165**, 756-762. 10.1001/archinte.165.7.756
48. Gatterer H, Haacke S, Burtscher M *et al.* (2015) Normobaric Intermittent Hypoxia over 8 Months Does Not Reduce Body Weight and Metabolic Risk Factors--a Randomized, Single Blind, Placebo-Controlled Study in Normobaric Hypoxia and Normobaric Sham Hypoxia. *Obes Facts* **8**, 200-209. 10.1159/000431157
49. Millet GP, Roels B, Schmitt L *et al.* (2010) Combining hypoxic methods for peak performance. *Sports Med* **40**, 1-25. 10.2165/11317920-000000000-00000
50. Clark SA, Quod MJ, Clark MA *et al.* (2009) Time course of haemoglobin mass during 21 days live high:train low simulated altitude. *Eur J Appl Physiol* **106**, 399-406. 10.1007/s00421-009-1027-4
51. Liu X, Yang C, Zhang X *et al.* (2024) Association between hemoglobin concentration and hypertension risk in native Tibetans at high altitude. *J Clin Hypertens (Greenwich)* **26**, 17-23. 10.1111/jch.14726
52. Neya M, Enoki T, Ohiwa N *et al.* (2013) Increased hemoglobin mass and VO2max with 10 h nightly simulated altitude at 3000 m. *Int J Sports Physiol Perform* **8**, 366-372. 10.1123/ijspp.8.4.366
53. Billett HH (1990) Hemoglobin and Hematocrit. In *Clinical Methods: The History, Physical, and Laboratory Examinations*, 3rd ed., https://www.ncbi.nlm.nih.gov/pubmed/21250102 [HK Walker, WD Hall and JW Hurst, editors]. Boston.
54. Alkhaldy HY, Awan ZA, Abouzaid AA *et al.* (2022) Effect of Altitude on Hemoglobin and Red Blood Cell Indices in Adults in Different Regions of Saudi Arabia. *Int J Gen Med* **15**, 3559-3565. 10.2147/IJGM.S358139
55. Yan Y, Mao Z, Jia Q *et al.* (2023) Changes in blood pressure, oxygen saturation, hemoglobin concentration, and heart rate among low-altitude migrants living at high altitude (5380 m) for 360 days. *Am J Hum Biol* **35**, e23913. 10.1002/ajhb.23913
56. Burtscher M, Pachinger O, Ehrenbourg I *et al.* (2004) Intermittent hypoxia increases exercise tolerance in elderly men with and without coronary artery disease. *Int J Cardiol* **96**, 247-254. 10.1016/j.ijcard.2003.07.021
57. Nishiwaki M, Kawakami R, Saito K *et al.* (2011) Vascular adaptations to hypobaric hypoxic training in postmenopausal women. *J Physiol Sci* **61**, 83-91. 10.1007/s12576-010-0126-7
58. Urdampilleta A, Gonzalez-Muniesa P, Portillo MP *et al.* (2012) Usefulness of combining intermittent hypoxia and physical exercise in the treatment of obesity. *J Physiol Biochem* **68**, 289-304. 10.1007/s13105-011-0115-1
59. Ladage D, Braunroth C, Lenzen E *et al.* (2012) Influence of intermittent hypoxia interval training on exercise-dependent erythrocyte NOS activation and blood pressure in diabetic patients. *Can J Physiol Pharmacol* **90**, 1591-1598. 10.1139/y2012-138
60. Jain T, Nikolopoulou EA, Xu Q *et al.* (2018) Hypoxia inducible factor as a therapeutic target for atherosclerosis. *Pharmacol Ther* **183**, 22-33. 10.1016/j.pharmthera.2017.09.003
61. Tremblay JC, Hoiland RL, Howe CA *et al.* (2019) Global REACH 2018: High Blood Viscosity and Hemoglobin Concentration Contribute to Reduced Flow-Mediated Dilation in High-Altitude Excessive Erythrocytosis. *Hypertension* **73**, 1327-1335. 10.1161/HYPERTENSIONAHA.119.12780
62. Zidek V, Fucikova A, Musilova A *et al.* (1999) Hematocrit and hemoglobin values are negatively correlated with insulin resistance in spontaneous hypertension. *Folia Biol (Praha)* **45**, 247-251. https://www.ncbi.nlm.nih.gov/pubmed/10732721
63. McKay AKA, Peeling P, Pyne DB *et al.* (2019) Chronic Adherence to a Ketogenic Diet Modifies Iron Metabolism in Elite Athletes. *Med Sci Sports Exerc* **51**, 548-555. 10.1249/MSS.0000000000001816
64. Yu JG, Javorschi S, Hevener AL *et al.* (2002) The effect of thiazolidinediones on plasma adiponectin levels in normal, obese, and type 2 diabetic subjects. *Diabetes* **51**, 2968-2974. 10.2337/diabetes.51.10.2968
65. Kutlu M, Sonmez A, Genc H *et al.* (2009) Relationship between hemoglobin and CD40 ligand in prediabetes. *Clin Invest Med* **32**, E244. 10.25011/cim.v32i6.10659

 doi:10.20944/preprints202501.0270.v1

66. Missiou A, Wolf D, Platzer I *et al.* (2010) CD40L induces inflammation and adipogenesis in adipose cells--a potential link between metabolic and cardiovascular disease. *Thromb Haemost* **103**, 788-796. 10.1160/TH09-07-0463
67. Thabane L, Ma J, Chu R *et al.* (2010) A tutorial on pilot studies: the what, why and how. *BMC Med Res Methodol* **10**, 1. 10.1186/1471-2288-10-1.