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ORIGINAL PAPER

Impact of intermittent hypoxia and exercise on blood pressure and metabolic features from obese subjects suffering sleep apnea-hypopnea syndrome

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Abstract Strategies designed to reduce adiposity and cardiovascular-accompanying manifestations have been based on nutritional interventions conjointly with physical activity programs. The aim of this 13-week study was to investigate the putative benefits associated to hypoxia plus exercise on weight loss and relevant metabolic and cardiorespiratory variables, when prescribed to obese subjects with sleep apnea syndrome following dietary advice. The participants were randomly distributed in the following three groups: control, normoxia, and hypoxia. All the subjects received dietary advice while, additionally, normoxia group was trained under normal oxygen concentration and Hypoxia group under hypoxic conditions. There was a statistically significant decrease in fat-free mass (Kg) and water (%) on the control compared to normoxia group ($p<0.05$ and $p<0.01$, respectively). Body weight, body mass index, and waist circumference decreased in all the groups after the study. Moreover, leukocyte count was increased after the intervention in hypoxia compared to control group ($p<0.05$). There were no statistically significant variations within groups in other variables, although changes in appetite were found after the 13-week period. In addition, associations between the variations in the leukocyte count and fat mass have been found. The hypoxia group showed some specific benefits concerning appetite and cardiometabolic-related measurements as exertion time and diastolic blood pressure, with a therapeutical potential.

Keywords Hypoxia · Obesity · Sleep apnea-hypopnea syndrome (SAHS) · Exercise · Weight management

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Introduction

The obesity condition is characterized by an adiposity excess determined by environmental factors such as energy consumption and expenditure, as well as by the individual's genetic makeup [30]. Indeed, there are a number of clinical strategies for obesity prevention and treatment based on nutritional interventions [1].

The conventional plans designed for this purpose have being based on calorie-controlled diets with different macronutrient distribution (fats, carbohydrates, and proteins), together with physical exercise in order to produce an energy deficit to decrease fat depots [26]. Given the frequent unsatisfactory short- or long-term outcomes achieved with these strategies, new approaches are explored [39]. In this context, newer approaches are focused not only on weight and fat loss but also on managing those adverse manifestations associated to obesity such as glucose intolerance, dyslipidemia, and cardiovascular disease [8].

Interestingly, there is now enough scientific evidence supporting that the excessive enlargement of the adipose tissue drives to cellular hypoxia, which participates in the subsequent process of inflammation. Thus, this inflammatory response involves up- and downregulation of more than 1000 genes, macrophage recruitment and infiltration, and overproduction of inflammatory adipokines in the adipose tissue [48]. Actually, inflammation may arise as a consequence of a hypoxic environment induced by adipose tissue expansion, cancer, pulmonary edema, and organ transplantation. On the other hand, the inflammatory condition may also be the cause of hypoxia due to infections, acute lung injuries, and colitis among others [13]. Furthermore, the metabolic importance of oxygen is clear, since some authors described this molecule as a frequently ignored nutrient [48].

Actually, the beneficial effects on appetite suppression and weight loss observed at high altitude due to hypoxic conditions has been reported [25]. Moreover, intermittent hypoxia could be useful in weight homeostasis by reducing fat deposition [22]. In this sense, different management patterns such as hypobaric, normobaric, or intermittent hypoxia have been devised to produce a low partial pressure of oxygen (PO_2), which could be used in various therapeutic approaches [32].

In addition, the association between sleep apnea-hypopnea syndrome (SAHS) and obesity has been widely described [34], as well as the implications of this sleep disorder in the development of inflammation-related chronic diseases, such as arterial hypertension, cardiovascular diseases, type 2 diabetes, some cancers, and metabolic syndrome [11]. Moreover, obesity is recognized as the main risk factor to develop SAHS [34]. During sleep, SAHS patients suffer from cycles of hypoxia and reoxygenation, upper airway occlusion, intrathoracic pressure swings, sleep fragmentation with arousals, and intermittent hypercapnia [11]. This phenomenon increases the oxidative stress, which could trigger an inflammatory response linking SAHS and metabolic disorders [37]. Furthermore, weight loss might counteract SAHS [49]. In this context, exercise increases the blood flow and oxygen consumption [20] and could be beneficial for SAHS treatment. In addition to this, hypoxic training could be beneficial for SAHS patients due to improvements both in weight loss [22] and in the respiratory system [32].

Therefore, the aim of this study was to analyze the potential metabolic benefits (mainly weight loss and muscle mass preservation), for obese subjects with SAHS, of exercising under hypoxic conditions following a healthy dietary pattern.

Materials and methods

Subjects

Patients with a body mass index (BMI) from 30 to 40 Kg/m^2 and an apnea-hypopnea index (AHI) greater than 15 events/h were selected from the Sleep Disorders Unit (Hospital Txagorritxu, University of the Basque Country, Vitoria, Álava, Spain). Subjects were excluded if they had cardiovascular disease, cardiac insufficiency, chronic obstructive pulmonary disease, forced respiratory vital capacity under 70 %, renal or hepatic dysfunction, infectious disease, and were under altitude sickness treatment or on a weight-loss diet during the past 2 months. Finally, 49 obese, suffering SAHS, male adults aged between 25 and 50 years old were randomized in three groups of study. Losses to follow up were due to absences when performing biochemical analyses or exercise testing (Fig. 1). This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethics and Clinical Trials Committee (code: HIPOXIA, 07/2012) and the

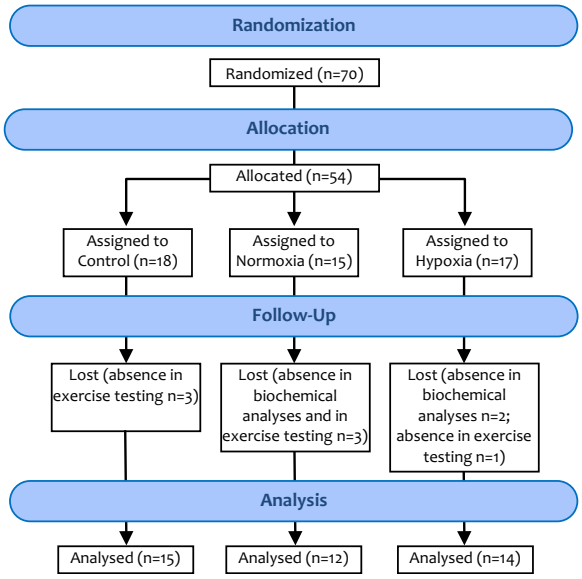

Fig. 1 Flow chart displaying the participants included in this study. The number of subjects can be found *inside the brackets*

Research Commission of the Txagorritxu Hospital, Vitoria, Spain. Written informed consent to participate in the intervention trial was obtained from all subjects.

Study protocol

The study lasted a total of 13 weeks and was implemented in three sequential stages. Baseline measurements were taken in the first week of the initial 4-week period. Normoxia and hypoxia groups were trained for six sessions in normal concentration of oxygen (normoxia) to adapt to the intensity of exercise, then a week without exercise and, the third period, all the groups followed their specific experimental protocol (Table 1). Control group (n=15) volunteers followed a healthy dietary pattern during 8 weeks. Normoxia group (n=12) volunteers followed the dietary advice plus 1 h training sessions twice a week in non-successive days. Hypoxia group (n=14) volunteers followed the dietary advice and exercise protocol, but the training was undertaken under hypoxic conditions. In the first 2 weeks of exercise, an altitude of 2150 m was simulated, with 16.0 % oxygen in the air breathed, and the rest of the weeks, 2750 to 3350 m were simulated, with 13.7–14.8 % oxygen in the air breathed. These hypoxic conditions were achieved with the hypoxia generator Hypoxicator Everest Summit II (Go2 Altitude Europe LTD, London, UK) and a set of exercise training in hypoxia (Go2 Altitude Europe LTD, London, UK). The amount of oxygen in the inspired air was monitored with an Expedition-X oxygen analyzer (OxyCheq, Florida, USA).

Exercise testing

Participants were recruited if they were physically capable when performing a submaximal exercise testing with electrocardiogram. All the tests were performed on a treadmill according to modified Bruce protocol [14]. Gas exchange was measured at rest and during exercise using a MetaSoft CPX testing cardiopulmonary exercise test analyzer (GE Medical Systems Information Technologies, Freiburg, Germany) with the software MetaLyzer 3B, Firmware Version 2.0 (Cortex, Leipzig, Germany). Data averaged in 20-s intervals were recorded. The system was calibrated before each test according to manufacturer specifications. During exercise testing, heart rate was constantly monitored with a CardioSoft v. 6.5 multiple-lead electrocardiographic system (GE Medical Systems Information Technologies, Freiburg,

Table 1 Study protocol

	Control	Normoxia	Hypoxia
Diet	Isocaloric 40 % CHO; 30 % protein; 30 % lipids		
Estimated altitude	No	525 m	(Sham) 2000–3350 m
FiO_2		20.0–20.5 %	16.7–13.7 %[a]
PA		50 % aerobic/50 % strength	
Duration		60 min/session	
Frequency		2/week; non-consecutive days	
Total study		16 sessions	

CHO carbohydrates, *FiO_2* inspirited oxygen fraction, *SaO_2* oxygen saturation, *PA* physical activity

[a] First 2 weeks 16.0 % of oxygen; last 6 weeks 13.7–14.8 % of oxygen

Germany), blood pressure was assessed with an automatic DS-45 Welch Allyn sphygmomanometer (Biotec Medica S.A.Valladolid, Spain), and blood oxygen saturation was monitored by a B-50DL pulse oximeter (Biosync, London, UK). All exercise testings were conducted under the supervision of a cardiologist to avoid any unexpected danger.

Before the initiation of the training period, every subject performed two maximal exercise tests on treadmill in two different sessions. They were randomly assigned to the exercise test either in normoxia or in hypoxia. Hypoxic conditions were the same as in the first 2 weeks of the study protocol (16 % O_2). The tests were consistent in terms of time of day, and they were performed on two alternating days. After the training period, the exercise test was performed only in normoxia. Subjects were instructed to perform the effort until exhaustion. Exhaustion was reached when two out of three of the following criteria were obtained: (a) heart rate approaching the maximal theoretical hearth rate (HR) (220–age), (b) oxygen uptake obtained a plateau even with an increase in external load, and (c) respiratory exchange ratio (RER) >1.1.

Anthropometric measurements

At baseline and at the endpoint of the 13-week intervention, nutritionists performed anthropometric measurements following the International Standards for Anthropometric Assessment [44]. Body weight was assessed to the nearest 0.1 Kg by using the BC 545 bioimpedance scale (Tanita Corporation, Tokyo, Japan). BMI was calculated as the body weight divided by the squared height (Kg/m^2). Total body fat mass (FM), fat-free mass (FFM), and water were evaluated with the BC 545 bioimpedance scale (Tanita Corporation, Tokyo, Japan) and skinfold measurement with the Tanner-Whitehouse skinfold caliper (Holtain Limited, Crosswell, UK) following the manufacturer instructions. Waist and hip circumferences were measured with the Harpenden anthropometric commercial tape (Holtain Limited, Crosswell, UK) following validated protocols, as previously described [44].

Dietary information

The healthy dietary advice slightly high in protein [1] was followed by all the participants of the study. Their daily energy intake was estimated according to their energy expenditure which was calculated using the Harris-Benedict formula and applying a 1.2 factor for physical activity (sedentary). Macronutrient distribution was as follows: 30 % protein, 40 % carbohydrates, and 30 % fat. They also received instructions of a food exchange system plan [1], to be independent in their food choices. Energy intake and macronutrient distribution were obtained by nutritionists analyzing 24-h questionnaires and food frequency questionnaires filled in by the volunteers at the beginning and at the end of the study, with the DIAL software (Alce Ingenieria, Madrid, Spain).

Training protocol

The volunteers who performed physical exercise in the study carried out 1 h training sessions twice a week in non-successive days, with alternation of 15′ aerobic exercise (stationary bicycle) and 15′ of strength exercise (lifting 4-Kg weights) to complete 1 h. During these sessions, heart rate and blood oxygen saturation were monitored by the B-50DL pulse oximeter (Biosync, London, UK) and the H7-Bluetooth 4.0 heart rate monitor (Polar Electro Ibérica, Barcelona, Spain), following validated procedures.

Biochemical and clinical parameters

Glucose, uric acid, total cholesterol, high-density lipoprotein (HDL), triglycerides (TG), total proteins, billirubin, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and gamma-glutamyl transpeptidase (GGT) serum concentrations were measured in an autoanalyzer (Architect c16000, Wiesbaden, Germany) with specific kits (Abbott, Wiesbaden, Germany), or by routine procedures (leukocyte). T&G index refers to Ln [triglyceride (mg/dL) * glucose (mg/dL)/2] [51]. Low-density lipoprotein (LDL) levels were calculated following the Friedewald formula: LDL-c=total cholesterol−HDL-c−TG/5 [16]. Blood pressure (BP) determinations were measured three times with a mercury sphygmomanometer, following the recommendations of the American Heart Association.

ELISA measurements

Serum insulin, leptin, adiponectin (AdipoQ), and C-reactive protein (CRP) were measured by commercial enzyme-linked immunosorbent assay kits (EZHI-14 K,

EZHL-80SK, EZHADP-61 K, and CYT298, respectively; Millipore, St Charles, Missouri, USA) following the manufacturer's specifications. All samples were measured in duplicate, and the mean was scored. Insulin resistance was assessed by the homeostasis model assessment index of insulin resistance (HOMA-IR), which was calculated as stated in the following formula: HOMA-IR=[glucose (mmol/L) × insulin (μU/mL)]/22.5, as described elsewhere [3]. Moreover, the leptin/adiponectin ratio (L/A) was calculated [45].

Statistical analyses

Mean values and standard deviations (SD) were reported for all the variables. Differences between the beginning and the end of the complete study and the analyses between groups (Control, Normoxia, and Hypoxia) were performed through repeated measures analysis of variance (RM-ANOVA). The percentage of change was analyzed by one-way ANOVA (homogeneity of variances assumed) followed by post hoc LSD test and Brown-Forsythe test (not homogeneous variances), followed by Tamhane's post hoc test. Pearson's *r* partial correlation was used to assess the association between leukocytes and FM, controlled by group. The SPSS 15.1 software for Windows (SPSS Inc., Chicago, USA) was used for all statistical analyses. Values of $p<0.05$ were considered as statistically significant, while values of $p<0.1$ were considered as trends.

Results

Anthropometric measurements

As expected after 3 months of intervention, all three groups lose weight, approximately 6 % of it ($p<0.01$ in all three groups), although there were no significant changes within groups. Consequently, the BMI decreased proportionally ($p<0.01$ in all three groups). In addition, FM (%) significantly decreased in the three groups. While FFM (Kg) decreased in all three groups, there was a statistically significant decrease in the control group compared to the normoxia group ($p<0.05$). In addition to this, water percentage (%) increased in the normoxia-trained volunteers ($p<0.05$) showing differences between the control and the normoxia groups ($p<0.01$). Hypoxia group showed a similar pattern to the normoxia group, although not significant.

Waist circumference (cm) was reduced in the control ($p<0.05$), normoxia ($p<0.01$), and hypoxia ($p<0.01$) groups, meanwhile hip circumference (cm) was only reduced in the last two groups (Table 2).

Dietary records

Energy intake was significantly reduced in the control group (−337 Kcal/day) and the hypoxia group (−651 Kcal/day) ($p<0.05$ and $p<0.01$, respectively), with only 239 Kcal/day of reduction in the normoxia group. Furthermore, macronutrient distribution (% energy/day) was analyzed, and no differences were found with baseline data and within groups (Table 2).

Training protocol

The volunteers that have followed the different protocols improved some of their exercise-related measurements (Table 3). There were no significant changes within groups, but resting DBP was significantly reduced after the hypoxia treatment ($p<0.01$), and there were no changes on SBP. RER max. was significantly reduced in both exercise groups, normoxia and hypoxia ($p<0.05$). On the other hand, dietary advice alone was able to reduce HR at rest ($p<0.05$), and exercise in hypoxia was able to increase the time to reach maximum heart rate (exertion time) ($p<0.05$).

Biochemical and clinical parameters

Different biochemical variables were measured in all experimental groups in the blood of the volunteers after the three interventions. Glucose did not show any significant change when comparing values before and after the intervention, and within groups (data not shown). Urate levels decreased in the control group after the intervention ($p<0.05$). Furthermore, the levels of total cholesterol ($p<0.01$) and LDL cholesterol ($p<0.05$) improved with dietary guidance alone and together with hypoxia training. Moreover, HDL cholesterol levels changed to healthier values ($p<0.05$) after following a program of exercise in normoxic conditions (Table 3). The percentages of change in hepatic markers such as bilirubin, GPT, GOT, and GGT followed the expected tendency associated to weight-lowering treatment. From all these parameters, only leukocyte count showed a significant difference ($p<0.05$) when comparing hypoxia and control group (Fig. 2). GPT showed a trend

Table 2 Anthropometric characteristics and dietary composition before and after the experimental treatments (13 weeks)

	Control		Normoxia		Hypoxia		*p* value
	Baseline	W13	Baseline	W13	Baseline	W13	
Weight (kg)	100.5 (9.7)	94.8 (10.3)**	101.1 (8.8)	95.2 (10.1)**	105.1 (11.6)	99.0 (10.8)**	NS
BMI (kg/m^2)	33.0 (2.1)	31.1 (2.4)**	33.7 (2.7)	31.8 (3.2)**	34.4 (2.8)	32.5 (2.9)**	NS
FM (%)	27.2 (3.5)	26.0 (4.8)*	27.8 (4.3)	25.7 (5.1)**	28.3 (4.7)	26.7 (4.2)*	NS
FFM (kg)	70.3 (5.0)	66.3 (5.4)**	68.0 (4.8)	66.9 (4.7)*	72.8 (3.7)	69.9 (3.5)**	0.038[a]
Water (%)	53.0 (2.1)	52.2 (2.4)	51.8 (3.8)	54.9 (5.3)*	51.5 (3.6)	52.5 (3.3)	0.005[a]
Waist (cm)	106.7 (6.1)	104.6 (5.9)*	107.9 (8.2)	103.9 (9.6)**	116.4 (10.0)	111.9 (10.3)**	NS
Hip (cm)	109.8 (6.0)	108.2 (6.6)	111.5 (8.1)	107.7 (9.2)**	118.8 (11.1)	114.7 (10.0)**	NS
Energy (Kcal/day)	2325 (365)	1988 (178)*	2301 (381)	2067 (141)	2505 (347)	1854 (264)**	NS
Prot (% Kcal/day)	20 (4)	22 (5)	20 (5)	24 (5)	19 (3)	23 (4)	NS
CHO (% Kcal/day)	32 (6)	33 (5)	33 (7)	32 (6)	38 (13)	31 (9)	NS
Lip (% Kcal/day)	44 (7)	43 (6)	42 (7)	42 (8)	38 (11)	44 (8)	NS

Values are expressed as means (SD); *p* value time*group (RM-ANOVA post hoc LSD test)

W13 week 13, *BMI* body mass index, *FM* fat mass, *FFM* fat-free mass, *WHR* waist-hip ratio, % *Prot* protein percentage, % *CHO* carbohydrate percentage, % *Lip* lipid percentage, *NS* non-significant

*$p<0.05$; **$p<0.01$ for the change from baseline to W13 within groups

[a] Control compared to normoxia group

Table 3 Blood sample markers and exertion test before and after the experimental treatments (13 weeks)

	Control		Normoxia		Hypoxia		*p* value
	Baseline	W13	Baseline	W13	Baseline	W13	
Urate (mg/dL)	6.46 (1.22)	6.04 (1.12)*	6.76 (1.32)	6.33 (1.61)	6.36 (1.31)	6.17 (1.27)	NS
Cholesterol (mg/dL)	218.56 (35.64)	196.56 (26.28)**	201.17 (44.18)	194.42 (52.90)	206.33 (36.53)	181.87 (29.65)**	NS
HDL (mg/dL)	46.88 (14.37)	46.25 (13.27)	44.75 (6.57)	48.33 (7.74)*	44.00 (10.79)	42.67 (11.09)	NS
TG (mg/dL)	123.27 (44.35)	97.87 (47.34)	124.00 (61.35)	92.91 (30.39)	156.14 (62.02)	125.21 (46.30)	NS
LDL (mg/dL)	144.00 (39.28)	129.94 (24.76)*	127.42 (48.61)	112.67 (55.95)	130.50 (35.08)	114.00 (23.41)*	NS
T&G index	8.67 (0.39)	8.52 (0.56)	8.63 (0.48)	8.63 (0.14)	8.63 (0.36)	8.76 (0.42)	NS
RER	0.87 (0.14)	0.90 (0.11)	0.89 (0.13)	0.84 (0.09)	0.85 (0.14)	0.78 (0.17)	NS
RER max.	1.20 (0.12)	1.18 (0.08)	1.20 (0.05)	1.13 (0.12)*	1.23 (0.09)	1.17 (0.11)*	NS
VO_2 (mL/kg/min)	2.94 (1.13)	2.75 (1.16)	3.17 (1.13)	3.33 (1.16)	3.07 (1.39)	2.71 (1.16)	NS
VO_2 max. (mL/kg/min)	26.88 (3.18)	27.50 (5.48)	30.83 (5.48)	29.25 (6.02)	28.43 (4.52)	29.43 (3.88)	NS
HR (beats/min^{-1})	72.73 (9.48)	67.47 (7.69) *	67.45 (13.00)	66.55 (13.89)	73.93 (9.85)	71.79 (12.45)	NS
HR max. (beats/min^{-1})	168.31 (9.29)	165.88 (7.74)	164.83 (11.78)	161.50 (11.64)	174.08 (13.98)	169.50 (11.28)	NS
Exertion time (s)	540.64 (71.21)	564.79 (80.53)	568.25 (125.24)	598.08 (134.61)	574.29 (82.65)	616.79 (116.59)*	NS
DBP (mm Hg)	78.00 (8.21)	76.63 (8.70)	77.73 (12.56)	76.00 (7.94)	82.50 (14.08)	73.83 (9.95)**	NS
SBP (mm Hg)	127.50 (16.67)	124.06 (17.04)	125.27 (20.06)	126.55 (15.20)	129.64 (15.49)	125.50 (15.72)	NS

Values are expressed as means (SD); *p* value time*group (RM-ANOVA post hoc LSD test)

W13 week 13, *HDL* high-density lipoprotein, *TG* triglycerides, *LDL* low-density lipoprotein, *T&G index* Ln[triglyceride (mg/dl) * glucose (mg/dl)/2], *RER* respiratory exchange ratio, *RER max*. maximum respiratory exchange ratio, *VO_2* oxygen consumption, *VO_2 max*. maximum oxygen consumption, *HR* heart rate, *HR max* maximum heart rate, *Exertion time* time spent to achieve maximum heart rate, *DBP* diastolic blood pressure, *SBP* systolic blood pressure, *NS* non-significant

*$p<0.05$; **$p<0.01$ for the change from baseline to W13 within groups

towards significance between normoxia and control groups (p=0.059). A positive association has been found between the changes in the number of leukocytes and FM controlled by group. This result (Fig. 3) suggests that an increase in leukocytes is accompanied by higher FM (r=0.39, p<0.05).

ELISA measurements

The inflammatory markers CRP, AdipoQ, Leptin, L/A ratio insulin, and HOMA-IR, shown in percentage of change, were not statistically different between groups (Fig. 4). However, HOMA-IR indicated a trend towards significance between hypoxia and control group (p=0.095).

Discussion

The current scientific efforts to understand and unveil the relationship between hypoxia and disease have a good rationale, since excessive adiposity is associated with poor tissue oxygenation leading to sleep disorders [21]. In addition to being a consequence of obesity, hypoxia could be a putative treatment [18]. In this context, intermittent hypoxia may be a tool to manage some obesity-associated risk factors such as cardiovascular disease [32]. Normoxic and hypoxic training have been related to important improvements in specific metabolic risk factors and exercise capacity [50]. Moreover, the stimulus of intermittent normobaric hypoxia, which

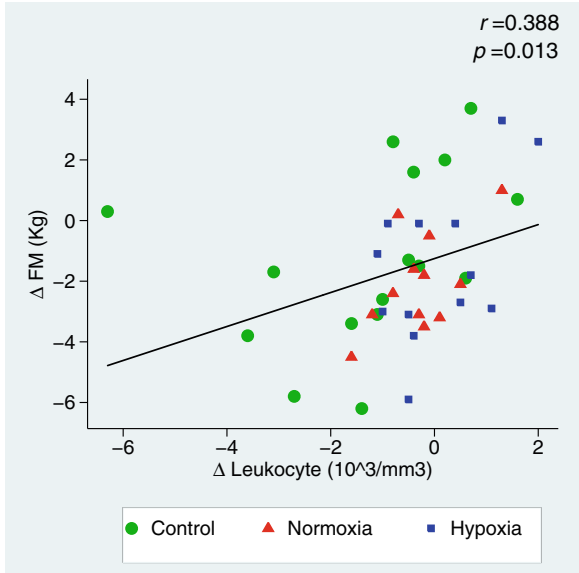

Fig. 3 Pearson's correlation analysis between changes (Δ= 13 weeks baseline) in leukocyte count with changes in FM (N= 42) controlled by groups. r correlation coefficient, *FM* fat mass. The *shapes* represent the subjects

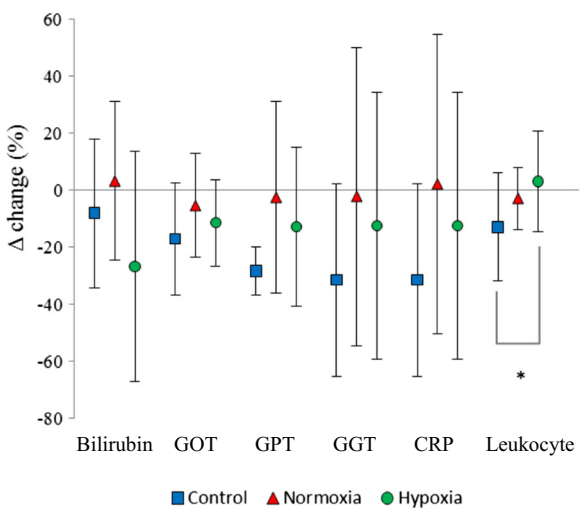

Fig. 2 Changes in percentage of hepatic enzymes and immune markers (Δ=13 weeks baseline). *GOT* glutamic oxaloacetic transaminase, *GPT* glutamic pyruvic transaminase, *GGT* gamma-glutamyl transpeptidase, *CRP* C-reactive protein; *p<0.05 between normoxia and control group (ANOVA and post hoc LSD test). The *shapes* represent the means and the *bars* standard deviations

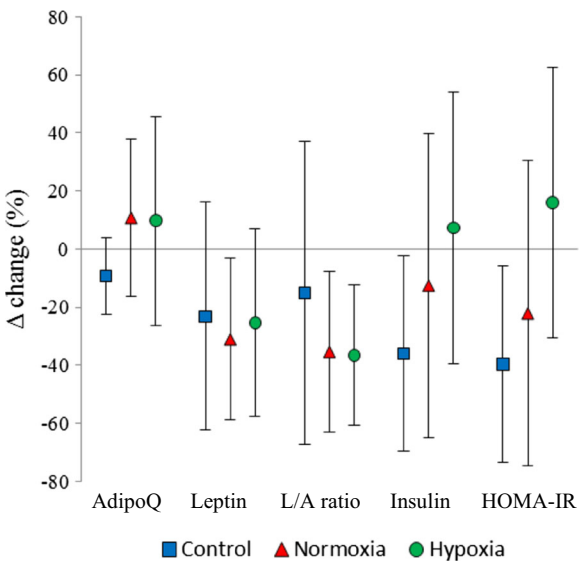

Fig. 4 Changes in percentage of adipokines and related ratios (Δ=13 weeks baseline). *AdipoQ* adiponectin, *LA* leptin-adiponectin ratio, *HOMA-IR* homeostasis model assessment index of insulin resistance. The *shapes* represent the means and the *bars* standard deviations

has been cited as an additive cardioprotective effect, may have relevant clinical implications affecting insulin sensitivity or inflammation [21], while an enhancement of leptin secretion under a hypoxia milieu may also be involved [50]. The underlying mechanisms may involve increases in angiogenesis, adrenergic activation, mitochondrial biogenesis, translocation of glucose transporters, and changes in metabolic pathways [50].

In this context, data from obese subjects suggested an increased adipose tissue oxygen tension in abdominal subcutaneous fat, which was accompanied by insulin resistance, impaired adipose tissue capillarization, and inflammation [19]. Other authors have shown that adipocyte respiration becomes uncoupled in overweight high-fat fed animals, leading to increased oxygen consumption and a state of relative adipocyte hypoxia. These changes trigger hypoxia inducible factor 1 (HIF-1)α induction, causing inflammation and insulin resistance [23]. Interestingly, some authors highlighted that intermittent hypoxia may be beneficial in stimulating the hypoxia inducible factor 1 (HIF-1)α pathway for the clinical management of diabetes and metabolic syndrome symptoms [17].

Indeed, hypoxia deeply affects the adipocyte functions and could be crucial for adipose tissue deregulation in the obese [48]. However, an experiment conducted in adipocytes [38] revealed that two different hypoxia-inducing conditions (100 μM $CoCl_2$ and 1 % O_2) mediated different effects in the expression of some genes, while it remained similar in others related to inflammation.

It should be noted that SAHS patients suffer from cycles of intermittent hypoxia and hypercapnia, which differs from the studied hypoxia exposure because it is accompanied by hypocapnia instead [43], which is similar to high-altitude atmosphere [2]. Indeed, altitude induces HIF activation, leading to changes in cellular metabolism and the activation of peripheral pathways and the central nervous system affecting energy expenditure and appetite pathways [35].

In this context, one experiment conducted in animals fed with a high-fat diet demonstrated that exposure to intermittent hypoxia leads to weight loss by reducing food intake and also muscle mass [39]. This unfavorable result concerning the lean tissue was hypothesized that could be overcome by exercise training and by providing a diet enriched in protein as was designed in the current trial [29]. Of value, the present experiment with obese subjects evidenced that all the interventions reduced body weight, BMI, and waist circumference as well as fat-free tissues. The body water content was increased in normoxia group comparing to control group, and hip circumference had better outcomes in programmed exercise intervention groups. Furthermore, an effect on energy intake was found in the control group and hypoxia group. The subjects following the hypoxic training were expected to decrease the energy intake in agreement with other investigations concerning hypobaric hypoxia in altitude that caused reduced appetite [24]. Also, a trial conducted in normobaric hypoxia training produced more weight loss and improved systolic pressure than normoxia training, both under calorie restriction, after a 4-week residential camp for obese young adults [22]. Other investigators found that low-intense activity in normobaric hypoxia circumstances lead to more weight loss in obese people as compared to normobaric sham hypoxia [33]. Physical exercise has been recommended for the treatment of obesity as a combined strength and endurance program [50], as carried out in this experimental trial.

Most assessed blood biochemical variables concerning carbohydrate, lipid, and protein metabolism followed the expected trends associated to weight loss in the three interventions (control, normoxia, and hypoxia) with minor differences, although some specific markers such as transaminase concentrations showed some distinctive patterns among the experimental groups. The differences in urate reduction in the control group, but not in the obese subjects on exercise, also could be speculated to be due to compensatory mechanisms in oxidative stress associated to dietary protein intake [9] and programmed physical activity [47].

Interestingly, markers of inflammatory processes such as CRP or leukocytes commonly associated to obesity [6] often are improved by weight loss [41]. In this intervention trial, leukocytes, but not CRP levels, were increased in the hypoxia versus control group, which could be explained by exercise as in a previous study [40] and by the intensity of the exercise [31].

SAHS is strongly associated with secondary hypertension [12], metabolic alterations [46], and obesity [34]. On the other hand, it has been reported that obesity impairs pulmonary functional capacities and causes airflow obstruction, poor arterial oxygenation, or hypoventilation [42], and it is related to SAHS [49]. All these conditions may benefit from weight loss induced by behavioral interventions [15] or bariatric surgery [27], and also by lifestyle strategies including dietary and physical activity interventions [4]. Indeed, one major aim of the current

trial was focused on analyzing in obese individuals with SAHS, the potential additive benefits of nutritional advices associated with regular exercise practice under hypoxia conditions. The expected achievements were hypothesized not only on body weight and composition improvements but also on fitness and cardiorespiratory outcomes trying to balance the typical FFM losses of energy restrictions with a compensatory physical activity. In this regard, other studies have shown the efficacy of intermittent hypoxia, caused by altitude, on increasing arterial oxygen saturation [36].

The cardiac stress test and the evaluation of cardiorespiratory fitness associated with the respiratory muscle function in the obese population is routinely performed to assess heart stimulation by exercise being useful to reflect the general physical condition of the assayed patient [5]. In the current trial, no adverse effects were reported after the cardiac stress test, whose outcomes evidenced small or subtle changes in all experimental groups with no relevant differences among them, but always in the direction of a general improvement of the cardiometabolic fitness as evidenced through different markers and assessments.

The respiratory exchange ratio (RER) is an indicator of the macronutrient proportions being metabolized to supply the body with energy. The computation of RER is commonly performed in combination with exercise tests such as the VO_2 max. test [28] and can be used as a sign of aerobic fitness and to set up the limits of the cardiorespiratory system. RER max. is the most accurate and reliable indicator of subjects effort, where a value >1.10 is generally considered an exercise effort. The values achieved by the hypoxia and normoxia as compared to those in the control group concerning RER max. suggest that less effort has been made for the same exercise intensity from those trained [7].

Globally, no major differences in physical functions were ascribed to the hypoxia treatment concerning the evaluation of physical functions and cardiorespiratory fitness, as was found in a report concerning weight loss associated with diet and exercise [52]. Furthermore, these results suggest that in a short-term hypoxic exposure does not seem to alter the anthropometric characteristics or fat content [10]. Another important finding from this intervention study is that changes in leukocyte levels evidenced direct statistical associations with variations in FM. These outcomes reveal that adiposity and inflammation are narrowly related and that further research in this scientific field is warranted.

Due to the small sample size of this study, a type II or β error could not be discarded, and some effects of the treatment could have been missed. Furthermore, the volunteers have followed dietary recommendations, which are subjected to individual interpretation, increasing the variability. In addition, 24-h questionnaires were used to assess calorie intake and macronutrient distribution, accepting some subjectivity of the method, despite validated procedures were applied.

Conclusion

Summing up, this investigation demonstrated that the muscle mass decrease commonly found under dietetic weight loss programs can be partly prevented by exercise. The hypoxia group has shown some specific cardiometabolic benefits improving exertion time and DBP. However, no changes in adipokines were found following our intermittent hypoxia protocol. These results suggest that a different or a more intense administration of hypoxia might be necessary to perceive effects on systemic inflammation.

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Conflict of interest The authors declare they have no conflict of interest.

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