NORMOBARIC HYPOXIA EXPOSURE ON SUBSTRATE OXIDATION PATTERN: SEX DIFFERENCES

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Abstract

Context. Hypoxic exposure has been associated with a metabolic perturbation that might affect basal energy expenditure (BEE).

Objective. The aim was to examine the metabolic response during hypoxic exposure of men and women adults.

Design. Crossover design with two experimental trials: normoxic and hypoxic exposure.

Subjects and Methods. Twenty-nine healthy subjects (14 women) participated in (1) control study (NOR), subjected first to normoxic exposure ($\text{FiO}_2 = 20.9\%$) and (2) after that, to passive normobaric hypoxic exposure study (HYP) ($\text{FiO}_2 = 15\%$). Respiratory gases and blood glucose samples were recorded every hour in hypoxia chamber (8 points in total), and blood lactate samples were collected at baseline, at 4 and 7 h to exposure.

Results. In females, basal energy expenditure was significantly higher at 2h, 4h, 6h and 7h compared with NOR group. Also, BEE was lower in females compared with men from 2h of hypoxia exposure. In the HYP group the blood lactate concentration increased significantly at 4h and 7 h relative to NOR group ($P < 0.05$) in males.

Conclusion. An exposure to moderate normobaric hypoxia did not alter metabolic response, but induced a different response on substrate oxidation in adults men and women.

Keywords: altitude, health status, hypoxia, metabolism, sex.

INTRODUCTION

Numerous lines of evidence indicate the effects of hypoxia exposure on performance and health parameters (1). Among these, the beneficial effects of hypoxic stimulation on metabolism is especially attractive as potential means to promote health (2). Although its underlying mechanism is not fully understood, hypoxia has been associated with metabolic perturbation that might affect fuel utilisation (3). Basic

knowledge regarding metabolic regulation via hypoxia has received considerable attention, and several recent studies have investigated the use of normobaric hypoxic exposure as a novel therapeutic strategy to reduce body fat (4-6). The beneficial effects of body fat reduction may be associated with increased post-exposition lipid oxidation (7). During exercise under conditions of hypoxia, a greater dependence on lipid sources *versus* glucose may occur (8). Second, oxygen debt, produced by use of non-oxidative mechanisms, increases the basal metabolic rate (BMR). The cost of this increase must be repaid during recovery, which enhances fat oxidation (9, 10). The mechanism is regulated with a metabolic switch controlled by hypoxia inducible factor (HIF), which regulates the expression of many genes involved in metabolic and substrate oxidation (11).

However, the response to acute hypoxia during exercise and relatively moderate hypoxia differs from the response to a more sustained and severe hypoxia (12).

Although a high-altitude environment has been the main setting used to study the physiological response to hypoxia, other contexts including confinement to normobaric hypoxia chambers have recently become common. Following prolonged stays at higher altitude, it is likely that individuals increasingly rely on carbohydrate oxidation to achieve an increased energy yield (13). Brooks *et al.*, (1991)(15) Roberts *et al.*, (1996)(14) and revealed that individuals depended on glucose as a fuel when exposed 4,300-m altitude at rest and during exercise compared with sea-level. Nevertheless, whether there is an increased dependency on glucose under normobaric hypoxic conditions *versus* sea level is a matter of some debate. At rest, a significant decrease in blood glucose concentrations was reported after 1 hour of exposure to moderate hypoxia (16). In contrast, another work revealed no significant change

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1 to 7 hours post-exposure to conditions of moderate hypoxia (1).

On the other hand, some investigations have suggested the presence of sexual dimorphism associated with metabolism at altitude (10). As discussed previously, HIF may induce a shift in metabolism favoring glucose utilisation that may be of greater magnitude in men compared with women. In the response to hypoxia in women, steroid hormones oestrogen and progesterone have been demonstrated to down regulate HIF activity, which may modulate its propensity for fat oxidation (17). The attenuation of HIF activation in females may account for sex differences in glucose utilisation and BMR changes highlighted previously (10).

In general, data used to investigate the influence of moderate hypoxia on metabolic responses are not conclusive. Evidence from several experimental models is necessary to explain metabolic economy in response to exposure to moderate normobaric hypoxia, and determine whether fuel oxidation is influenced by sex (18). Developing a better understanding of hypoxia-induced changes in substrate utilisation may facilitate the development of nutritional and physical strategies for combating different metabolic disorders (19). To our knowledge, it is the first study that has analysed substrate oxidation throughout normobaric hypoxia exposure in both sexes. Hence, the aim of the present study was to examine the metabolic response during hypoxic exposure in male and female adults. We hypothesised that moderate hypoxia may induce a degree of greater glucose utilisation than exposure to normoxia, and that these changes would be observed predominantly in the male sex.

MATERIALS AND METHODS

Participants

Participants were recruited via personal or mass invitations in a community setting, either via e-mail or through online social networks. Inclusion criteria included: (1) women or men aged 18 years or older; and (2) individuals that had not travelled above 1,500m during the 3-month period that preceded the study. The following exclusion criteria were applied: (1) use of any prescribed medications; (2) smoking; (3) respiratory problems; (4) heart disease; (5) hypertension; (6) chronic or acute illness; (7) anxiety disorder; (8) drug and alcohol abuse. Study participants attended an orientation session. After verifying inclusion and exclusion criteria using a general questionnaire to collect

medical and demographic data, participants were asked to sign a declaration of consent to participate in the research. They were also informed about the equipment and experimental design. Anthropometric measurements (using standard methods) and physical activity levels (using the International Physical Activity Questionnaire) of participants were obtained. Fourteen healthy females (27.1 ± 7.0 years, 1.6 ± 0.1 m, 56.4 ± 4.5 kg, 21.1 ± 1.7 kg/m²) and 15 healthy males (22.9 ± 4.1 years, 1.8 ± 0.1 m, 72.2 ± 9.5 kg, 23.4 ± 2.1 kg/m²) participated in the study. All procedures were performed in accordance with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Furthermore, the study design was approved by the Bioethical and Biosecurity Commission of the University of Extremadura.

Experimental design

In a crossover design, two experimental studies separated by seven days were performed. Individuals were placed in a hypoxic chamber (CAT 310, Louisville, Colorado, USA) built in our laboratory (459m altitude, 24°C and 40% relative humidity). The studies were carried under two different conditions: (1) control (NOR) group, individuals were subjected to normoxic conditions (sea level: $\text{FiO}_2 = 20.9\%$) followed by a (2) passive normobaric hypoxic exposure (HYP) study ($\text{FiO}_2 = 15.0\%$, corresponding to simulated altitude of 3,000 m).

Throughout a 7 h period, participants were exposed to either hypoxic or normoxic conditions following an overnight fast (at least 10 h) (1). All experimental sessions were conducted at the same time of the day, from 8:00 am to 3:00 pm. Throughout experimental trials, participants were comfortably seated in a normobaric hypoxia room and were free to read, to listen to music or to work/study as desired. They were allowed to leave the hypoxia room for a short period to use the toilet. If systolic blood pressure increased more than 30 mmHg from baseline, heart rate increased or decreased more than 20 beats per minute from baseline (individual values from baseline) or an increase in acute mountain sickness (AMS) score above 3 points was observed, the participant was required to terminate hypoxic exposure for safety reasons. Standard meals were provided after baseline and 5 h after entering the environmental chamber, and subjects were instructed to consume the meals within 7 min. The first meal consisted of 68.4% carbohydrates, 10.1% protein, and 21.5% fat (745 kcal). The second meal consisted of 66.9% carbohydrates, 10.1% protein, and 23.0% fat (731 kcal).

Experimental trial day measurements

Symptoms of AMS using the Lake Louis Scoring (LLS) questionnaire (15), arterial oxygen saturation (SpO₂) (by pulseoximetry: Onyx II 9550, NONIN, USA), systolic (SBP), and diastolic (DBP) blood pressure (M4 Plus, OMRON, Japan) values were obtained. Respiratory gas and blood glucose sample content were recorded every hour when individuals were in the hypoxia chamber (8 data collection times total). In addition, blood lactate samples were collected at baseline and at 4 and 7 h post-exposure (Fig. 1). Periodically, a researcher entered the chamber and performed measurements / harvested samples.

Following an overnight fast, the participants visited the department in the morning and rested before the first evaluation. Participants were subjected to metabolic rate measurements using an indirect calorimetric technique that employed an automatic gas analyser (Metalyzer 3b; CORTEX Biophysik GmbH). To determine each participant's basal metabolic rate (baseline), they were requested to comply with the following criteria: engage in no exercise during the preceding 36 hours; ingest no caffeine or alcohol during the preceding 24 hours; consume a last meal

before 10:00 p.m. the prior evening and drink only water afterward; travel to the laboratory using a car or public transportation. Upon arrival to the laboratory, participants were placed in a comfortable position in a quiet environment in preparation for metabolic rate measurement. Throughout data collection, participants were instructed to remain quiet and relax, but stay awake. Appropriate calibrations of O₂ and CO₂ sensors and the volume transducer were performed before each measurement. Metabolic rate measurements were determined during a 10-min period within a 20 min data collection period. The first and last 5-min portions of the procedure were discarded in order to nullify any metabolic rate fluctuations that occurred due to facemask familiarisation and the expected termination of data collection. The remaining 10-min segment was integrated and normalised over time. From respiratory gas samples, oxygen consumption (VO₂), carbon dioxide production (VCO₂) and respiratory quotient (RQ) were determined. Basal energy expenditure (BEE) in kilocalories (kcal), and oxidation of glucose and fat (g × min⁻¹) were calculated from VO₂ (oxygen consumption) and VC CO₂ (carbon dioxide production), using stoichiometric equations, as follows (20, 21):

Basal Energy Expenditure = $(3.9 \times \text{VO}_2 \text{ (L/min)}) + (1.1 \times \text{VC CO}_2 \text{ (L/min)}) \times 1,440$,

Fat oxidation = $[(1.67 \times \text{VO}_2 \text{ (L/min)}) - (1.67 \times \text{VC CO}_2 \text{ (L/min)})]$,

Carbohydrate oxidation = $[(4.55 \times \text{VC CO}_2 \text{ (L/min)}) - (3.21 \times \text{VO}_2 \text{ (L/min)})]$.

Blood glucose and lactate were measured from arterialised capillary blood (finger) and were determined using an automated glucose analyser (FreeStyle Optium Neo, Abbott Diabetes Care Ltd., UK) and lactate analyser (Lactate Scout, SensLab GmbH, Germany).

Statistical analysis

Statistical analyses were performed using the SPSS v.20 for MAC statistical package (IBM, New York, USA). Data are presented as mean ± standard deviation. Standard statistical methods were used for the calculation of the mean and standard deviations. Kolmogorov–Smirnov tests were performed to show the distributions of variables studied and Levene's test was performed to assess homogeneity of variance. Three-way analysis of variance (ANOVA) with repeated measures was used to test the interaction between trial, time and sex. When ANOVA revealed a significant interaction or main effect, Bonferroni post hoc analysis was used to identify where changes occurred. In all analyses, values of $P < 0.05$ were considered statistically significant.

Figure 1. Overview of the study design. HR: heart rate; SpO₂: oxygen saturation.

Figure 2. Time courses of changes in SPO₂ over 7 h. SPO₂: arterial oxygen saturation. F-hypoxia: female in hypoxia trial; F-normoxia: female in normoxia trial; M-hypoxia: male in hypoxia trial; M-normoxia: male in normoxia trial. #Significant differences compared with normoxia trial. 🍏: meals.

RESULTS

Figure 2 shows changes in SpO₂ values over a time course of 7 hours. There was a significant interaction observed between study and time with regard to SpO₂. The HYP study had significantly lower

SpO₂ values at all timepoints ($P < 0.05$) in female participants, and at all times points > 30 min in male participants ($P < 0.05$) compared with the NOR study and baseline values. In the NOR study, this parameter did not significantly change at past the 7 h experimental period. Average SpO₂ values recorded 1–7 h after

Figure 3. Time courses of changes in basal energy expenditure (a) and respiratory quotient (b) over 7 h. BEE: basal energy expenditure; * Significant differences compared with normoxia trial. \$ Significant differences compared with males 🍏: meals.

Table 1. AMS score, diastolic blood pressure and systolic blood pressure over 7-hour

		Baseline	1h	2h	3h	4h	5h	6h	7h
Male									
AMS	HYP	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.26	0.07 ± 0.26	0.07 ± 0.26
	NOR	0.07 ± 0.25	0.00 ± 0.00	0.07 ± 0.26	0.00 ± 0.00	0.13 ± 0.35	0.07 ± 0.26	0.07 ± 0.26	0.07 ± 0.26
SBP, bpm	HYP	115.7 ± 8.3	113.0 ± 9.7	111.3 ± 10.5	109.9 ± 10.4	110.1 ± 10.8	109.7 ± 11.7	115.9 ± 8.6	116.3 ± 12.5
	NOR	116.3 ± 8.6	116.6 ± 9.5	114.9 ± 9.2	113.2 ± 13.3	113.9 ± 9.4	113.7 ± 9.1	115.9 ± 10.2	116.7 ± 11.1
DBP, bpm	HYP	69.4 ± 2.7	69.2 ± 5.6	67.7 ± 7.8	69.7 ± 7.1	69.3 ± 6.4	66.6 ± 8.6	69.0 ± 5.2	69.7 ± 8.0
	NOR	69.9 ± 6.8	68.6 ± 7.5	67.0 ± 7.7	67.5 ± 7.0	68.6 ± 5.9	69.9 ± 6.6	66.4 ± 6.6	67.3 ± 5.2
Female									
AMS	HYP	0.07 ± 0.26	0.00 ± 0.00	0.07 ± 0.26	0.00 ± 0.00	0.13 ± 0.35	0.07 ± 0.26	0.07 ± 0.26	0.07 ± 0.26
	NOR	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.21 ± 0.43	0.21 ± 0.43
SBP, bpm	HYP	116.3 ± 8.5	116.7 ± 9.5	115.0 ± 9.2	113.2 ± 13.3	113.9 ± 9.4	113.7 ± 9.1	115.9 ± 10.2	116.7 ± 11.1
	NOR	103.6 ± 7.0	103.2 ± 7.8	101.0 ± 6.5	100.7 ± 6.1	102.3 ± 6.9	101.4 ± 7.2	102.8 ± 7.6	99.4 ± 6.8
DBP, bpm	HYP	69.9 ± 6.8	68.6 ± 4.9	67.0 ± 7.9	67.5 ± 10.0	68.6 ± 5.9	69.9 ± 6.6	66.4 ± 6.6	67.3 ± 5.2
	NOR	66.9 ± 6.8	63.9 ± 6.2	64.6 ± 6.5	64.4 ± 5.9	65.4 ± 5.4	65.3 ± 7.4	63.8 ± 6.9	62.8 ± 5.9

AMS: acute mountain sickness; SBP: systolic blood pressure; DBP: diastolic blood pressure. HYP: hypoxia trial; NOR: normoxia trial. Mean ± SE.

entering the chamber were $98.47 \pm 0.80\%$ and $98.50 \pm 0.56\%$ for the NOR study and $95.09 \pm 2.63\%$ and $96.17 \pm 1.62\%$ for the HYP study in female and male participants, respectively. No significant interactions between SBP, DBP and AMS were observed (Table 1). No serious adverse health effects of exposure were reported by the subjects in the LLS questionnaire.

Respiratory gas parameters

Basal energy expenditure (BEE) and respiratory quotient (RQ) values are shown in Figure 3. BEE was only significantly elevated after 2h, 4h, 5h and 7h compared with NOR trial in females. However, a significant interaction between time of exposure and sex was observed after 2 h of normoxic exposure. Females had lower BEE values than males.

Substrate utilisation

Figure 4 shows the rate of fat and carbohydrates oxidation over the 7-hour period considered. No significant interaction was observed between fat oxidation and carbohydrate oxidation ratio.

Blood glucose and lactate

Figure 5 shows changes in blood glucose and blood lactate levels over the 7-h time course. There was a significant interaction (time x trial) observed for the blood glucose response ($P < 0.05$). Glucose values were significantly higher in HYP group at 3h ($P < 0.05$) compared with NOR group in females. In HYP group, men had significantly higher glucose values at baseline, 4h and 5h compared with NOR group ($P < 0.05$). Regarding lactate levels, a significant interaction between time and study group was observed in males. HYP group blood lactate concentrations were significant after 4 h and 7 h exposure relative to NOR group values ($P < 0.05$).

DISCUSSION

To the best of our knowledge, this is the first study that has examined metabolic responses during hypoxic exposure in adult men and women. This study indicated that metabolic responses were not significantly affected by passive normobaric hypoxic

Figure 4. Time courses of changes in fat (a) and carbohydrates (b) oxidation over 7 h. 🍏: meals.

exposure in healthy adults. However, our hypothesis was partially confirmed, since normobaric moderate hypoxia may induce a different substrate oxidation response in adult men and women.

Although some data suggest that hypoxia may stimulate a shift in substrate use to favour increased dependence on glucose, the duration and degree of hypoxic exposure are critical factors that affect the metabolic response (12). Severe doses of hypoxia have been suggested to elicit a more severe environmental condition than moderate hypoxia, and that leads to different physiological adaptations (22), which likely have an increased influence on the metabolic response (23). However, similar to findings reported in this study, healthy young men exposed to 7 h of moderate normobaric hypoxia did not display an altered glucose response compared with individuals exposed to normoxia (1). Physiological effects of hypoxia were confirmed by comparing SpO₂ levels of both trials in both sexes. SpO₂ was significantly lower in the hypoxia trial ($95.09 \pm 2.63\%$ and $96.17 \pm 1.62\%$ in females and males, respectively) than in the normoxia trial (98.47

$\pm 0.80\%$ and $98.50 \pm 0.56\%$ in females and males, respectively). However, levels were modest, even in the hypoxia trial, and thus, they may have been insufficient to augment glucose uptake. In fact, Morishima & Goto., (2014)(1) reported that moderate hypoxic exposure does not affect endocrine and metabolic responses. Chen *et al.*, (2009) reported that glucose uptake was significantly promoted by hypoxia when levels of SpO₂ were below 80%. Other potential mechanisms likely underlie effects of hypoxia to enhance use of glucose as an energy substrate (8). The combination of exercise and hypoxic exposure resulted in a greater energy deficit and increased weight loss. This finding suggested that a combination of hypoxia and exercise may be an increasingly potent metabolic stimulus than either individual factor. Future studies that combine different hypoxic protocols with controlled activity/inactivity and diet could facilitate the elucidation of mechanisms that underly metabolic changes (12).

On the other hand, previous evidence has supported sexual dimorphism in metabolism at altitude, and has suggested that a shift in metabolism

Figure 5. Time courses of changes in blood glucose (a) and blood lactate (b) over 7 h. * Significant differences compared with normoxia trial. \$ Significant differences compared with male. 🍏 : meals.

that favors increased glucose utilisation occurs in men (10). In females, normobaric hypoxic exposure also leads to an increase in basal metabolic rate and the augmentation of lipid oxidation. The lack of a comparable shift in substrate metabolism in women suggests that sex differences are mediated by their differing impact on carbohydrate and lipid use. Consistent with sex differences observed in response to other metabolic stressors (25), women may be more efficient at conserving energy using preferentially fat as substrate (18, 26). Specifically, oestrogens have been demonstrated to down-regulate HIF activity (10), mediating fat oxidation propensity (17). Considering the high prevalence of obesity in women, knowledge of sex-based differences may provide a promising strategy for fat reduction with less energy restriction. Conversely, increased lactate concentrations from hour 4 of hypoxic exposure were observed in men. The production of the metabolite lactate increases under hypoxic conditions (27). During early stages of hypoxia, HIF-1-mediated up-regulation of anaerobic glycolysis (9) and increased lactate concentrations, a common product of anaerobic metabolism, are observed (28). These findings likely indicate that ATP formation occurs *via* anaerobic glycolysis in males. However, since respiratory gas parameters did not show significant changes, further research will be necessary to clarify whether elevated lactate concentrations could indicate increased levels of anaerobic glycolysis in males subjected to normobaric hypoxic conditions.

Despite the importance of findings determined in the present study, some limitations must be mentioned. Blood parameters that were not measured may have helped to explain sex differences in fat oxidation. First, hormonal factors and physiological determinants of substrate oxidation were not quantified. Therefore some physiological mechanisms were difficult to elucidate (19). In this sense, authors are aware that the lack of control of the menstrual cycle is a relevant limitation of this study. Therefore, the effect of these factors on basal metabolic rate changes in women during hypoxia exposure may be of interest in future research. Second, indirect calorimetry measures net lipid oxidation, which is regulated by both plasma free fatty acid and insulin levels. Therefore, plasma free fatty acid and glycerol measurements may enhance our knowledge of lipid oxidation (29). Finally, our sample size was limited due to logistic restrictions (availability of rooms, devices, etc.). Since the sample size of the present investigation was relatively small, differences observed require confirmation using large-scale studies.

In conclusion, exposure to moderate normobaric hypoxia did not alter the metabolic response in healthy adults. However, exposure altered levels of substrate oxidation differently in adult men and women. While female displayed increased basal metabolic rates along with an enhanced tendency to undergo lipid oxidation, males displayed increased lactate levels. However, these findings require further confirmation using large-scale studies.

Conflict of interest

The authors declare that they have no conflict of interest.

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