



Article

Physiological Responses of Anemic Women to Exercise under Hypoxic Conditions

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Abstract: When combining two conditions of reduced oxygen availability, anemia and hypoxia, human physiological responses are highly challenged to maintain arterial oxygen delivery, especially during whole-body exercise. The aim of this study was to compare the cardiorespiratory responses of mildly anemic women with those of healthy controls, while cycling in normobaric hypoxia. Two groups of young females were matched for age, weight, height, and involvement in physical activity, one with normal hemoglobin, hematocrit, and ferritin levels and another suffering from mild iron deficiency anemia ($10 < \text{Hb} < 12 \text{ g/dL}$, $34 < \text{Hct} < 37\%$, ferritin $< 15 \mu\text{g/L}$). They cycled to exhaustion under normoxia and hypoxia (FIO_2 0.21 and 0.14), and their physiological responses were compared at 40, 80, and 100% $\text{VO}_{2\text{max}}$ of the specific condition. The two groups differed ($p < 0.05$) mainly at the higher exercise intensities; the anemic participants exhibited similar heart rate but lower oxygen pulse than their control counterparts, as well as a larger drop in maximal oxygen uptake. However, they sustained maximal effort by employing the anaerobic metabolism to a larger extent, which stimulated a greater ventilatory response. It appears that iron deficiency anemia of mild severity, which is commonly observed in young athletic females, impacts physiological responses during whole-body exercise in the presence of moderate hypoxia.

Keywords: anemia; hypoxia; women; cycling exercise; submaximal; maximal; $\text{VO}_{2\text{max}}$; cardiovascular; respiratory; response



Citation: Koskolou, M.D.; Komboura, S.; Zacharakis, E.; Georgopoulou, O.; Keramidas, M.E.; Geladas, N. Physiological Responses of Anemic Women to Exercise under Hypoxic Conditions. *Physiologia* **2023**, *3*, 247–258. <https://doi.org/10.3390/physiologia3020017>

Academic Editor: Daniel Marinho

Received: 8 March 2023

Revised: 28 March 2023

Accepted: 12 April 2023

Published: 22 April 2023



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1. Introduction

Hypoxia (low partial pressure of oxygen; PO_2) and anemia (low hemoglobin concentration; Hb) are two conditions of reduced oxygen availability which induce perturbations in human physiological functions in an effort to maintain arterial oxygen delivery to the cells of various organs and tissues. During exercise under these conditions, the different links in the chain of oxygen transport may all contribute to supplying the working muscles with a sufficient amount of oxygen.

It is well documented that acute exposure to altitude or the inspiration of gas mixtures with low oxygen fractions (FIO_2) augments the ventilatory response and elevates cardiac output and regional blood flow to compensate for the reduced arterial oxygen content (CaO_2), thereby maintaining oxygen delivery and uptake at rest and during submaximal exercise [1,2]. At maximal physical effort, however, hyperventilation is still present, whereas systemic and muscular blood flow may be reduced. Such a response depends on the mode of exercise and the degree of hypoxia; for instance, during maximal exercise engaging large muscle groups, the hypoxia-related respiratory and circulatory compensatory responses are insufficient, and result in impaired oxygen delivery and proportional reductions in peak

oxygen uptake and power output [2–5]. Moreover, reductions in O₂ supply exacerbate lactate production, which may be associated with the rate of development of fatigue [6,7].

Compensatory responses to the reduced oxygen availability have also been observed when CaO₂, and, therefore, the oxygen-carrying capacity of blood, is reduced due to anemia. When Hb is lowered, for a given submaximal oxygen uptake (VO₂), ventilation, cardiac output, and leg blood flow are relatively elevated in comparison to a normal Hb state, thus partly compensating for impaired oxygen delivery during whole-body exercise [8–10] or maintaining oxygen delivery during two-leg dynamic knee extensor exercise [11,12]. In contrast, oxygen delivery cannot be compensated for at maximal effort, and, hence, VO₂ is decreased in proportion to the Hb reduction [11]. In addition, blood lactate accumulation is greater for a given exercise intensity, thereby accelerating fatigue [12,13].

When anemia is combined with hypoxia, the system is further challenged, and oxygen delivery to the tissues may be insufficient, especially during exercise. For instance, acute experimentally induced anemia elicited circulatory responses adequate to maintain oxygen delivery and uptake during submaximal, but not maximal, knee-extension exercise under hypoxic conditions [14,15]. There is, however, a scarcity of data regarding the physiological responses of anemic individuals during exercise performed under hypoxia that involves large muscle mass (e.g., cycling or running), in which the cardiorespiratory system is heavily taxed.

Anemia is a common health problem worldwide, affecting approximately one-third of the world's population; it affects a considerable percentage (12–18%) of apparently healthy and fit women, with iron deficiency estimated to contribute to ~50% of all anemia cases in women [16,17]. Physically active females often participate in sports and demanding physical activities performed in hypoxic conditions. Therefore, the aim of the present study is to compare the cardiorespiratory responses of mildly anemic women with those of healthy controls while cycling to exhaustion under normobaric hypoxia. It was hypothesized that at higher exercise intensities, the compensatory responses occurring to offset the shortfall in oxygen availability induced by hypoxia would be less efficient in the anemic group.

2. Materials and Methods

The procedures employed in the present study were in accordance with the Declaration of Helsinki, and had the approval of the School of Physical Education and Sport Science Ethics Committee for human experimentation.

2.1. Participants

The experiments were conducted on two groups of young, non-smoking, physically active women, selected from volunteers ranging in age from 19 to 32 years. The first group (C) consisted of 8 participants with normal hemoglobin (Hb > 12 g/dL), hematocrit (Hct > 37%), and ferritin levels (>15 µg/L), and the other group (A) consisted of 8 participants suffering from mild iron deficiency anemia (10 < Hb < 12 g/dL, 34 < Hct < 37%, ferritin < 15 µg/L) [18,19]. The diagnosis of anemia was verified by a specific hematological test conducted in a state hospital within the two-week period before the commencement of the study. The two groups were matched for age, weight, and height, as well as for their degree of involvement in physical activity, which was assessed with a questionnaire. Participants were asked to abstain from physical exercise for 24 h before each experiment, as well as from food and caffeine intake at least 3 h prior to reporting to the laboratory. To ensure that participants maintained similar nutritional routines before each testing session, they were also instructed to record the food they consumed. All the procedures and possible risks were thoroughly explained to the participants, and their written consent was obtained.

2.2. Experimental Design and Procedure

During a preliminary visit, the participants reported to the laboratory for measurement of their anthropometric characteristics and to become familiarized with the equipment

and the experimental procedure. Thereafter, and on two different days, participants performed, in a counterbalanced order, two incremental exercise tests to exhaustion while breathing either room air (FIO_2 0.21; normoxia) or a hypoxic gas mixture (FIO_2 0.14; ambient simulated altitude of ~3300 m). For each individual participant, the two experimental sessions (normoxic and hypoxic) were run within one week during the same phase of her menstrual cycle, which was based on participant's self-report, allowing at least a 24-h interval between trials. Prior to the initiation of each exercise trial, participants were unaware of the breathing condition; however, no further blinding measures were employed. Hematocrit and Hb levels were also measured in our lab on each experimental day.

2.3. Protocol

After the participant was seated on the cycle ergometer, capillary blood samples were taken from the finger of one hand, whereas the other hand was immersed in a pot containing warm water to precondition it for the measurement of hemoglobin saturation with a good pulse signal. The subject was then connected to the cardiac telemetry and ergospirometry systems for the purpose of recording resting heart rate values (HR) and respiratory variables for 5 min. Subsequently, the participant, blinded to the experimental condition, began breathing the respective gas from a Douglas bag via a two-way Hans–Rudolph breathing valve. The Douglas bag was continuously filled either with room air (FIO_2 0.21), using a pump, or with hypoxic gas (FIO_2 0.14; i.e., normobaric hypoxia), using a hose connected to a tank with 14% O_2 in N_2 ; the hose contained a small quantity of water to humidify the hypoxic gas passing through it. The pulse oximeter was then applied on the index finger of the preheated hand, and new resting values for all variables were recorded for another 5 min in the normoxic condition and for as long as the SaO_2 value dropped below 94% in the hypoxic condition. The incremental test was performed on an electrically braked cycle ergometer (Lode, Groningen, Holland) at a cadence of 60 rpm. The initial workload was 30 watts, which was increased by 15 watts/min until exhaustion. Criteria for terminating the test were: (a) plateau in VO_2 , (b) $\text{HR} \geq 90\%$ of age-predicted HR_{max} , (c) respiratory exchange ratio (RER) > 1.15 , and (d) inability to continue exercise. Heart rate, SaO_2 , and respiratory variables were continuously recorded during the exercise test. Capillary blood sampling was repeated at the last minute of exercise to determine Hb and Hct, and at the 5th minute of recovery to measure blood lactate.

2.4. Measurements and Calculations

Hemoglobin concentration was measured using the photometric cyanmethemoglobin method at a wavelength of 560 nm (Dr. Lange miniphotometer LP2, Oststeinbek, Germany) using 10 μL samples of capillary blood and specific reagents. Duplicate capillary blood samples, collected in 75 μL capillary tubes, were centrifuged for 4 min at 11,500 rpm (Micro Haematocrit Mk5 Centrifuge, Hawksley, UK), and Hct values were obtained via a Haematocrit Reader (Hawksley Inc., Hawksley, UK). Blood lactate was determined enzymatically with reflectance photometry at a wavelength of 660 nm using a drop of capillary blood taken from a fingertip and applied to lactate test strips (BM-Lactate Accusport Analyzer, Roche, Indianapolis, IN, USA). Oxygen saturation was monitored with a pulse oximeter attached to the index finger (Nellcor Symphony N-3000-I10, Minneapolis, MN, USA). Arterial oxygen content was calculated as $\text{CaO}_2 = [\text{Hb}] \times 1.34 \times \text{SaO}_2$. Heart rate was measured using a telemetry system (Sport Tester, PE3000, Vantaa, Finland). A metabolic cart (CPX-D MedGraphics Max Series II, Saint Paul, MN, USA), calibrated before each test with a known air volume and two different gas mixtures, was used for the measurement of the respiratory variables (oxygen uptake, VO_2 ; carbon dioxide production, VCO_2 ; respiratory exchange ratio, RER; minute ventilation, VE ; respiratory rate, RR; tidal volume, VT; end-tidal oxygen tension, PETO_2 ; end-tidal carbon dioxide tension, PETCO_2). PETO_2 and PETCO_2 were used as indices of hyperventilation. The ventilatory equivalents for oxygen and carbon dioxide (VE/VO_2 and VE/VCO_2) were calculated and used as indices of ventilatory efficiency. The oxygen pulse,

which is considered an indication of stroke volume, was calculated by dividing VO_2 by HR. The maximal workload attained was calculated as $W_{\text{max}} = \text{LW} + (\text{Tf} * \text{W})$, where LW is the workload at the last completed minute of the incremental test, Tf is the exercise time at the last stage of the incremental test, and W is the workload increment per minute [20].

2.5. Statistical Analysis

To compare baseline differences between groups, *t*-tests for independent samples were performed. A two-way ANOVA (group \times breathing condition) with repeated measures on the second factor was performed to separately evaluate the resting and exercise responses obtained at three exercise intensities (relative to the specific condition's VO_2 max): 40% of VO_2 max (Submax₁), 80% of VO_2 max (Sub max₂), and maximal exercise (Max). Post hoc comparisons were conducted using Tukey's test, and the level of significance was set at $p \leq 0.05$. Values are reported as means \pm sd.

3. Results

3.1. Participants

The participants' characteristics and the hematological data used for the diagnosis of anemia and assignment of the participants to the corresponding study group are presented in Table 1. The two groups of participants were matched ($p > 0.05$) for age, weight, and height, and differed in hematocrit ($p < 0.001$), hemoglobin ($p < 0.001$), and ferritin ($p < 0.05$) concentration.

Table 1. Subjects' characteristics and hematological data (mean \pm sd (range)) used for the formation of the two study groups.

	Control	Anemic
Age (y)	22.1 \pm 3.4 (19–27)	23.4 \pm 4.6 (19–32)
Weight (kg)	59.9 \pm 7 (46–74)	61.8 \pm 6.19 (57–76)
Height (cm)	169.4 \pm 5.3 (159–177)	168.6 \pm 8.4 (160–188)
Hematocrit (%)	40.1 \pm 1.7 (38.3–41.8)	36.1 \pm 0.6 (35.1–36.8) **
Hemoglobin (g/dL)	13.2 \pm 0.7 (12.4–14.2)	10.5 \pm 0.3 (10.0–10.9) **
Ferritin ($\mu\text{g/L}$)	42.5 \pm 28.5 (18–90)	7.8 \pm 2.4 (5–11) *

Significantly different from control * ($p < 0.05$), ** ($p < 0.001$).

3.2. Hematological Variables

Regardless of the group and breathing condition, exercise increased Hct and Hb ($p < 0.001$) (Tables 2 and 3). Hypoxia reduced SaO_2 ($p < 0.001$) similarly in C and A at rest (Table 2) and throughout exercise (Table 3). Arterial oxygen content was affected both by the Hb level ($p < 0.001$) and hypoxia ($p < 0.01$) at rest, and increased from rest to maximal exercise ($p < 0.001$) in normoxia. It was lower in A than in C at rest ($p < 0.001$) and at maximal exercise ($p < 0.05$) under normoxia, and was decreased by hypoxia in the C group, both at rest ($p < 0.01$) and at maximal exercise ($p < 0.05$) (Figure 1 and Table 2).

3.3. Respiratory Variables

The resting RR was higher ($p = 0.05$) in A than in C under normoxia (Table 2). Resting VE was increased ($p < 0.05$) in all participants under hypoxia (Table 2), whereas maximal VE was not affected either by the level of Hb or by hypoxia (Figure 2a). At both submaximal exercise intensities, VE was increased ($p < 0.05$) by hypoxia only in the control group (Figure 2a). VO_2 was reduced by hypoxia in both groups at rest ($p < 0.05$) (Table 2) and during exercise (Table 3). The drop was more pronounced in the A ($p < 0.001$) than the C group ($p < 0.05$) at maximal intensity, and, consequently, in the two relative submaximal exercise levels as well (Table 3). VE/VO_2 was increased by hypoxia in both groups at rest ($p < 0.005$) (Table 2) and during submaximal exercise (both at 40% and 80% of $\text{VO}_{2\text{max}}$) (Figure 2c). At the maximal intensity, however, it was increased by hypoxia only in the A group ($p < 0.01$) (Figure 2c). In both groups, PETO_2 was suppressed by hypoxia at rest

(Table 2) and throughout exercise ($p < 0.001$) (Table 3). VCO_2 was affected by hypoxia only at the higher exercise intensities. The A group exhibited decreased VCO_2 at 80% $\text{VO}_{2\text{max}}$ ($p < 0.001$) and at maximal exercise ($p < 0.001$), whereas the C group showed this effect only at maximal exercise ($p < 0.05$) (Table 3). VE/VCO_2 was increased by hypoxia only in the control group at rest ($p < 0.05$) (Table 2); in both groups during submaximal exercise ($p < 0.001$), with the A group exhibiting higher values than C at 40% $\text{VO}_{2\text{max}}$ ($p < 0.05$); and only in the A group at maximal intensity ($p < 0.001$) (Figure 2d). PETCO_2 was reduced by hypoxia during exercise in both groups at 80% $\text{VO}_{2\text{max}}$ ($p < 0.001$), but only in A at 40% $\text{VO}_{2\text{max}}$ and at Max ($p < 0.01$) (Figure 2b).

Table 2. Resting values (mean \pm sd) for physiological variables in the two study groups (control, $n = 8$; anemic, $n = 8$) under normoxia (FIO_2 0.21) and hypoxia (FIO_2 0.14).

	Control		Anemic	
	Normoxia	Hypoxia	Normoxia	Hypoxia
Hb (g/dL)	13.0 \pm 1.1	13.0 \pm 1.2	10.9 \pm 0.2 #	11.1 \pm 0.4 #
Hct (%)	40.5 \pm 1.8	41.3 \pm 2.1	36.1 \pm 1.2 #	36.3 \pm 1.2 #
SaO ₂ (%)	99 \pm 1	93 \pm 1 ¥	99 \pm 1	93 \pm 2 ¥
CaO ₂ (mL/dL)	17.3 \pm 1.4	16.2 \pm 1.5 ‡	14.5 \pm 0.3 #	13.8 \pm 0.5 #
VE (L/min)	7.9 \pm 1.34	9.2 \pm 1.80	9.1 \pm 1.69	10.2 \pm 1.24
RR	16 \pm 2	18 \pm 4	19 \pm 2 *	19 \pm 3
VT (mL)	689 \pm 82	680 \pm 87	660 \pm 87	722 \pm 88
VO ₂ (mL/kg/min)	5.43 \pm 0.87	4.22 \pm 0.83 †	5.32 \pm 0.78	4.51 \pm 0.73 †
VE/VO ₂	31.5 \pm 5.24	48.8 \pm 6.41 †	36.1 \pm 4.43	49.5 \pm 4.74 †
VCO ₂ (mL/min)	264 \pm 32	254 \pm 48	277 \pm 42	285 \pm 30
VE/VCO ₂	39.0 \pm 6.19	46.5 \pm 4.42 †	41.8 \pm 5.07	44.8 \pm 5.82
PET _O ₂ (mmHg)	104 \pm 7	69 \pm 7 ¥	112 \pm 5	74 \pm 10 ¥
PET _{CO} ₂ (mmHg)	40 \pm 4	38 \pm 3	39 \pm 3	39 \pm 3
HR (bpm)	76 \pm 5	83 \pm 9 †	83 \pm 9	87 \pm 9 †
O ₂ pulse (mL/beat)	4.23 \pm 0.62	3.07 \pm 0.92 ‡	3.90 \pm 0.66	3.20 \pm 0.53
Lactate (mmol/L)	1.76 \pm 1.19	1.27 \pm 0.36	1.55 \pm 0.81	2.00 \pm 1.57

* $p < 0.05$, # $p < 0.001$: different from control in the same condition; † $p < 0.05$, ‡ $p < 0.01$, ¥ $p < 0.001$: different from normoxia in the same group.

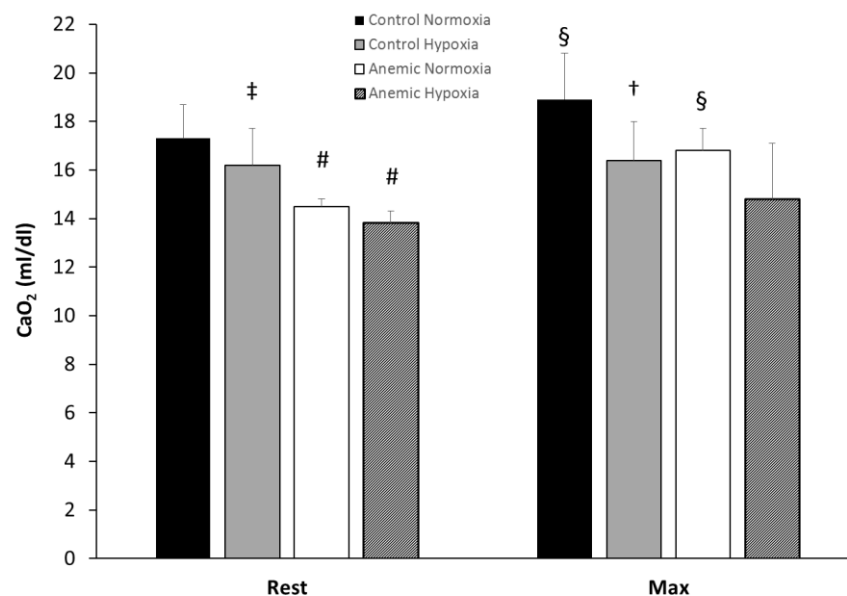


Figure 1. Mean (\pm sd) values of arterial oxygen content (CaO_2) at rest and at maximal exercise in the two study groups (control, $n = 8$; anemic, $n = 8$) under normoxia (FIO_2 0.21) and hypoxia (FIO_2 0.14). # $p < 0.001$: different from control in the same condition; † $p < 0.05$, ‡ $p < 0.01$: different from normoxia in the same group; § $p < 0.001$: different from rest in the same group and condition.

Table 3. Physiological and performance variables in the two study groups (control, $n = 8$; anemic, $n = 8$) at submaximal (Submax 1: at 40% of the condition's VO_2 max; Sub max2: at 80% of the condition's VO_2 max) and maximal (Max) exercise under normoxia (FIO_2 0.21) and hypoxia (FIO_2 0.14).

	Normoxia			Hypoxia		
	Sub _{max1} 40% $\text{VO}_{2\text{max}}$	Sub _{max2} 80% $\text{VO}_{2\text{max}}$	Max	Sub _{max1} 40% $\text{VO}_{2\text{max}}$	Sub _{max2} 80% $\text{VO}_{2\text{max}}$	Max
Control						
Hb (g/dL)	- *	- *	14.5 ± 1.6	- *	- *	14.7 ± 1.4
Hct (%)	- *	- *	43.6 ± 2.1	- *	- *	44.6 ± 2.5
SaO ₂ (%)	99 ± 1	98 ± 1	97 ± 1	89 ± 2 ¥	84 ± 3 ¥	83 ± 3 ¥
RR	24 ± 5	34 ± 3	50 ± 7	25 ± 4	36 ± 3	48 ± 8
VT (mL)	1056 ± 330	1680 ± 160	1821 ± 335	1163 ± 275	1815 ± 110	1926 ± 180
VO_2 (mL/kg/min)	16.3 ± 1.5	32.5 ± 2.9	40.8 ± 3.6	14.7 ± 1.6 †	29.2 ± 3.4 †	36.7 ± 4.1 †
VCO_2 (mL/min)	806 ± 197	2101 ± 359	2981 ± 377	813 ± 167	2037 ± 295	2706 ± 319 †
PET _{O2} (mmHg)	99 ± 8	107 ± 8	116 ± 6	56 ± 6 ¥	64 ± 5 ¥	72 ± 6 ¥
HR (bpm)	118 ± 10	170 ± 8	185 ± 8	127 ± 5 †	170 ± 7	183 ± 5
Power output (Watts)	59 ± 18	143 ± 26	183 ± 27	52 ± 11	133 ± 26	159 ± 18
Exercise time (s)	-	-	706 ± 107	-	-	610 ± 77 †
Lactate (mmol/L)			11.67 ± 2.61			10.70 ± 1.83
Anemic						
Hb (g/dL)	- *	- *	13.1 ± 0.7 #	- *	- *	13.4 ± 1.3 #
Hct (%)	- *	- *	39.4 ± 1.3 #	- *	- *	39.6 ± 1.4 #
SaO ₂ (%)	99 ± 1	98 ± 1	96 ± 1	92 ± 1	84 ± 4	82 ± 4
RR	26 ± 8	34 ± 3	52 ± 11	25 ± 8	36 ± 3	54 ± 13
VT (mL)	1091 ± 212	1710 ± 102	1762 ± 296	1147 ± 312	1700 ± 48	1745 ± 294
VO_2 (mL/kg/min)	15.4 ± 2.1	30.6 ± 4.4	38.2 ± 5.3	12.5 ± 1.6 † #	24.9 ± 3.1 † #	31.1 ± 3.9 † #
VCO_2 (mL/min)	803 ± 187	2217 ± 415	3043 ± 550	716 ± 85	1835 ± 267 ¥	2485 ± 331 ¥
PET _{O2} (mmHg)	104 ± 12	111 ± 9	120 ± 7	60 ± 8 ¥	66 ± 5 ¥	73 ± 3 ¥
HR (bpm)	122 ± 10	169 ± 8	184 ± 7	125 ± 9 †	167 ± 7	180 ± 5
Power output (Watts)	53 ± 23	142 ± 39	174 ± 43	41 ± 12	120 ± 23	151 ± 35
Exercise time (s)	-	-	666 ± 179	-	-	579 ± 123 †
Lactate (mmol/L)			11.01 ± 1.22			12.69 ± 1.46

$p < 0.001$: different from control in the same condition; † $p < 0.05$, ¥ $p < 0.001$: different from normoxia in the same group. * Blood samples were not collected during exercise at 40% VO_2 max or 80% VO_2 max.

3.4. Cardiovascular Variables

There was an increase in HR ($p < 0.05$) under hypoxia in the two groups at rest (Table 2) and at 40% $\text{VO}_{2\text{max}}$, but not at 80% $\text{VO}_{2\text{max}}$ or Max (Table 3). Oxygen pulse was reduced by hypoxia at rest only in the C group ($p < 0.01$) (Table 2), and at 40% VO_2 max in both groups ($p < 0.001$) (Figure 3). However, at 80% $\text{VO}_{2\text{max}}$ and maximal exercise, the drop was greater in A ($p < 0.001$) than in C ($p < 0.05$), resulting in lower maximal O_2 pulse values in A than in C under hypoxia ($p < 0.05$) (Figure 3).

3.5. Maximal Work Variables

The resting values of lactate were similar under normoxia and hypoxia in both groups (Table 2). After maximal exercise, lactate values increased under hypoxia only in A ($p < 0.05$), resulting in higher maximal lactate values in the A compared to the C group ($p < 0.05$) (Figure 4a). Power output was reduced with hypoxia at 80% VO_2 max only in the A group ($p < 0.05$), but at maximal effort, power output was reduced ($p < 0.05$) in both groups (Figure 4b). Exercise time was reduced ($p < 0.05$) by hypoxia similarly in the two groups (Table 3).

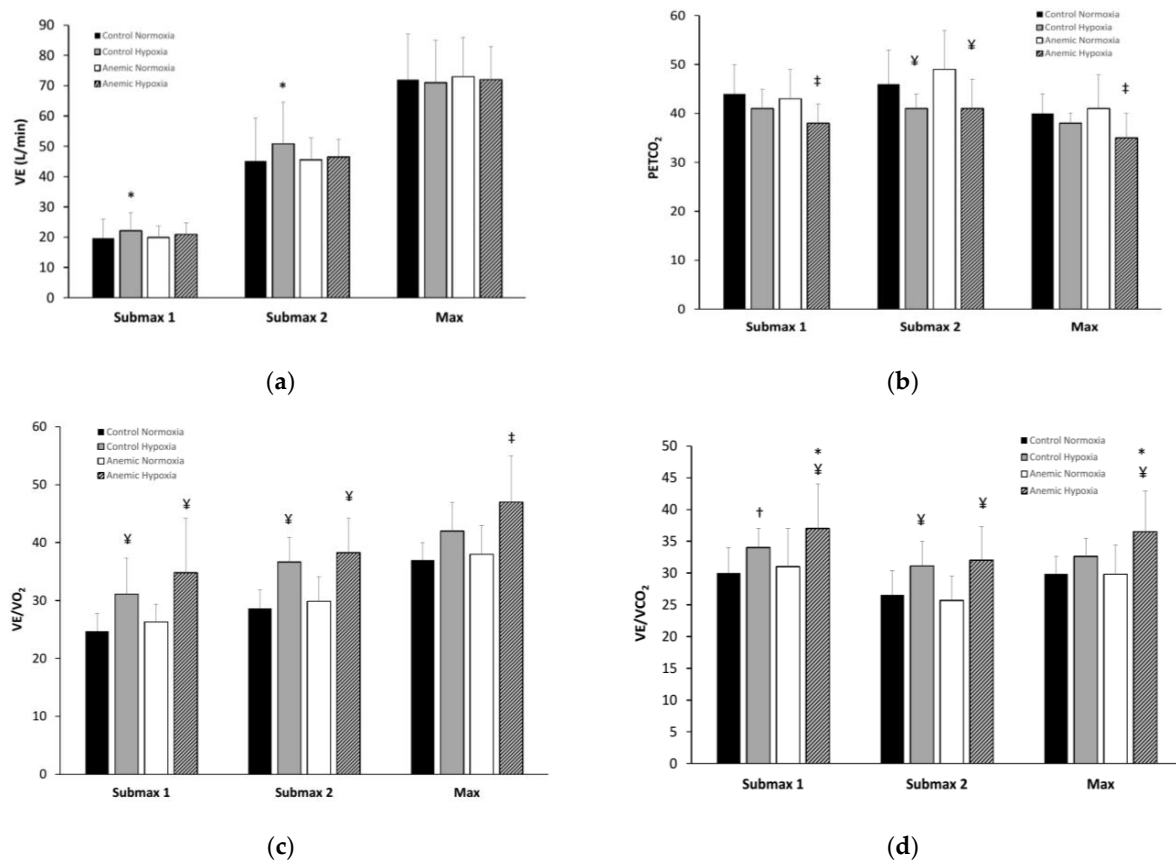


Figure 2. Mean (\pm sd) values of ventilatory variables ((a) VE, (b) PETCO₂, (c) VE/VO₂, and (d) VE/VCO₂) in the two study groups (control, $n = 8$; anemic, $n = 8$) at submaximal (Sub max1: at 40% of the condition's VO₂ max; Sub max2: at 80% of the condition's VO₂ max) and maximal (Max) exercise under normoxia (FIO₂ 0.21) and hypoxia (FIO₂ 0.14). * $p < 0.05$: different from control in the same condition; † $p < 0.05$, ‡ $p < 0.01$, ¥ $p < 0.001$: different from normoxia in the same group.

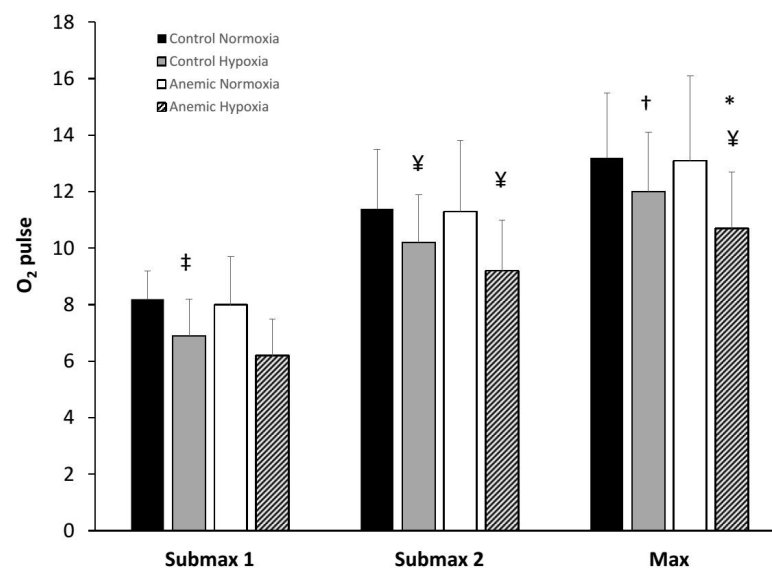


Figure 3. Mean (\pm sd) O₂ pulse in the two study groups (control, $n = 8$; anemic, $n = 8$) at submaximal (Sub max1: at 40% of the condition's VO₂ max; Sub max2: at 80% of the condition's VO₂ max) and maximal (Max) exercise under normoxia (FIO₂ 0.21) and hypoxia (FIO₂ 0.14). * $p < 0.05$: different from control in the same condition; † $p < 0.05$, ‡ $p < 0.01$, ¥ $p < 0.001$: different from normoxia in the same group.

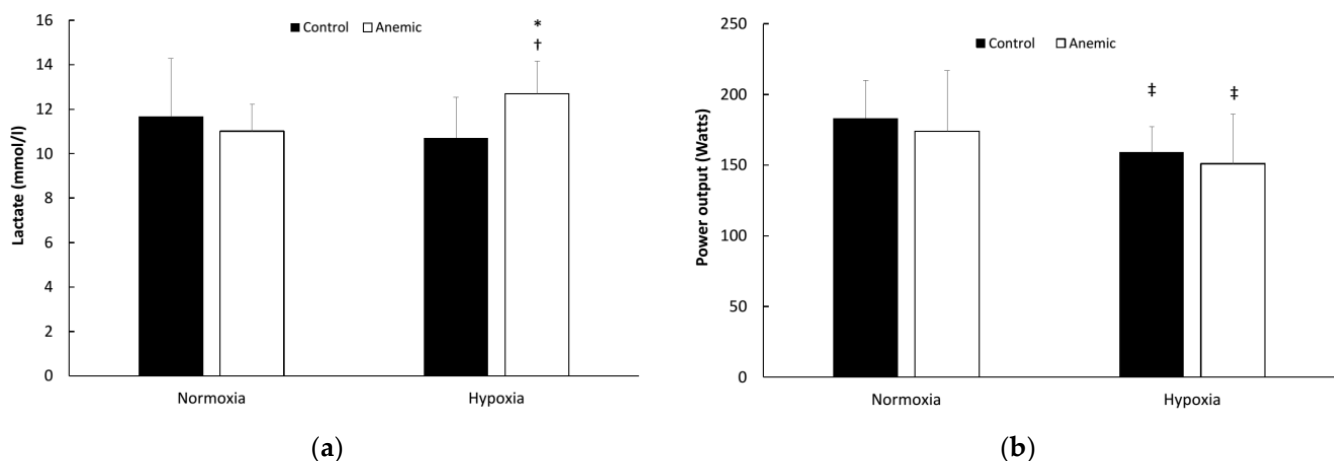


Figure 4. Mean (\pm sd) values of (a) lactate after maximal exercise and (b) power output produced at maximal effort in the two study groups (control, $n = 8$; anemic, $n = 8$) under normoxia (FIO_2 0.21) and hypoxia (FIO_2 0.14). * $p < 0.05$: different from control in the same condition; † $p < 0.05$, ‡ $p < 0.01$: different from normoxia in the same group.

4. Discussion

In this study, females with mild iron deficiency anemia were studied under normoxic and hypoxic conditions and compared to matched controls, at rest as well as at submaximal and maximal intensities of whole-body exercise. Despite the similar degrees of arterial hemoglobin desaturation induced by hypoxia at rest and during exercise, the arterial oxygen content differed between the two groups, seemingly exerting a detrimental effect on some, but not all, physiological responses of the anemic individuals.

There is evidence that iron deficiency anemia impairs aerobic work capacity [21]. Even small reductions in Hb may result in decreased performance during maximal or near-maximal exercise [22,23]. In the present study, a ~16% difference in CaO_2 between the anemic participants and the controls was associated with inter-group differences of 6.5% in VO_{2max} and 5% in power output produced during maximal cycling exercise under normoxia.

Hypoxia-induced reductions in arterial partial pressure of oxygen, and, thus, in hemoglobin SaO_2 , are aggravated during exercise because of a diffusion limitation attributed mainly to lower alveolar PO_2 and enhanced pulmonary blood flow [4,24]. The hypoxic stimulus employed in the present study (i.e., FIO_2 0.14, corresponding to an altitude of ~3300 m) reduced SaO_2 at the point of exhaustion to a value of 83% in the controls and 82% in the anemic participants. These values appear to correspond well with those (SaO_2 : ~83%) reported previously, in which untrained individuals exercised at 3050 m [25].

The hypoxic increase in HR at the two higher exercise intensities was similar in the two groups. There appeared to be considerable inter-individual variation in the HR response during exercise at or above the anaerobic threshold under FIO_2 0.15 hypoxia [26]. Moreover, it has been suggested that any hypoxia-related reduction in maximal HR may require hours of exposure [27]. It is worth mentioning, however, that the significant differences observed in HR at an absolute submaximal workload disappear when data are expressed at similar relative workloads [28], as in the present study. Regarding the oxygen pulse, used as an index of stroke volume, it was reduced with hypoxia to a similar extent in the two groups during submaximal exercise, but was more profoundly reduced in anemic women at the point of exhaustion. This data complements previous observations of decreased oxygen pulse in subjects with low Hb [8,29]. The amount of oxygen delivery to be shared by tissues other than the active muscles, i.e., the myocardium, the respiratory muscles, and the brain, is much lower during exercise involving large muscle mass [4]. Thus, the likelihood of an imbalance between oxygen demand and delivery in these critical regions of the anemic individuals cycling under hypoxia is high; thus, their

myocardial muscles were presumably less able than those of the controls to respond to severe stress. In addition, in light of the similar HR and SaO₂ values observed between groups engaging in maximal hypoxic exercise, we could assume that keeping maximal cardiac output at low levels would serve as an advantage for anemic individuals in terms of maintaining SaO₂. This is due to the avoidance of a diffusion limitation in the lungs in a condition of maximal whole-body exercise under hypoxia, during which the limits of the human pulmonary system may be exceeded [1].

Under normoxia, the anemic group exhibited higher RR, but similar VT compared to the controls, a finding which was also reported in an early study with participants suffering from more severe anemia [30]. At submaximal exercise intensities, the hypoxic stimulus enhanced VE by ~12% in the control individuals. It is noteworthy, however, that, no such hypoxia-related increase in ventilation was noted in the anemic group. A similar blunting of exercise ventilation has previously been observed in anemic individuals while exercising with less muscle mass (i.e., two-leg knee extension) under more severe hypoxic circumstances (FIO₂ 0.11) [14]. At maximal exercise, hypoxia did not modify the exercise ventilatory responsiveness of the control group. In the anemic participants, however, it augmented VE/VO₂ and VE/VCO₂ and attenuated PETCO₂ (as indicated by the within-group comparisons in Figure 2b–d). These differential ventilatory responses might be associated with the higher post-exercise lactate values in the anemic group, conceivably inducing an increased stimulation of peripheral chemoreceptors. The lower CaO₂ in the anemic individuals might have decreased the diffusion gradient of oxygen from the blood to the mitochondria more rapidly, leading to early anaerobic metabolism, as a critically low PO₂ could be reached at a reduced VO₂ [31]. Notably, the two experimental groups in the present study exhibited similar hypoxia-induced drops (~13%) in maximal power output and exercise time, despite greater reductions in VO₂ being observed in anemic participants (~19%) than in the controls (~10%). This suggests that the anemic group could sustain maximal effort under hypoxia with greater reliance on anaerobic metabolism.

In chronically anemic individuals, there is a compensatory rise in 2,3-DPG in proportion to the severity of anemia, which facilitates oxygen delivery to the tissues [13,30] and explains the exercise tolerance and lack of symptoms often experienced by patients with mild anemia [32]. Of interest, however, is that in a recent study [33] performed in nonanemic cyclists, the 2,3-DPG concentration appeared to be reduced after intense exercise in moderate (FIO₂ = 16.5%) hypoxia primarily due to exercise-induced acidosis, as was indicated by the higher (by 1.71 mmol/L) lactate concentration in hypoxia than in normoxia. In the present study, a similar inter-condition magnitude of difference (i.e., 1.68 mmol/L) in lactate was observed in the anemic group. We, therefore, speculate that the role of 2,3-DPG was conceivably limited, if even present, during maximal hypoxic exercise for the mildly anemic individuals participating in the present study.

In anemia, reduced buffering capacity can be expected to be associated with the decreased hemoglobin mass. However, the significant increase in the arteriovenous oxygen difference during exercise with anemia results in maximal operation of the Haldane effect, allowing for unimpeded removal of carbon dioxide from the tissues of the anemic. During vigorous exercise, however, when the supply of oxygen to working muscle cells is sharply limited despite maximal utilization of all available hemodynamic adjustments, the production of CO₂ is also curtailed [30]. In the present study, the anemic group had decreased VCO₂ in hypoxia already at 80% VO_{2max}, and at maximal exercise, they exhibited a more profound drop in VCO₂ than the control group did.

Iron deficiency, even without any anemia being apparent, can cause a decrease in aerobic metabolism since many enzymes involved in energy production through the oxidative processes, such as the mitochondrial cytochromes, rely on iron content. There were no measurements of oxygen utilization in the muscle tissues in the present study, but since our anemic participants were diagnosed as iron-deficient anemic according to the criteria set by the World Health Organization (ferritin < 15 µg/L; Hb < 12 g/dL), it is reasonable to assume that they had severe limitations in increasing oxygen extraction

during high-intensity exercise under hypoxia. This, however, needs to be confirmed by direct studies.

Red blood cells can cause an NO-dependent increase in local blood flow by releasing ATP, which stimulates endothelial NO formation [34,35]. This effect seems to be enhanced when exercise is performed under hypoxia, since the erythrocyte releases ATP in proportion to the offloading of O₂ from hemoglobin, and the greater venous deoxygenation in hypoxia would result in a greater release of ATP [36]. There are no measurements to support the involvement of this mechanism in the present study, but it may have contributed, to some extent, to the compensatory effort for oxygen transport, maintaining oxygen delivery at rest and mild exercise, but not at maximal effort, where performance was decreased with decreasing CaO₂.

In conditions with limited oxygen availability, several of the mechanisms discussed above might contribute to optimizing oxygen transport at rest, during submaximal exercise, or even at peak effort with small muscle mass. However, they are insufficient at high exercise intensities performed with greater muscle mass, resulting in decreases in VO₂ and exercise capacity along with decreasing levels of arterial oxygen [3,12].

In summary, this study showed that during incremental exercise using large muscle mass, women with iron deficiency anemia presented dissimilar physiological responses compared to the matched controls. At high exercise intensities (>80% VO_{2max}), particularly, the two groups differed in the degree and type of compensatory responses occurring to offset the shortfall in oxygen availability induced by hypoxia as well as to avoid extreme arterial oxygen desaturation. The anemic women exhibited similar heart rate response, but lower oxygen pulse, than their control counterparts, in addition to a larger drop in maximal oxygen uptake. However, the impairment of the power output and exercise time of the anemic participants was less pronounced, as they sustained maximal effort by recruiting the anaerobic metabolism to a larger extent. This, in turn, stimulated a greater ventilatory response. It appears that iron deficiency anemia of mild severity, as is commonly observed in the population of young, athletic females, impacts physiological responses during exercise in the presence of moderate hypoxia.

Author Contributions: Conceptualization, M.D.K., S.K., M.E.K. and N.G.; methodology, M.D.K., S.K., M.E.K. and N.G.; analysis, M.D.K., S.K. and N.G.; investigation, M.D.K., S.K., E.Z., O.G., M.E.K. and N.G.; data curation, M.D.K., S.K., E.Z., O.G., M.E.K. and N.G.; writing—original draft preparation, M.D.K., S.K., M.E.K. and N.G.; writing—review and editing, M.D.K., S.K., M.E.K. and N.G.; funding acquisition, S.K. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by Hellenic General Secretariat for Research and Technology, the European Union and the Research Council of the University of Athens.

Institutional Review Board Statement: The procedures employed in the present study were in accordance with the Declaration of Helsinki and had the approval of the School of Physical Education and Sport Science Ethics Committee for human experimentation.

Data Availability Statement: The data are not publicly available due to privacy and ethical concerns.

Conflicts of Interest: The authors declare no conflict of interest.

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