

Hematological status and endurance performance predictors after low altitude training supported by normobaric hypoxia: a double-blind, placebo controlled study

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ABSTRACT: The benefits of altitude/hypoxic training for sea level performance are still under debate. This study examined the effects of low altitude training supported by normobaric hypoxia on hematological status and endurance performance predictors in elite female cyclists. Twenty-two female cyclists trained for 3 weeks at low altitude (<1100 m) and 2 weeks near sea level. During the first 3 weeks, 15 subjects stayed in hypoxic rooms simulating an altitude of 2200 m (+NH group, $n = 8$) or 1000 m (placebo group, $n = 7$), and 7 (control group) stayed in regular rooms. Significant increases in total hemoglobin mass (tHb-mass: $p = 0.008$, $p = 0.025$), power at 4 mmol·l⁻¹ lactate (PAT4: $p = 0.004$, $p = 0.005$) (in absolute and relative values, respectively) and maximal power (PF: $p = 0.034$) (in absolute values) were observed. However, these effects were not associated with normobaric hypoxia. Changes in tHb-mass were not associated with initial concentrations of ferritin or transferrin receptor, whereas changes in relative tHb-mass ($r = -0.53$, $p = 0.012$), PF ($r = -0.53$, $p = 0.01$) and PAT4 ($r = -0.65$, $p = 0.001$) were inversely correlated with initial values. Changes in tHb-mass and PAT4 were positively correlated ($r = 0.50$, $p = 0.017$; $r = 0.47$, $p = 0.028$). Regardless of normobaric hypoxia application, low altitude training followed by sea-level training might improve hematological status in elite female cyclists, especially with relatively low initial values of tHb-mass, which could translate into enhanced endurance performance.

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INTRODUCTION

Maximal and peak oxygen uptake, peak power output and power output at lactate threshold are the main endurance performance predictors (EPP) in cycling; therefore, these indices are commonly used to assess training effectiveness in both track and road cyclists [1]. Since indices of endurance performance are well correlated with total hemoglobin mass (tHb-mass) [2–4], many endurance athletes, including cyclists, regularly stay and train in the mountains, or make use of artificial hypoxia to replicate altitude, as a way to achieve improvements in hematological status and thus sea-level performance.

Exposure to a natural or simulated altitude of 2100–2500 m for 3 weeks (>12 h daily), is considered the minimum dose to attain a hematological acclimatization effect [5]. However, some authors argue that this altitude range is too low to stimulate significant

erythropoietic adaptation [6,7]. On the other hand, significant erythropoietic effects were observed in athletes exposed to much lower than recommended altitudes, both natural [8–10] and simulated [11,12]. Significant erythropoietic effects were also observed after endurance training conducted at sea level, but only in previously untrained individuals or recreational athletes [13–15]. It does appear that changes in hematological variables after exposure to hypoxia depend, in part, on their initial level [6,16,17]. Additionally, a meta-analysis suggested that positive effects of natural or artificial hypoxia may be associated with the placebo effect [18], a topic few studies have examined to date [19,20].

Another crucial question is whether improvements in hematological indices translate into enhancement of physical performance. Most authors, although not all [21], did not find any significant cor-

relations between changes in tHb-mass and changes in VO_2max [12,20,22], maximal performance, or performance at 4 $\text{mmol}\cdot\text{l}^{-1}$ lactate concentration [23]. Nevertheless, others have reported a strong correlation between the changes in tHb-mass and running performance after low altitude training (<1200 m), supported by natural or simulated moderate altitude (2250 m) at rest [11]. Some discrepancies might be explained by a transient impairment in performance, given that about 2 weeks of near-sea level training is necessary to obtain maximal performance gains [10,22,24,25]. Although the benefits of altitude training are still controversial, particularly in elite athletes [16,26], this form of training aid is widely applied in cycling and other endurance-based sports.

These inconsistencies motivated us to conduct a double-blind, placebo-controlled study, which aimed to assess the effects of 3-week low altitude training (<1100 m) supported by normobaric hypoxia at rest (2200 m), followed by 2-week near sea-level training on hematological status and values of EPP in elite female cyclists. We also investigated the relationship between changes in the examined variables relative to their initial values. We hypothesized that additional exposure to moderate normobaric hypoxia would increase training efficiency, especially in athletes with relatively low initial values of tHb-mass and EPP.

MATERIALS AND METHODS

Participants

This study constituted part of the training process supervised by the Polish Cycling Federation. A group of 22 endurance event track and road female cyclists (age 20.5 ± 2.9 years, training experience in cycling 7.3 ± 3.1 years, height 170 ± 5 cm, body mass 60.2 ± 6.6 kg, and body fat $20.7 \pm 3.8\%$) were studied at the beginning of the competitive phase of the annual training cycle. All athletes were members of the national team and included both Olympic Games participants and European Championship medalists. The study was approved by a local Ethics Committee (KEBN-14-2-DS) and performed in accordance with the 1964 Declaration of Helsinki and its later amendments. Prior to signing consent, all subjects were fully informed of the benefits and risks of the investigation. Prior to, during, and after the study they completed detailed medical examinations.

Design

The procedures accompanied the coach-prescribed training of high-level female athletes. To ensure comparable distribution of track and road cyclists, the subjects were semi-randomly assigned to 3 groups: +NH ($n = 8$; 4 track and 4 road cyclists), placebo ($n = 7$; 3 track and 4 road cyclists) and control ($n = 7$; 4 track and 3 road cyclists). During the 3-week training camp, the first two groups trained at altitudes of 700–1100 m and were accommodated in hypoxic rooms (with controlled O_2 and CO_2 compositions) in which they were asked to spend at least 13 h a day. The oxygen concentration in these rooms was 17.5% (+NH group) or 20.4% (placebo group). The hotel was situated at about 900 m altitude, and thus partial oxygen

pressure was, on average, 122 mm Hg (simulated altitude about 2200 m; +NH group) or 141 mm Hg (simulated altitude about 1000 m; placebo group). None of the cyclists had previous experience with normobaric hypoxia (hypoxic rooms or tents); however, in the past (but not less than 8 months preceding the study) they participated in several camps at low and moderate natural altitudes. They all submitted daily report questionnaires about the time spent in their rooms, general wellness and any symptoms of maladaptation. The assignment of subjects to +NH and placebo groups was known only to the supervising physician, dedicated hotel technician, and director from the cycling federation, who was not a coach of the athletes.

The remaining 7 subjects who formed the control group participated in a training camp about 300 km away at a similar altitude (accommodated at 900 m, trained at 700–1050 m). All cyclists were subjected (at about 90 m altitude) to laboratory examinations on two occasions: 2–6 days before (Pre) and 13–15 days after the training camp (Post). Those examinations included health status evaluation, blood analyses, basic anthropometry testing, a graded exercise test on a cycle ergometer, and measurement of total hemoglobin mass. The post-treatment assessment was set according to the coaches' assumptions on maximal performance gains after the altitude training camp.

Procedures

On the night preceding each examination the subjects were accommodated in a dormitory in the laboratory. At 7:30–8:00 a.m. (in a fasted state), following a 15-min rest in a seated position, 2 ml of blood was withdrawn from the antecubital vein for hematological assay using the Advia 120 analyzer (Bayer, Germany). At the first examination (in an additional 2 ml blood sample), the soluble transferrin receptor (sTfR) was assayed by immunoassay (Ramco, USA), and C-reactive protein and ferritin concentrations were measured using a Pentra 400 device (Horiba Medical, France). All cyclists had sTfR, C-reactive protein concentrations and leukocyte counts within normal limits, but 3 subjects from the +NH group and 4 from the placebo group had ferritin concentrations below $35 \text{ ng}\cdot\text{ml}^{-1}$ and therefore throughout the 3 weeks of the training camp were orally supplemented with iron (Chela-Ferr Forte containing 28 mg of ferrous bisglycinate, one tablet daily). No athletes from the control group were iron supplemented at that time.

At least two hours following a light standard breakfast the subjects performed a graded exercise test (GXT), which was preceded by a medical examination by a physician. The cyclists performed the GXT on their own bicycle frame mounted on a Cyclus2 cycle ergometer (Avantronic, Germany). The bicycle chain was set on the largest front sprocket and on the 58 mm diameter sprocket of the ergometer. All subjects were well familiarized with the test, as part of their normal testing procedures. The initial load was $1.5 \text{ W}\cdot\text{kg}^{-1}$ body mass and thereafter increased every 3 min by $0.65 \text{ W}\cdot\text{kg}^{-1}$ until volitional exhaustion (or cycling cadence < 70 rpm). According

to the following formula, maximal power output (PF) was computed employing the time of sustaining the last load applied:

$$PF = P_{pfin} + (t_{fin} * (P_{fin} - P_{pfin})) / 180$$

where: P_{pfin} = power at the pre-final step, P_{fin} = power at the final step, t_{fin} = time of sustaining power at the final step.

During the GXT, oxygen uptake (VO_2) was continuously recorded (MetaMax 3B analyzer; Cortex, Germany) and the highest values for any 1-min period (VO_{2peak}) were determined. Within the last 15 s of every load increment, blood samples (20 μ l) from fingertips were collected for assessing lactate concentration (LA) using the Super GL2 analyzer (Dr. Müller, Germany). Power at fixed blood lactate of 4 $mmol \cdot l^{-1}$ (PAT4), considered as the most reliable criterion of lactate threshold [27], was determined. Pre-Post changes in PF and PAT4 within the ranges $\pm 3.9\%$ and $\pm 4.2\%$, respectively, were considered as trivial, above as positive, and below as negative [28].

At least an hour following the exercise testing, total hemoglobin mass (tHb-mass) was determined by applying an optimized carbon monoxide (CO) rebreathing method, as described elsewhere [29]. All measurements were performed by the same experienced investigators. During the test the administered gas mixture consisted of CO (0.8 $ml \cdot kg^{-1}$ body mass) and 99.5% O_2 . A CO sensor (Pac 7000, Dräger, Germany) was used to check potential leaks. Blood ($\sim 105 \mu$ l) was sampled from hyperaemized earlobes (Finalgon, Boehringer Ingelheim, Germany) in the following order: just before the test (4 samples), and in the 6th (2 samples) and 8th minutes (3 samples) from the beginning of inhalation of the gas mixture (lasting 2 min). The samples served to determine the percentage value of carboxy-hemoglobin (HbCO%) using a CO oximeter (ABL 80 Flex, Radiometer, Denmark). Computer software (Blood Volume Measurements: SpiCO; Blood tec, Bayreuth, Germany) was employed to calculate tHb-mass. The typical error (TE) assessed before the study from averaged duplicate measurements of tHb-mass (24–48 h time lag between tests) was 1.9%; thus, Pre-Post changes in tHb-mass within the range $\pm 1.9\%$ were considered as trivial, above as positive, and below as negative.

Training

Subject training was typical for the competitive phase of the annual training cycle. During the week they performed, on average, 4 to 5 continuous training sessions and 2 interval sessions. Additionally, resistance training sessions at a sports gym (track cyclist) or on the bike with a relatively low cadence and high force (road cyclists) were performed. On the basis of training registration, conducted daily by coaches, the training volume (time) and intensity were controlled. Three intensity zones (Z1 – extensive aerobic endurance, Z2 – intensive aerobic endurance, Z3 – speed/anaerobic endurance) were distinguished. During the observation period, the cyclists raced in 3 competitions (all at an altitude below 360 m). The first competition took place after 11 days of the training camp (athletes of +NH and

placebo groups spent one night outside the hypoxic rooms), the second immediately after the training camp, and the third 11 days after training camp termination. In the first two, all cyclists competed on the road, but in the third they competed according to their specialization (road or track).

Statistical analysis

The results are presented as means with standard deviations (\pm SD) and expressed in absolute values and relative to body mass. A two-way analysis of variance (ANOVA) with repeated measures was applied to analyze the main effects (group, time) and interaction (group x time). Partial eta-squared (η^2_p) effect sizes were determined for all significant main effects revealed by the ANOVA. Values for $\eta^2_p = 0.02$, 0.13, and 0.26 were considered as small, moderate, and large, respectively. Kruskal-Wallis one-way analysis of variance and Welch's test were used to evaluate between-group differences in training load and time spent in hypoxic rooms, respectively. Relationships between variables were tested using Pearson's correlation coefficients. The level of $p < 0.05$ was considered significant. Analyses were performed using Statistica 13.1 (Dell Inc., USA).

RESULTS

No serious health problems (e.g. injuries, severe infections or inflammation) were detected across this study, nor a lack of intolerance to hypoxia or training. Nevertheless, two cyclists (one from the +NH and one from the placebo group) claimed absence of > three consecutive menses. Mean time spent in the hypoxic rooms was not different ($p = 0.140$) for the +NH and placebo groups, recorded at 288 ± 21 h and 276 ± 5 h, respectively. Based on daily report questionnaires submitted by the subjects staying in hypoxic rooms, and from the interviews conducted on the last day of the study, all subjects were convinced they were assigned to the +NH group. However, the technical reports confirmed that the values of hypoxia pre-set for both groups were maintained.

No differences in training volume ($p = 0.824$) or percentage contribution of training in the three intensity zones ($p = 0.771$ – 0.919) were found. Overall training time and percentage distribution of intensity zones were as follows: 74.2 ± 8.4 h, $68 \pm 7\%$ (Z1), $27 \pm 6\%$ (Z2), $5 \pm 1\%$ (Z3); 72.6 ± 7.8 h, $67 \pm 7\%$ (Z1), $28 \pm 6\%$ (Z2), $5 \pm 1\%$ (Z3); 78.9 ± 1.3 h, $65 \pm 2\%$ (Z1), $30 \pm 2\%$ (Z2), $5 \pm 1\%$ (Z3), in +NH, placebo and control groups, respectively.

Total hemoglobin mass and EPP (PF, VO_{2peak} , and PAT4) before (Pre) and after the period of observation (Post) in the three groups of female cyclists are presented in Table 1.

A significant main effect of time was found for tHb-mass ($p = 0.008$, $\eta^2_p = 0.319$; $p = 0.025$, $\eta^2_p = 0.238$) and PAT4 ($p = 0.004$, $\eta^2_p = 0.355$; $p = 0.005$, $\eta^2_p = 0.344$), in absolute and relative values, respectively, as well as for PF ($p = 0.034$, $\eta^2_p = 0.216$) in absolute values only. There was no significant effect of time for VO_{2peak} . For all variables there was no significant group effect and no group x time interaction.

TABLE 1. Total hemoglobin mass and endurance performance predictors before (Pre) and after the period of observation (Post) in the three groups of female cyclists. Values are means \pm SD.

		+NH		Placebo		Control		ANOVA $p =$		
		Pre	Post	Pre	Post	Pre	Post	Group	Time	Interaction
tHb-mass	(g)	677 \pm 104	692 \pm 108	626 \pm 65	646 \pm 61	622 \pm 51	643 \pm 58	0.362	0.008	0.911
tHb-mass	(g·kg ⁻¹)	11.0 \pm 1.1	11.2 \pm 1.0	10.9 \pm 1.7	11.1 \pm 1.3	10.3 \pm 0.8	10.7 \pm 0.9	0.563	0.025	0.839
PF	(W)	291 \pm 21	299 \pm 28	276 \pm 16	292 \pm 21	279 \pm 18	285 \pm 41	0.489	0.034	0.595
PF	(W·kg ⁻¹)	4.75 \pm 0.46	4.86 \pm 0.35	4.79 \pm 0.64	5.03 \pm 0.38	4.63 \pm 0.18	4.69 \pm 0.41	0.472	0.075	0.586
VO ₂ peak	(l·min ⁻¹)	3.63 \pm 0.37	3.69 \pm 0.39	3.44 \pm 0.30	3.58 \pm 0.34	3.57 \pm 0.21	3.57 \pm 0.37	0.683	0.115	0.365
VO ₂ peak	(ml·kg ⁻¹ ·min ⁻¹)	58.9 \pm 3.8	59.7 \pm 3.1	59.5 \pm 5.4	61.6 \pm 5.3	59.2 \pm 2.0	58.9 \pm 4.5	0.741	0.220	0.371
PAT4	(W)	223 \pm 21	229 \pm 20	216 \pm 24	229 \pm 16	208 \pm 19	220 \pm 18	0.449	0.004	0.666
PAT4	(W·kg ⁻¹)	3.64 \pm 0.43	3.73 \pm 0.31	3.75 \pm 0.53	3.96 \pm 0.40	3.46 \pm 0.40	3.65 \pm 0.31	0.361	0.005	0.600

Group: +NH – staying at simulated altitude of 2200 m, placebo – staying at simulated altitude of 1000 m, control – without altitude simulation; tHb-mass – total hemoglobin mass, PF – maximal power output, VO₂peak – peak oxygen consumption, PAT4 – power output at blood lactate of 4 mmol·l⁻¹. Statistically significant p-values are bolded.

Individual percentage changes in absolute values of tHb-mass, PF and PAT4 in the Pre-Post period (iron-supplemented athletes and athletes with amenorrhea are marked) are presented in Figure 1A-C.

Changes in absolute and relative tHb-mass did not differ with respect to iron supplementation (supplementation \times time interaction: $p = 0.792$ and $p = 0.865$) and were not significantly correlated (pooled data) with initial levels of ferritin ($r = 0.119$, $p = 0.599$ and $r = 0.026$, $p = 0.091$) or sTfR ($r = -0.131$, $p = 0.562$ and $r = -0.084$, $p = 0.709$).

In pooled data, changes in relative tHb-mass ($r = -0.53$, $p = 0.012$), PF ($r = -0.53$, $p = 0.01$) and PAT4 ($r = -0.65$, $p = 0.001$) were inversely correlated with the initial values observed at Pre (Figure 2A-C).

Positive correlations between percentage changes in tHb-mass and PAT4 (Figure 3) were found ($r = 0.50$, $p = 0.017$ and $r = 0.47$, $p = 0.028$, in absolute and relative units, respectively). Correlations between percentage changes in tHb-mass and PF ($r = 0.07$, $p = 0.755$ and $r = 0.09$, $p = 0.690$) or VO₂peak ($r = 0.003$, $p = 0.990$ and $r = 0.05$, $p = 0.830$) were not significant.

DISCUSSION

The study results partly confirmed our hypothesis, since the observed increases in tHb-mass, PF and PAT4 were related to their initial

values, but exposure to moderate hypoxia (2200 m) in a total dose of 288 ± 21 hours had no additional effect. Nevertheless, high-level athletes can promote beneficial effects, both hematological and performance, by training even at low altitude, which is consistent with recent studies. For instance, Robach *et al.* [9] investigated differences in changes of aerobic performance and hematological status in well-trained male and female cross-country skiers who spent 26 nights either at low (1035 m) or moderate natural altitude (2207 m), and trained together daily at a common location ranging from 550 to 1500 m (82% of training time < 1100 m). It is worth noting that tHb-mass changes in this work [9] were similar to our study (on days 13–15 after the training camp); $3.0 \pm 4.5\%$, $2.2 \pm 3.6\%$, $3.4 \pm 5.7\%$, and $3.4 \pm 4.6\%$ in all subjects together, and separately in the +NH, placebo, and control group, respectively. In cross-country skiers, sleeping at a moderate altitude also did not boost improvement in VO₂max or time trial performance. Similarly, no significant between-group differences in changes of tHb-mass and performance were reported in triathletes who trained at <1200 m and slept at altitudes of 2250 or 1200 m [11].

In accordance with a previous study [9], we did not find an additional effect of moderate altitude acclimatization on examined variables. Thus, the changes observed herein could reflect a general training effect. This view is supported by Garvican *et al.* [30], who

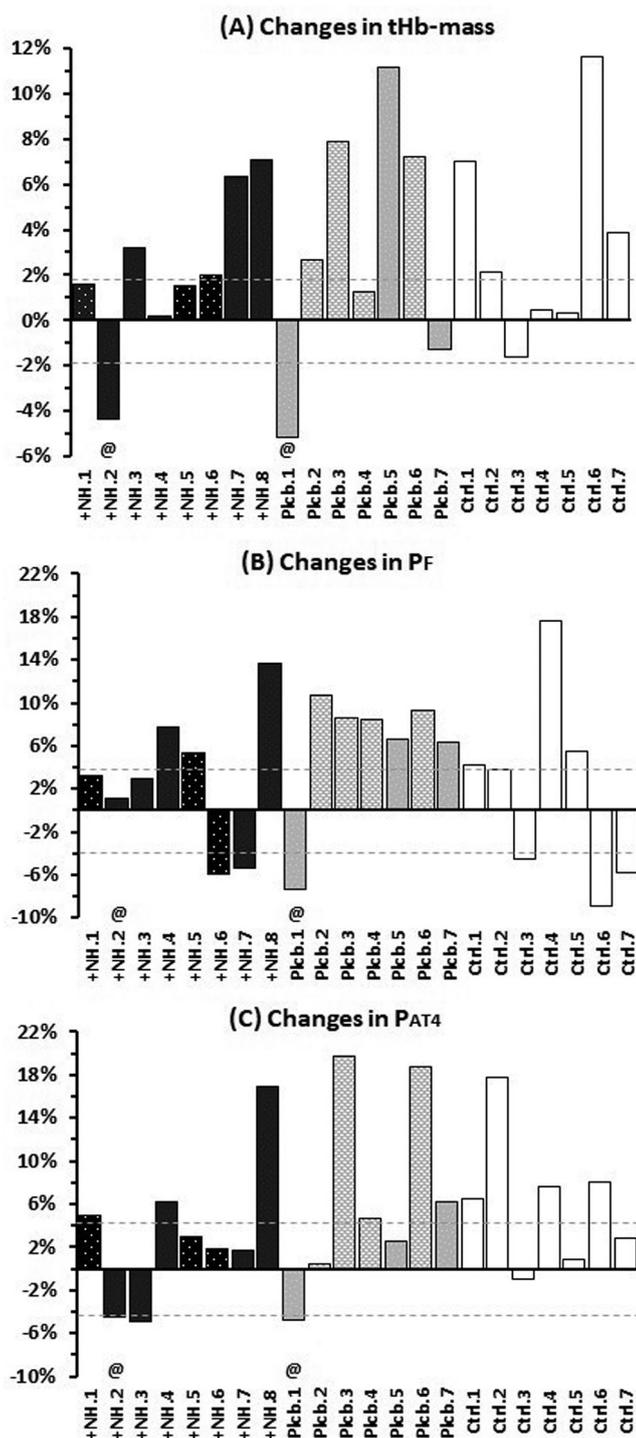


FIG. 1A-C. Percentage changes in absolute values of: (A) – total hemoglobin mass (tHb-mass), (B) – maximal power output (PF), and (C) – power at blood lactate concentration of 4 mmol·l⁻¹ (PAT4) in female cyclists belonging to the +NH (staying at simulated altitude of 2200 m), placebo (Pcb – staying at simulated altitude of 1000 m), and control (Ctrl – without additional altitude simulation) group. Dotted bars – iron supplemented athletes, @ – amenorrhagic athletes. Dotted lines express borders between positive, trivial, and negative changes.

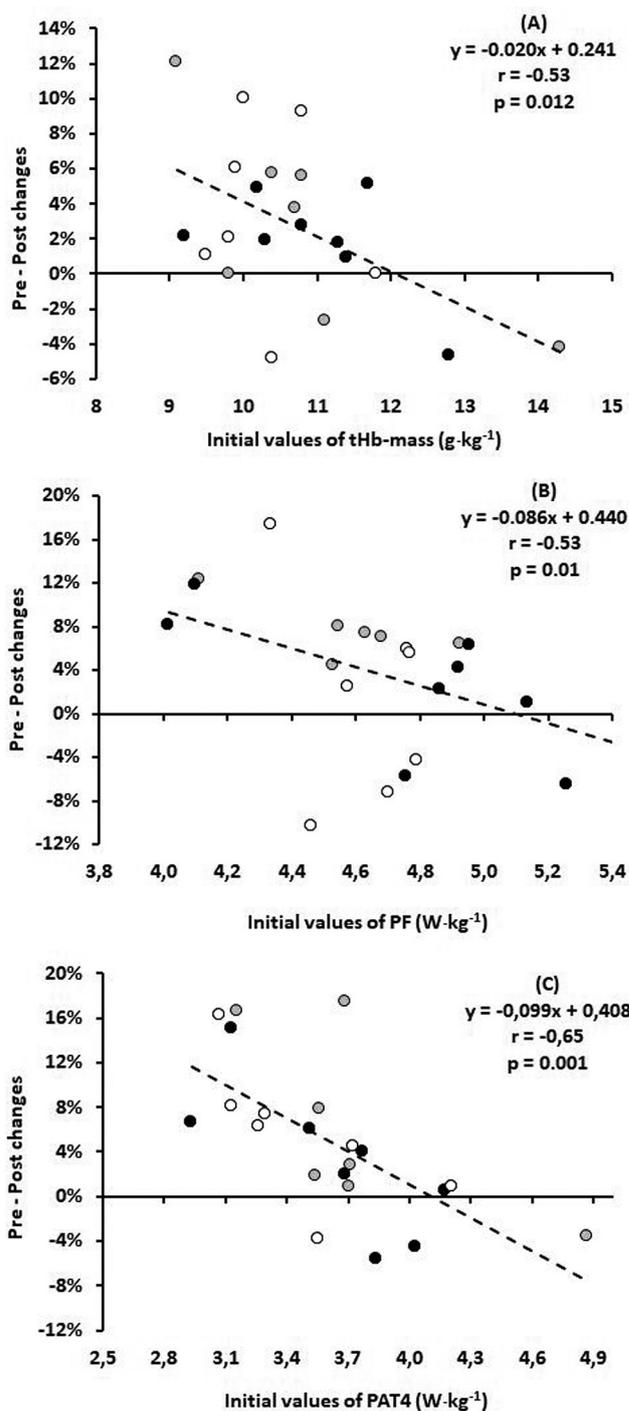


FIG. 2A-C. Percentage changes in relative values of: (A) – total hemoglobin mass (tHb-mass), (B) – maximal power output (PF), and (C) – power at fixed blood lactate concentration of 4 mmol·l⁻¹ (PAT4) in relation to the initial values. Black, grey, and white circles denote athletes from the +NH (staying at simulated altitude of 2200 m), placebo (staying at simulated altitude of 1000 m), and control (without additional altitude simulation) group, respectively.

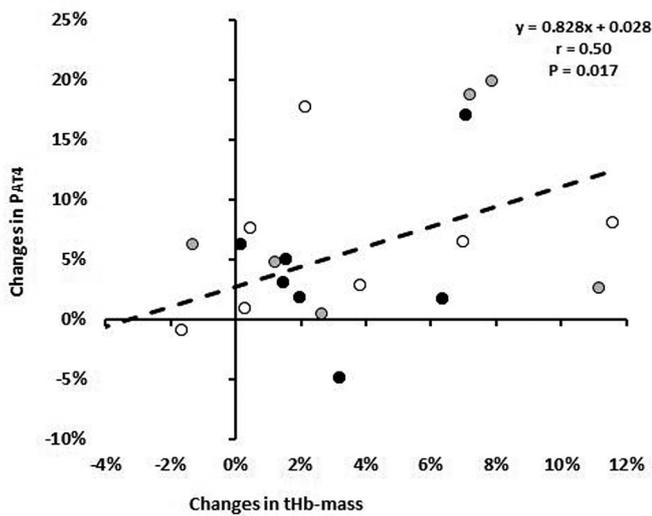


FIG. 3. Relationship between changes in absolute values of total hemoglobin mass (tHb-mass) and power at blood lactate concentration of $4 \text{ mmol}\cdot\text{l}^{-1}$ (PAT4). Black, grey, and white circles denote athletes from the +NH (staying at simulated altitude of 2200 m), placebo (staying at simulated altitude of 1000 m), and control (without additional altitude simulation) group, respectively.

repeatedly measured tHb-mass in elite female cyclists during a competitive season; tHb-mass varied by 3.3% throughout the 6-month period and this was linked to changes in training load. Furthermore, it has been demonstrated that a reduction in training load due to injuries or illness is associated with a significant reduction in tHb-mass [31,32]. These data indicate that the training load is an important stimulus for maintenance of tHb-mass, although another study examining intra-individual variance of tHb-mass in elite athletes during a training year found no significant relationship between changes in training volume and tHb-mass [15].

According to information obtained from our coaches, there were no excessive quantitative differences in training volume during the observation period and in the week preceding the altitude camp. However, the training-camp effect should be considered as a potential factor amplifying the training response in our cyclists. Admittedly, in comparison to the training-intensity distribution reported in elite male cyclists [33,34], the contribution of Z3 in our female cyclists was not particularly high, but Z2 (intensive aerobic endurance exercises) was much higher. Since the altitude camp was held at the beginning of the competitive phase, the physiological adaptations to training were not yet completed, thereby allowing room for additional changes in PAT4 and PF. This viewpoint is also in line with the relatively low initial value for tHb-mass ($10.7 \pm 1.2 \text{ g}\cdot\text{kg}^{-1}$). Many studies [6,16,17] have demonstrated that initial values play a significant role in the magnitude of the hematological response to altitude training, as confirmed by the negative correlation between percentage changes in relative tHb-mass and initial values. Similar associations were observed for EPP, such as PF and PAT4. The nature of these relation-

ships (all negative) are indicative of a biological ceiling effect (i.e. lower/higher responsiveness to training stimuli when initial adaptation is close to/far from the potential maximal).

In this study, we did not find either placebo/nocebo [18] or location effects on the study variables [9,35]. Since most altitude-based studies use only matched control groups, the placebo/nocebo effect of altitude training remains elusive. In one of the few placebo-controlled studies [19], elite race walkers trained for 3 weeks at 600 m altitude and were exposed (14 h/day) to a simulated altitude of 3000 m. Increases in tHb-mass and VO_2peak were observed in the hypoxia-exposed group, but the improvement in treadmill performance did not exceed the training camp effect. In another placebo-controlled study [20], the athletes spent 4 weeks (minimum 16 h/day) in hypoxic rooms flushed with either normal ambient air (altitude 1135 m) or normobaric hypoxia corresponding to an altitude of 3000 m, combined with 4 weeks training at altitude (<1200 m). After the intervention period, tHb-mass remained unchanged in both groups, whereas endurance performance (expressed as average power output during a 26.15 km time trial performed at an altitude of 1020 m) tended to increase in all subjects. However, there was no effect of the normobaric hypoxia intervention on time-trial performance. Similarly, other placebo-controlled studies have reported a lack of an additional effect of hypoxia on performance indices in well-trained endurance athletes [36–38]. Collectively, the cited results indicate that even a more hypoxic dose than that applied in our study might not be sufficient for boosting altitude training effectiveness.

Hematological effects of altitude training could be compromised by insufficient iron stores [39]. However, in our athletes the initial concentrations of sTfR were in the normal range, which may indicate that even those with ferritin below $35 \text{ ng}\cdot\text{ml}^{-1}$ had at most the first stage of iron deficiency. However, it should be emphasized that we did not find an associations between initial values of ferritin or sTfR and changes in tHb-mass. Moreover, changes in tHb-mass did not differ with respect to iron supplementation. It suggests that iron stores in studied subjects were adequate for requirements. A lack of association between pre-altitude camp serum ferritin concentration and changes in tHb-mass has also been reported by others [40,41].

No serious health problems were detected in our athletes during the observation period, but amenorrhea was reported in two athletes. These athletes also showed the worst reaction to training, especially with respect to tHb-mass; being the only athletes who exhibited a negative change. It is also noteworthy that amongst all participants, the same two athletes exhibited two of the lowest initial percentages of body fat (16.3 and 12.4%), which could reflect long lasting energy deficiency and, in turn, might explain menstrual disturbances [42]. Higher risk score for low energy availability in female endurance athletes showed a trend to correlate with less favourable changes in tHb-mass following altitude training [16]. However, relatively high initial values of tHb-mass (12.8 and $14.3 \text{ g}\cdot\text{kg}^{-1}$) – the highest among all our cyclists – may also be the reason for the lack of positive changes in tHb-mass.

The most important issue for scientists, coaches, and athletes considering altitude training is whether improvements in hematological indices translate into enhancement of physical performance. Although tHb-mass was reported to be strongly related to VO_2max [43], only a weak correlation between percent changes in these two indices was found [44]. In our study, no relationship between changes in tHb-mass and VO_2peak was observed, but changes in tHb-mass were significantly correlated with those in PAT4 – an index which may be a better predictor of endurance performance in female cyclists than VO_2peak [45]. A significant correlation between tHb-mass and PAT4 was reported in male and female cyclists [3] and in male rowers [4]. Conversely, in elite junior swimmers there was no significant correlation between changes in tHb-mass and performance at $\text{LA} = 4 \text{ mmol}\cdot\text{l}^{-1}$ after 3 weeks of training at a moderate natural altitude [23]. To our knowledge, this study is the first to show a significant correlation between post-training changes in tHb-mass and PAT4, which might demonstrate a direct contribution of hematological factors to enhancement of endurance abilities after training at low altitude.

In this study we had a unique opportunity to observe effects of training in elite female cyclists. However, studies on elite athletes have inherent restrictions [46], such as an inability to modify training and competition activities. Moreover, because of cyclist participation in actual competitions, we had no opportunity to conduct performance testing. Nevertheless, many studies on cyclists [47,48], including females [45], show strong relationships of time-trial performance with power at lactate thresholds or peak power output during exercise testing. The increases in PAT4 and PF in our cohort suggest a likely improvement in time-trial performance. Although

food intake was not controlled in our study, we can assume the cyclists had proper and comparable diets, because the training camps were supported by qualified sport nutritionists. Also, we had no possibility to inspect the intake of pharmacological substances (e.g. nonsteroidal anti-inflammatory drugs, oral contraceptives). Still, these athletes were regularly subjected to national and international doping control, both in- and out-of-competitions, so we did not anticipate any misuse of banned substances.

CONCLUSIONS

Regardless of the application of normobaric hypoxia ($288 \pm 23 \text{ h}$ during 3 weeks at a simulated altitude of 2200 m), low altitude training followed by near sea-level training might improve hematological status in elite female cyclists, especially those athletes with relatively low initial values of tHb-mass, which could then translate into enhanced endurance performance.

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The authors declare no conflicts of interest.

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