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Hemoglobin mass and performance responses during 4 weeks of normobaric "live high–train low and high"

Oona Kettunen[1] | **Antti Leppävuori**[1] | **Ritva Mikkonen**[1] | **Juha E. Peltonen**[2,3] | **Ari Nummela**[4] | **Bettina Wikström**[1] | **Vesa Linnamo**[1]

[1]Sports Technology Unit, Faculty of Sport and Health Sciences, University of Jyväskylä, Vuokatti, Finland

[2]Helsinki Sports and Exercise Medicine Clinic (HULA), Foundation for Sports and Exercise Medicine, Helsinki, Finland

[3]Department of Sports and Exercise Medicine, Clinicum, University of Helsinki, Helsinki, Finland

[4]Finnish Institute of High Performance Sport KIHU, Jyväskylä, Finland

Correspondence
Oona Kettunen, Sports Technology Unit, Faculty of Sport and Health Sciences, University of Jyväskylä, Vuokatti, Finland.
Email: oona.e.kettunen@jyu.fi

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Purpose: To investigate whether 4 weeks of normobaric "live high–train low and high" (LHTLH) causes different hematological, cardiorespiratory, and sea-level performance changes compared to living and training in normoxia during a preparation season.

Methods: Nineteen (13 women, 6 men) cross-country skiers competing at the national or international level completed a 28-day period (~18 h day^{-1}) of LHTLH in normobaric hypoxia of ~2400 m (LHTLH group) including two 1 h low-intensity training sessions per week in normobaric hypoxia of 2500 m while continuing their normal training program in normoxia. Hemoglobin mass (Hb_{mass}) was assessed using a carbon monoxide rebreathing method. Time to exhaustion (TTE) and maximal oxygen uptake (VO_{2max}) were measured using an incremental treadmill test. Measurements were completed at baseline and within 3 days after LHTLH. The control group skiers (CON) (seven women, eight men) performed the same tests while living and training in normoxia with ~4 weeks between the tests.

Results: Hb_{mass} in LHTLH increased 4.2 ± 1.7% from 772 ± 213 g (11.7 ± 1.4 g kg^{-1}) to 805 ± 226 g (12.5 ± 1.6 g kg^{-1}) ($p < 0.001$) while it was unchanged in CON ($p = 0.21$). TTE improved during the study regardless of the group (3.3 ± 3.4% in LHTLH; 4.3 ± 4.8% in CON, $p < 0.001$). VO_{2max} did not increase in LHTLH (61.2 ± 8.7 mL kg^{-1} min^{-1} vs. 62.1 ± 7.6 mL kg^{-1} min^{-1}, $p = 0.36$) while a significant increase was detected in CON (61.3 ± 8.0–64.0 ± 8.1 mL kg^{-1} min^{-1}, $p < 0.001$).

Conclusions: Four-week normobaric LHTLH was beneficial for increasing Hb_{mass} but did not support the short-term development of maximal endurance performance and VO_{2max} when compared to the athletes who lived and trained in normoxia.

KEYWORDS
altitude training, cross-country skiing, endurance performance, hemoglobin mass, maximal oxygen uptake, normobaric hypoxia

1 | INTRODUCTION

Many competitive endurance athletes undertake training camps in hypoxic conditions to increase hemoglobin mass (Hb_{mass}) in order to improve oxygen carrying capacity and endurance performance.[1,2] Training and living at moderate altitude (1800–2500 m) has often been used as a hypoxic stimulus for potential performance benefits.[3] In addition, generators can be used to simulate a normobaric hypoxic environment at sea level, which provides a logistically convenient option for transitioning between normoxic and hypoxic conditions.[3,4] Indeed, living in normobaric hypoxia (e.g., in hypoxic apartments) enables relatively easy use of the "live high–train low" method (LHTL), which is commonly used to stimulate hematological and performance adaptations. The LHTL-method enables athletes to avoid decreasing training intensity due to reduced oxygen availability.[3–5] Nevertheless, some evidence suggests that LHTL combined with intermittent training in hypoxia (namely "live high–train low and high", LHTLH in the present study) may elicit greater enhancement in the physiological capacities than more traditional LHTL.[6,7]

Altitude training is commonly used among cross-country (XC) skiers as they attempt to improve their performance.[8,9] Increased performance following altitude training is attributed to an increase in hemoglobin mass (Hb_{mass}) that is associated with an increase in maximal oxygen uptake (VO_{2max})[10] and exercise performance, assuming that the athlete has been able to maintain a normal pace during high intensity training.[5] In addition, altitude training may increase other physiological capabilities, such as exercise economy and muscle buffering capacity,[3,11] which are important factors for XC skiing performance.[9] In contrast, training at moderate altitude may decrease training intensity and/or expose athletes to excessive stress that may decrease training quality thus reducing the performance benefits of altitude training.[3] Unfortunately, several major countries in XC skiing (e.g., Nordic countries) have limited possibilities for natural altitude training, and traveling to altitude (i.e., a mountainous region) may place additional stress on the athletes. Therefore, normobaric LHTL and LHTLH may be potential methods for XC skiers to maintain training quality while obtaining the physiological benefits of altitude acclimatization.

Although altitude training theoretically increases endurance performance, scientific evidence is controversial.[2,12,13] In addition, research on normobaric LHTLH is limited, even though it is implemented by athletes. Thus, the aim of this study is to investigate whether 4 weeks of normobaric LHTLH during a preparation season causes different hematological, cardiorespiratory, and sea-level performance changes in national and international level XC skiers compared to the skiers who live and train in normoxia.

2 | METHODS

2.1 | Participants

A total of 34 national to international level[14] XC skiers and biathletes (age 22 ± 4 years) participated in this study during their preparation season (the first part of the annual training cycle in the spring/early summer). All participants had baseline ferritin levels >35 μg L^{-1} suggesting no iron deficiency[15] and did not have any symptoms of illness or injury. All participants lived and trained at normoxia in the 6 months prior to the study with the exception of two athletes from LHTLH and one athlete from CON who had short (<2 weeks) stays at moderate altitude 4 months prior the study due to competitions. Participants provided written informed consent prior to their involvement in the study and were allowed to drop out of the study at any time. The ethical committee of the University of Jyväskylä approved the study (29/13.00.04.00/2021) and the study was conducted in accordance with the Declaration of Helsinki.

2.2 | Design

In this longitudinal study, 19 (13 women, 6 men) athletes completed a 28-day period of LHTLH (LHTLH group) while 15 (7 women, 8 men) athletes formed a control group (CON) that lived and trained in normoxia. Due to limited availability of the altitude apartments, the LHTLH period started either in late April ($n = 8$) or in late May ($n = 11$). The CON period started in early ($n = 7$) or late ($n = 8$) May. LHTLH spent 18.1 ± 1.2 h day^{-1} performing activities of daily living or at rest in normobaric hypoxia of 2250–2500 m (fraction of inspired oxygen 15.9%–15.4%) and performed two 1 h low-intensity training (LIT) sessions each week at a simulated altitude of 2500 m while otherwise continuing their normal training program in normoxia. Both groups lived near the altitude apartments during the study period and trained according to their individual training programs. Training was monitored but not controlled. The LHTLH group completed hematological and performance measurements at baseline within 6 days before starting LHTLH (pre) and within 3 days after LHTLH (post). Hematological and performance testing were completed on separate days. CON performed hematological and performance measurements at pre and post with 27 ± 4 and 30 ± 2 days between measurements, respectively.

2.3 | Normobaric hypoxia

Normobaric hypoxia was generated in hypoxic apartments of the Olympic Training Centre (Vuokatti Sport, ~150 m above sea level) and continuously monitored with a Hypoxico K2-2500 device (Hypoxico). The target altitude was 2250 m for the first week and 2500 m for weeks 2–4. Participants recorded daily mean altitude and the time spent in the hypoxic room in research logs. Hypoxic training sessions were performed by running on the treadmill at low intensity (blood lactate <2.5 mmol L^{-1}) and wearing a JAY-10H hypoxic generator mask (Longfian). The total hypoxic dose was calculated as "kilometer hours".[16]

2.4 | Hematological measurements

The optimized carbon monoxide (CO) rebreathing method[17] was used to calculate Hb_{mass} and blood volume. In brief, subjects rebreathed a dose of CO based on body mass (1.1 mL kg^{-1} for men and 0.9 mL kg^{-1} for women) and ~3 L pure oxygen for 2 min via closed circuit spirometer (SpiCO, Blood tec). The fraction of carboxyhemoglobin (%HbCO) from fingertip capillary blood was analyzed before CO rebreathing and 6 and 8 min after rebreathing using the ABL90 FLEX blood gas analyzer (Radiometer Medical ApS). Hb_{mass} calculations were based on the change in %HbCO from baseline to the 6 and 8 min samples after CO rebreathing.[17] A typical error reported for the method is 1.1%–1.7%.[17,18] Hemoglobin concentration and hematocrit were measured before rebreathing by obtaining blood from an antecubital vein into EDTA tubes (Greiner-Bio-One GmbH) and analyzing with Sysmex XN-1000 (SysmexCo.). Hb_{mass} and blood volume were presented as absolute values as well as normalized for body mass, which was measured prior to hematological measurements (Inbody 770, Biospace Co.). For the analysis of baseline ferritin, blood was drawn into Vacuette EDTA gel serum tubes (Greiner-Bio-One GmbH). The tubes were centrifuged at 3600 rpm for 10 min to collect serum and analyzed at using Roche CobasPro e801 (Roche Diagnostics).

2.5 | Endurance performance

Endurance performance was measured using an incremental test, which was performed by walking or running with poles on a treadmill (Telineyhtymä). The inclination and/or the speed of the treadmill was increased every third minute so that oxygen demand calculated using the equation by Balke and Ware[19] was 20 mL kg^{-1} min^{-1} in the first stage and increased 6 mL kg^{-1} min^{-1} for each subsequent stage. The test was continued until voluntary exhaustion and time to exhaustion (TTE) was used as a measure of endurance performance.

Respiratory variables were measured continuously using a mixing chamber system (Medikro 919 Ergospirometer, Medikro Oy). Volume and gas calibration of the ergospirometer were completed prior to each measurement. VO_{2max}, maximal ventilation (VE_{max}), and maximal respiratory exchange ratio (RER_{max}) were defined as the highest 60 s average. Heart rate was monitored using a Polar H10 heart rate belt (Polar Electro Oy), and the maximal heart rate (HR_{max}) was recorded. Blood lactate samples were obtained from a fingertip 1, 4, and 7 min after the test and collected into capillary tubes (20 µL), which were placed in a 1 mL hemolyzing solution and analyzed using Biosen C-line analyzer (EKF diagnostics). The highest lactate level (LA_{max}) was recorded.

2.6 | Training monitoring

Training was monitored during the intervention using an electronic training log (eLogger, eSportwise Oy). Total training volume in hours and intensity distribution were recorded. The training was divided into low-intensity training (LIT, target blood lactate <2.5 mmol L^{-1}), moderate-intensity training (MIT, target blood lactate 2.5–4 mmol L^{-1}), high-intensity training (HIT, target blood lactate >4 mmol L^{-1}),[9] speed and strength training, and other training.

2.7 | Statistical analyses

Statistical analyses were conducted using SPSS Statistics 26 (IBM). Data are presented as mean ± SD. Shapiro–Wilk indicated that data were normally distributed with the exception of absolute Hb_{mass} at pre and post (total group $p = 0.029$; $p = 0.021$, respectively) and absolute VO_{2max} at pre and post (total group $p = 0.005$, $p = 0.003$, respectively). Nevertheless, as the statistical methods used are not very sensitive to violations in normality, and skewness and kurtosis were <1.2 in both variables, data were analyzed with parametric tests. Between- and within-group differences were analyzed with a mixed ANOVA with group and sex as a between-subjects factors and measurement point (time) as the within-subjects factor. When a significant interaction existed, simple effects with Bonferroni correction were analyzed. Forward multiple regression analyses were performed to investigate if training volume, intensity distribution and/or changes in body mass explained changes in Hb_{mass}. In addition, forward regression analyses were performed to investigate if training volume, intensity distribution, and/or changes in Hb_{mass}, blood

volume, and/or plasma volume explained changes in TTE or VO_{2max}. Pearson's correlation coefficient was used to show relationships between the variables in Figure 3. Statistical significance was defined as $p < 0.05$.

3 | RESULTS

Total hypoxic exposure was 507 ± 41 h (18.1 ± 1.2 h day^{-1}) in a mean altitude of 2419 ± 38 m resulting the mean hypoxic dose of 1227 ± 105 km h. A total of four participants from LHTLH (two due to air flush during CO rebreathing, one due to unexpectedly high (13.9%) increase in Hb_{mass}, and one due to long interval between pre and post) and one participant from CON (due to unexpectedly high (7.0%) increase in Hb_{mass}) were excluded from hematological analyses. Two participants from LHTLH (one due to injury prior post testing and one due to long interval between pre and post) and one participant from CON (due to injury prior post testing) were excluded from the performance analyses. The final number of the participants are shown in Tables 1 and 2.

Table 1 shows hematological measures and body mass at pre and post. There was a significant time × group interaction on absolute and relative Hb_{mass} ($F = 39.3$, $p < 0.001$, $F = 21.3$, $p < 0.001$, respectively). Analyses of simple effects showed that LHTLH increased both absolute and relative Hb_{mass} (4.2 ± 1.7%, $F = 56.2$, $p < 0.001$; 6.8 ± 2.6%, $F = 77.9$, $p < 0.001$, respectively) while CON increased relative (2.0 ± 3.2%, $F = 6.0$, $p = 0.02$) but not absolute Hb_{mass} (0.8 ± 2.3%, $F = 1.6$, $p = 0.21$). Figure 1A shows the percentual changes in absolute Hb_{mass} and Figure 1B in relative Hb_{mass}. There was a significant time × group interaction for absolute ($F = 11.8$, $p = 0.002$) and relative ($F = 9.3$, $p = 0.005$) plasma volume. Analyses of simple effects showed that both absolute and relative plasma volume decreased in LHTLH ($F = 22.5$, $p < 0.001$; $F = 13.8$, $p = 0.001$, respectively) but not in CON ($F = 0.003$, $p = 0.96$; $F = 0.3$, $p = 0.58$, respectively). There was a significant main effect for time on body mass ($F = 64.4$, $p < 0.001$) but no time × group interaction ($F = 2.5$, $p = 0.13$) showing that body mass decreased regardless of the group.

There was a significant main effect for time on TTE ($F = 24.9$, $p < 0.001$) and VE_{max} ($F = 9.8$, $p = 0.004$). No time × group interactions were detected suggesting that those performance variables improved regardless of the group. Consequently, the 3.3 ± 3.4% increase in TTE in LHTLH was similar to the 4.3 ± 4.8% increase in CON (Figure 2A). There was a significant time × group interaction for relative VO_{2max} ($F = 4.6$, $p = 0.04$). Analyses of simple effects showed that CON increased VO_{2max} from pre to post ($F = 15.5$, $p < 0.001$) while LHTLH did not ($F = 0.9$, $p = 0.36$) (Figure 2B). There were significant time × group and time × sex interactions for LA_{max}. Analyses of simple effects showed that LA_{max} increased in CON ($F = 5.4$, $p = 0.03$) while no significant changes were detected in LHTLH ($F = 2.3$, $p = 0.14$), women ($F = 1.5$, $p = 0.023$) or men ($F = 3.5$, $p = 0.07$).

The mean training volume in LHTLH was 16.7 ± 2.4 h week^{-1} (81% LIT; 5% MIT; 1% HIT; 12% speed and strength training; 1% other) and in CON 15.2 ± 2.6 h week^{-1} (79% LIT; 7% MIT; 2% HIT; 11% speed and strength training; 1% other). There were no group differences in training volume or intensity distribution.

When forward regression was performed none of the variables explained changes in absolute Hb_{mass}, TTE, or VO_{2max} in LHTLH, CON, or when the groups were analyzed together. The relationship between the changes in Hb_{mass} and TTE are shown in Figure 3A and the relationship between the changes in Hb_{mass} and VO_{2max} in Figure 3B. Increase in relative Hb_{mass} was explained by the decrease in body mass in LHTLH ($R^2 = 0.47$, $p = 0.009$), CON ($R^2 = 0.48$, $p = 0.009$), and when the groups were analyzed together ($R^2 = 0.47$, $p < 0.001$).

4 | DISCUSSION

The present study provided novel information on the effects of normobaric LHTLH on Hb_{mass} and endurance performance during a preparation period in national and international level XC skiers. The main findings of the study were that a 4 week LHTLH period increased Hb_{mass} 4.2% while living and training in normoxia did not. Although increased Hb_{mass} should theoretically be beneficial for endurance performance,[10] positive changes in endurance performance compared to CON were not detected immediately after the intervention. Notably, only CON improved relative VO_{2max} during the study.

Four-weeks (507 h/18 h day^{-1} hypoxia exposure) of LHTLH increased Hb_{mass} 4.2%, which is quite well in line with a previous meta-analysis suggesting that Hb_{mass} increases at a mean rate of 1.1% per 100 h of exposure at simulated or natural altitude.[20] The similar rate of Hb_{mass} increase is also supported by a study using normobaric LHTL at an altitude of 2250 m.[21] In addition, the Hb_{mass} increase of 4.2% with a hypoxic dose of 1227 ± 105 km h is in line with the 4.4% calculated by the exponential model presented by Garwican-Lewis.[16] Thus, the detected Hb_{mass} increase in LHTLH was expected based on previous literature. Notably, the 0.8% increase in CON was not statistically significant or higher than the typical 1.1%–1.7% CO rebreathing method measurement error.[17,18] Although relative Hb_{mass} increased 2.0% in CON, the change was explained by the decreased body mass. These findings

TABLE 1 Hematological measures and body mass before (pre) and after (post) a 4-week period either in normobaric hypoxia (LHTLH) or in normoxia (CON).

	Whole group				Women				Men				Mixed ANOVA					
	LHTLH ($n = 15$)		CON ($n = 14$)		LHTLH ($n = 10$)		CON ($n = 7$)		LHTLH ($n = 5$)		CON ($n = 7$)		Main effects			Interactions		
	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post	Time	Group	Sex	Time × Group	Time × Sex	Time × Group × Sex
Hb_{mass} (g)	772 ± 213	805 ± 226***	844 ± 166	850 ± 164	644 ± 72	670 ± 78	695 ± 58	703 ± 62	1028 ± 153	1076 ± 167	1008 ± 106	1030 ± 118	<0.001	0.84	<0.001	<0.001	0.24	0.07
Hb_{mass} (g kg^{-1})	11.7 ± 1.4	12.5 ± 1.6***	11.6 ± 1.5	11.8 ± 1.6*	10.8 ± 0.8	11.6 ± 0.7	10.3 ± 0.7	10.5 ± 0.7	13.5 ± 0.4	14.5 ± 0.6	12.8 ± 0.9	13.1 ± 1.0	<0.001	0.03	<0.001	<0.001	0.08	0.61
Blood volume (mL)	5898 ± 1229	5709 ± 1147	6401 ± 996	6427 ± 1045	5242 ± 662	5119 ± 565	5535 ± 477	5551 ± 500	7200 ± 1057	6985 ± 883	7266 ± 414	7303 ± 573	0.14	0.24	<0.001	0.05	0.93	0.79
Blood volume (mL kg^{-1})	90.3 ± 5.3	89.8 ± 5.9	87.9 ± 7.7	89.2 ± 9.2	88.2 ± 5.0	87.5 ± 5.9	82.3 ± 4.2	82.8 ± 5.4	94.5 ± 2.6	94.3 ± 2.6	93.4 ± 6.4	95.7 ± 7.6	0.53	0.20	<0.001	0.24	0.44	0.64
Plasma volume (mL)	3568 ± 657	3228 ± 542***	3707 ± 498	3703 ± 570	3282 ± 484	2986 ± 431	3348 ± 316	3300 ± 327	4140 ± 609	3712 ± 415	4066 ± 368	4106 ± 467	0.002	0.27	<0.001	0.002	0.84	0.31
Plasma volume (mL kg^{-1})	55.0 ± 3.5	50.9 ± 4.2**	51.8 ± 4.1	52.4 ± 5.8	55.0 ± 3.5	51.3 ± 5.1	49.7 ± 2.6	49.2 ± 4.0	54.4 ± 1.6	50.2 ± 1.7	53.8 ± 4.5	55.5 ± 5.9	0.03	0.64	0.13	0.005	0.59	0.36
Hb concentration (g L^{-1})	142 ± 10	153 ± 14***	145 ± 9	146 ± 8	135 ± 7	144 ± 10	138 ± 7	139 ± 7	154 ± 4	169 ± 5	152 ± 6	151 ± 6	<0.001	0.58	<0.001	<0.001	0.36	0.14
Hematocrit (%)	42.8 ± 3.1	47.5 ± 3.9***	45.1 ± 2.7	45.8 ± 2.7	41.0 ± 1.9	45.5 ± 3.0	43.4 ± 2.5	44.6 ± 2.4	46.2 ± 1.5	51.3 ± 1.5	46.6 ± 2.0	46.9 ± 2.5	<0.001	0.82	<0.001	<0.001	0.93	0.27
Body mass (kg)[n]	65.2 ± 10.8	63.9 ± 10.3	74.1 ± 8.3	73.0 ± 7.6	59.7 ± 6.7	58.7 ± 6.7	67.3 ± 4.5	67.1 ± 3.6	76.1 ± 9.0	74.2 ± 8.2	79.3 ± 6.2	77.9 ± 6.8	<0.001	0.02	<0.001	0.13	0.03	0.73

Note: Hb_{mass} change (%), hemoglobin mass; Hb, hemoglobin; [n] subject number: LHTHL $n = 18$ (12 women, 6 men) and CON $n = 15$ (seven women, eight men).

*Significant simple effect/significantly different from pre $p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

TABLE 2 Maximal and submaximal performance measures from incremental treadmill test before (pre) and after (post) a 4-week period either in normobaric hypoxia (LHTLH) or in normoxia (CON).

	Whole group				Women				Men				Mixed ANOVA					
	LHTLH (n = 15)		CON (n = 14)		LHTLH (n = 10)		CON (n = 7)		LHTLH (n = 5)		CON (n = 7)		Main effects			Interactions		
	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post	Time	Group	Sex	Time × Group	Time × Sex	Time × Group × Sex
TTE (min)	23.2 ± 2.8	24.0 ± 2.9	23.1 ± 2.8	24.0 ± 3.1	22.2 ± 2.2	22.9 ± 1.9	20.8 ± 2.1	21.4 ± 1.8	25.5 ± 3.0	26.6 ± 2.7	24.8 ± 2.1	26.0 ± 2.1	<0.001	0.22	<0.001	0.82	0.08	0.71
VO_{2max} (L min^{-1})	3.9 ± 1.0	4.0 ± 1.0	4.6 ± 0.9	4.7 ± 0.9	3.4 ± 0.2	3.4 ± 0.2	3.7 ± 0.4	3.8 ± 0.3	5.3 ± 0.9	5.3 ± 0.8	5.3 ± 0.3	5.4 ± 0.5	0.08	0.29	<0.001	0.05	0.45	0.26
VO_{2max} (mL kg^{-1} min^{-1})	61.2 ± 8.7	62.1 ± 7.6	61.3 ± 8.0	64.0 ± 8.1***	57.3 ± 5.0	58.7 ± 3.7	53.6 ± 5.3	55.8 ± 3.2	70.4 ± 8.9	70.3 ± 8.6	67.1 ± 3.0	70.2 ± 3.8	0.002	0.19	<0.001	0.04	0.72	0.22
VE_{max} (L min^{-1})	148 ± 32	154 ± 31	168 ± 29	175 ± 35	131 ± 15	142 ± 12	141 ± 19	142 ± 12	188 ± 24	194 ± 17	188 ± 13	200 ± 23	0.004	0.15	<0.001	0.33	0.23	0.21
RER_{max}	1.15 ± 0.04	1.13 ± 0.04	1.14 ± 0.04	1.13 ± 0.06	1.16 ± 0.04	1.13 ± 0.05	1.15 ± 0.03	1.13 ± 0.06	1.13 ± 0.03	1.14 ± 0.04	1.13 ± 0.04	1.13 ± 0.06	0.02	0.17	0.69	0.39	0.001	0.36
HR_{max} (bpm)	191 ± 9	194 ± 9	197 ± 8	197 ± 7	190 ± 11	191 ± 11	196 ± 8	194 ± 7	193 ± 5	193 ± 6	198 ± 9	199 ± 7	0.84	0.07	0.43	0.09	0.62	0.11
LA_{max} (mmol L^{-1})	11.4 ± 2.4	10.3 ± 2.5	9.2 ± 2.2	10.3 ± 2.9*	11.3 ± 2.7	9.8 ± 2.7	9.7 ± 2.4	10.2 ± 3.7	11.5 ± 1.3	11.8 ± 1.2	8.9 ± 2.1	10.4 ± 2.5	0.56	0.21	0.66	0.01	0.04	0.45

Abbreviations: HR_{max}, maximal heart rate; LA_{max}, maximal blood lactate; RER_{max}, maximal exchange ratio; TTE, time to exhaustion; VE_{max}, maximal ventilation; VO_{2max}, maximal oxygen uptake.
*Significant simple effect/significantly different from pre $p < 0.05$; ***$p < 0.001$.

FIGURE 1 Changes in hemoglobin mass (Hb_{mass}) (A) and Hb_{mass} in relation to body mass (B) during 4-week period either in stimulated altitude (LHTLH) or in normoxia (CON). The circles represent individual values and the lines represent group means. Open circles represent LHTLH and closed circles represent CON. Gray zone shows the typical measurement error of the carbon monoxide rebreathing method.[17] Significant interaction between LHTLH and CON ***$p < 0.05$.

suggest that an increase in Hb_{mass} was due to normobaric hypoxic exposure and not training, per se.

According to the experiments of Schmidt and Prommer,[10] a 1 g change in Hb_{mass} causes a VO_{2max} change of 4 mL min^{-1}. In addition, a pooled study by Sauders et al.[1] found that a 1% increase in Hb_{mass} after altitude training resulted in a 0.6%–0.7% increase in VO_{2max} in elite athletes. Nevertheless, the present findings did not indicate significant associations between Hb_{mass} changes and VO_{2max} changes and despite the increase in Hb_{mass}, LHTLH did not increase VO_{2max}. In contrast, CON increased VO_{2max} (in mL kg^{-1} min^{-1}) by 4.6% although Hb_{mass} did not

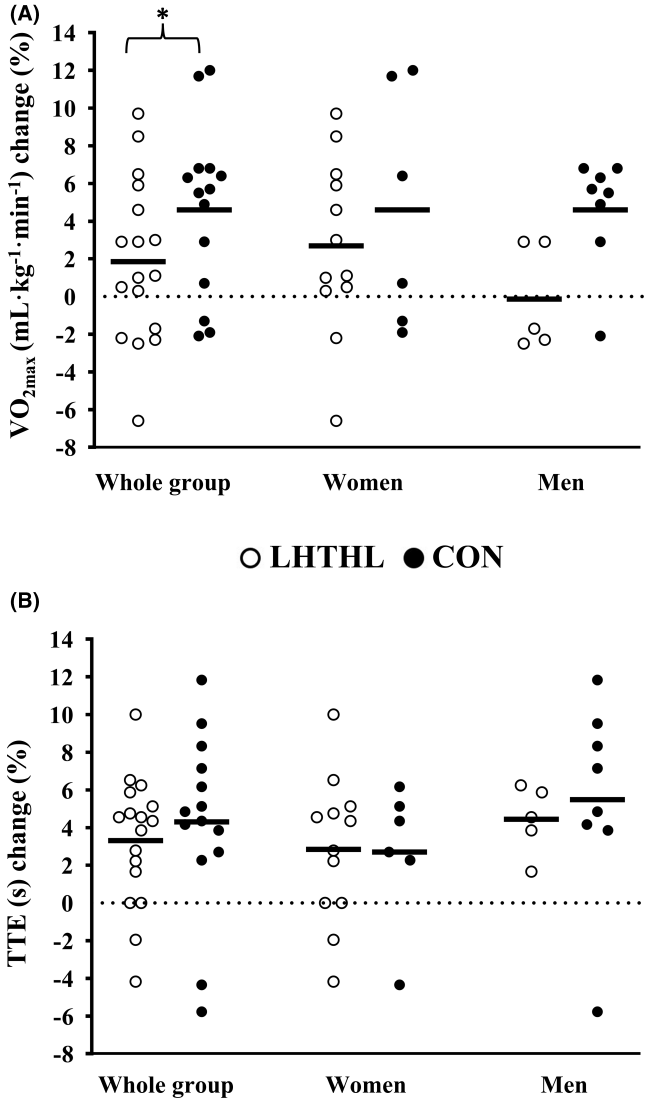

FIGURE 2 Changes in maximal oxygen uptake (VO_{2max}) (A) and time to exhaustion (TTE) (B) during 4-week period either in normobaric hypoxia (LHTLH) or in normoxia (CON). The circles represent individual values and the lines represent group means. Open circles represent LHTLH and closed circles represent CON. Significant interaction between LHTLH and CON $*p < 0.05$.

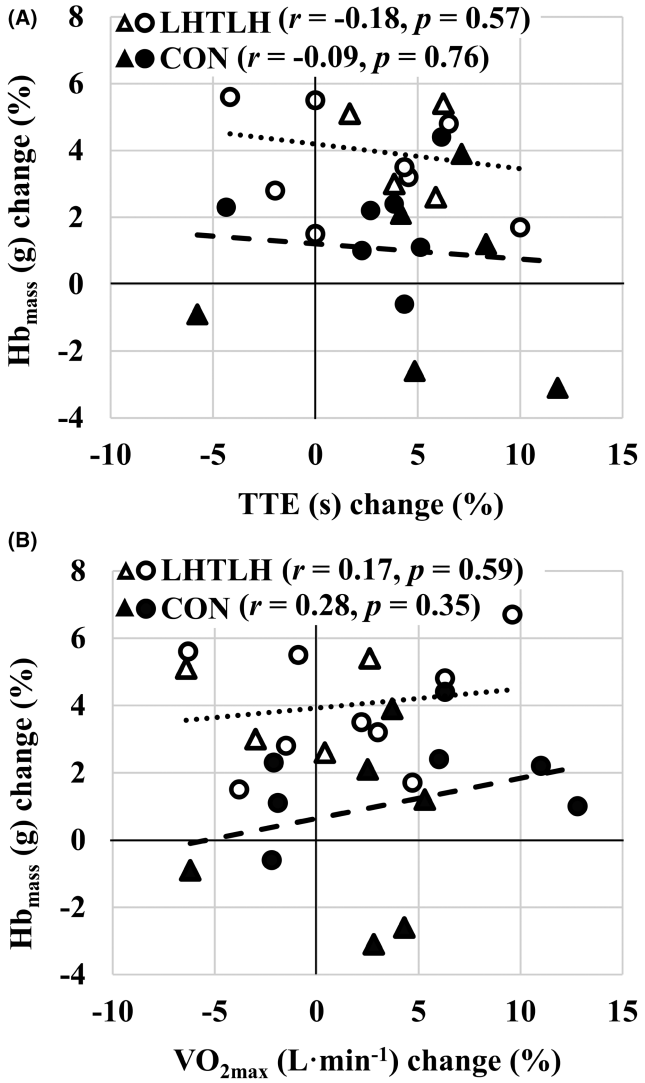

FIGURE 3 Correlations between changes in hemoglobin mass in L min^{-1} (Hb_{mass}) and time to exhaustion (TTE) (A) and between changes Hb_{mass} and maximal oxygen uptake in L min^{-1} (VO_{2max}) (B). Women are represented by circles and men by triangles. Open circles/triangles represent LHTLH and closed circles/triangles represent CON. Dotted linear line for LHTLH and dashed linear line for CON.

increase. These findings suggest that despite the positive effects of LHTLH on Hb_{mass}, this training method did not promote short-term VO_{2max} development. A potential explanation for our findings is that plasma volume significantly decreased during LHTLH, which may have affected VO_{2max}[22] and further limited the positive performance benefits of increased Hb_{mass}. Indeed, Siebenmann et al.[23] suggested that daily confinement to hypoxic apartments may lead to unusually long periods of inactivity as well as a reduction in plasma volume. Nevertheless, in the present study changes in plasma volume did not explain changes in TTE or VO_{2max}.

In contrast to VO_{2max}, TTE in the incremental treadmill test increased during the intervention with no significant group differences between the 3.3% increase in LHTLH and the 4.3% increase in CON. These results suggest that LHTLH did not provide an additional short-term benefit in endurance performance when compared to training and living in normoxia. Despite several studies having reported the effectiveness of LHTL on endurance performance, results remain controversial.[2,13] Our findings are in line with previous research that suggests that LHTL does not increase exercise performance and should not be recommended for endurance athletes.[12,13,23,24] Nevertheless, other literature describes the performance benefits of LHTL.[2] For example, Hauser et al.[25] found that 3 km running performance increased more in male triathletes

during 3 weeks of normobaric LHTL (2.2%) compared to a 3-week training period in normoxia (1.2%). As training and living in normoxia induced similar performance improvements in the present study, the positive changes after LHTLH may be explained by something other than hypoxic exposure whereas other mechanisms influencing performance beyond VO_{2max} may be involved.

The time-course of the post performance measurements may have affected the results of the present study where the performance tests were executed within 3 days after LHTLH. Although observational findings by coaches suggest that athletes generally perform well during the first 2–4 days after altitude training, scientific evidence is limited.[3] Indeed, Wachsmuth et al.[26] found that swimming competition performance was impaired during the first 2 weeks after natural altitude training while the best performance was observed 25–35 days after altitude exposure. Similarly, Gough et al.[27] found decreased competition performance on Days 1 and 7 after normobaric LHTL despite a 4% increase in Hb_{mass}. In their study, a return to baseline performance was achieved 14 and 28 days after altitude.[27] In contrast, several studies have found an increase in time trial performance and/or VO_{2max} when performance was measured within 3 days after normobaric LHTL.[7,25,28] Taken together, although the timing of post performance measurements were in line with the general experiences of "good timing" for post-altitude performance,[3] some scientific evidence suggests that athletes perform better during the third and fourth week after altitude exposure.[26,27] As such, it is possible that the positive performance effects of LHTLH appeared later than measured in the present study.

The present study was performed during an annual preparation period, which may affect the results. Indeed, most XC skiers significantly decrease their training load during the regeneration period (i.e., active recovery period) prior to the preparation period,[29] which may decrease both Hb_{mass} and endurance performance. For example, several studies have shown a significant decrease in Hb_{mass} when training load is reduced due to illness or injury.[30,31] In addition, Garvican et al.[32] reported that an increase in training load of 36% over 4 weeks may result in an increase of 2.7% in Hb_{mass}. Similarly, endurance performance and VO_{2max} may decrease already during 2 weeks of detraining after a training season,[33] and it has been shown that sport specific endurance performance progressively increases in XC skiers as the season progresses.[34] Therefore, the timing of the study may partly confound the positive changes in Hb_{mass}, VO_{2max}, and endurance performance. Nevertheless, both LHTLH and CON completed the study during preparation period, and it is therefore unlikely that the phase of the season would explain the differences observed between the groups.

As shown in Figures 1–3, the individual variation in Hb_{mass}, TTE, and VO_{2max} as well as in the relationships between these variables was high, which is a typical finding in altitude training studies.[25] Notably, all athletes in LHTLH had higher or similar increase in Hb_{mass} (1.5%–6.9%) than the typical 1.1%–1.7% measurement error of the CO rebreathing method,[17,18] while athletes in CON had both negative and positive changes. Thus, the results suggest that all athletes in LHTLH had atleast a minor positive Hb_{mass} response to altitude training that may be supported, in part, by the fact that participants had adequate ferritin levels ($>35\,\mu g\,L^{-1}$)[15] prior LHTLH[35] and reported no infection during LHTLH.[26,30] As none of the variables explained individual Hb_{mass} changes, it is difficult to assess what factors determined the within group differences in Hb_{mass} changes. One potential explanation may be that athletes with smaller Hb_{mass} increases had higher pre-altitude Hb_{mass} compared with their individual mean pre-altitude levels,[36] but this cannot be concluded based on the current study protocol. Furthermore, low energy availability[37] and excessive total stress[38] may prevent optimal Hb_{mass} changes. In addition to individual hematological responses, changes in TTE and VO_{2max} varied significantly between the participants regardless of the magnitude in their Hb_{mass} changes. Consequently, the management of total stress and training quality may have been factors affecting performance changes[39] and should be prioritized when planning the implementation of training camps and training, in general.

The scientific literature regarding LHTLH is limited. Therefore, the LHTL protocol is mainly used for reference in the present study. As the LHTL and LHTH methods have previously been shown to have a similar effect on Hb_{mass} and performance,[25] it is likely that the LHTLH method does not significantly differ from those methods. Nevertheless, Robertson et al.[6] showed that 3-week normobaric LHTLH including 14 h day^{-1} living at 3000 m and 4–5 h $week^{-1}$ LIT–HIT training at 2200 m elicited increases in Hb_{mass}, time trial performance, and VO_{2max}. Notably, LHTLH resulted in greater enhancement in VO_{2max} than "live low, train high" and LHTL methods.[6,7] Regardless, it is difficult to conclude, whether 2 h $week^{-1}$ of LIT in normobaric hypoxia, that was used in the present study, had any additional effect on Hb_{mass} or endurance performance compared to the LHTL training. Although technological developments have made the implementation of normobaric LHTLH training quite easy, while reducing travel stress, more data is needed from the combination of living and training in normobaric hypoxia.

A limitation of the present study was that we did not control training or dietary intake, which both have an important role in regulating Hb_{mass} and performance.[29,32,37] Nevertheless, analyses of the training logs did not reveal significant group differences in training volume or intensity

distribution. In addition, athletes were encouraged by sport nutritionist to consume adequate energy, fluid, and iron. Although decreased body mass suggests negative energy and/or fluid balance, the decrease was similar in both groups. As another limitation, the sex distribution between LHTLH and CON was inequal. We acknowledge that this imbalance may have affected group-level results, however, additional analyses were performed for women and men separately showing no time × sex or time × group × sex interactions in hematological or performance variables. In addition, Figures 1 and 2 show that changes from pre to post were quite similar regardless of the sex. These finding suggest that sex distribution had a minor effect on pre to post changes in hematological and performance measures. Unfortunately, the number of women and men including in the study was inadequate to reliably examine group differences within sex or sex differences within group. The present study was limited to investigate TTE and VO_{2max} from an incremental treadmill test, although several other factors influence XC skiing performance.[9] Nevertheless, it has been recently shown that both VO_{2max} and TTE from an incremental running test have a strong positive association with XC skiing competition performance.[40] Finally, the absence of duplicate Hb_{mass} measurements increases the risk of error in hematological measurements. To limit the risk of error, outliers were removed from the analyses.

5 | PERSPECTIVE

The results of the present study suggest that four-week normobaric LHTLH during the preparation period is beneficial for endurance athletes aiming to increase their Hb_{mass}. Nevertheless, LHTLH did not support the development of maximal endurance performance and VO_{2max} when compared to the athletes who lived and trained in normoxia. As performance adaptations are the main goal of training, the present results highlight the importance of future studies to investigate the optimal implementation of LHTLH for endurance performance.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.The data are not publicly available due to privacy or ethical restrictions.

CONSENT STATEMENT

Participants provided written informed consent prior to their involvement in the study.

ORCID

Oona Kettunen https://orcid.org/0000-0002-8185-4942
Ritva Mikkonen https://orcid.org/0000-0002-5668-2202
Juha E. Peltonen https://orcid.org/0000-0002-4733-8784
Ari Nummela https://orcid.org/0000-0002-7965-3092

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