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INFLUENCE OF LIVE HIGH TRAIN HIGH ON PHYSIOLOGICAL TRAINING ADAPTATIONS AND ATHLETIC PERFORMANCE IN TRAINED RUNNERS: AN OBSERVATIONAL COHORT CASE STUDY

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Abstract Introduction: Live high-train high (LHTH) is suggested to improve performance, especially maximal aerobic capacity through the hypoxic adaptation responses. The present study aims to determine the impact of LHTH protocol on cardio-respiratory variables, lactate response, iron profile indices, and lung function capacities to the performance enhancement of middle-long distance (MLD) runners. Methods: Seven MLD runners were trained LHTH for six weeks at 8000ft altitude. Cardio-respiratory variables ($\dot{V}O_{2max}$, VO_2/HR, VE_{max}, HR response), lactate response, iron profile indices (Hb, iron, UIBC, TIBC, transferrin, EPO, ferritin), and lung function parameters (FVC, FEV1, FEV1/FVC, PEF, and VC) were measured via standard protocols. Result: Hemoglobin (Hb, 7.8%), iron concentration (36.7%), and TIBC (8.2%) were found to be significantly increased after LHTH. On the other hand, $\dot{V}O_{2max}$ (6.4%) and PEF (10.7%) were found to be significantly ($p<0.05$) increased after camp. But HR_{rest} (1.8%), HR_{rec} (5min and 10min with 2.3% and 4.7% respectively), and lactate peak (7.9%) significantly decreased after LHTH condition. Discussion: All participating athletes improved performances after LHTH (overall group improved by 1.7%, individual increase up to 2.6%). Specific training load with hypoxic stress at high altitude induces peripheral chemoreceptor activation and stimulates sympathetic drive to initiate adaptive cascade leading to $\dot{V}O_{2max}$ improvement (improved group mean 6.4%, individually up to 16.7%) which might be supported by enhanced erythropoiesis, iron metabolism, Hb concentrations, peak expiratory flow, glycolytic enzyme activity, enhanced oxidative capacity, improved resting and recovery HR response and peak lactate tolerance.

Key words: live high-train high, erythropoiesis, endurance performance, running performance, iron metabolism, lung functioning

Introduction

A high-altitude environment causes physiological stress in the body, which often happens at high altitudes (>3000 m) and moderate altitudes (2000 m to 3000 m). Hypobaric hypoxia is a condition caused by a drop in atmospheric oxygen pressure and a corresponding fall in barometric pressure as elevation rises (Vargas-Pinilla, 2014; Vissers, 2014). Along with hypoxia, many additional challenges, including strong sun radiation, low temperatures, low humidity, high winds, a limited dietary basis, hard terrain, etc., are significant elements that stress human physiological conditioning under high-altitude conditions (Vargas-Pinilla, 2014). It is necessary for people to adjust to the physiological stress of high-altitude exposure due to the reduced partial pressure of oxygen. Numerous variables influence these responses, including altitude-induced hypoxia, exposure length, exercise intensity, and individual conditions (Vargas-Pinilla, 2014). The body must undergo some adaptations and modifications at high elevations in order for the oxygen delivery system to make up for the hypoxia and keep the tissue oxygen levels sufficient for metabolism (Vargas-Pinilla, 2014; Vissers, 2014).

Hypoxic conditions are thought to activate glycolysis, up-regulate lactate and hydrogen ion transport, down-regulate the sodium-potassium pump, enhance capillary density, and increase pyruvate availability (Vissers, 2014; Wyatt, 2014). Where rapid fluid loss makes a person susceptible to dehydration, ventilation keeps becoming worse (Wyatt, 2014). Higher altitudes showed increased sympathetic activity, which eventually causes the HR_{max} to fall and the vagal tone to rise, down-regulating the beta receptors (Vissers, 2014). This comprehensive view aids in acclimatization and promotes cardiorespiratory adaptation to make up for the negative effects of higher elevations, which may be seen as an improvement upon returning to sea level. Although Saunders et al. (2009) have suggested that to get maximal benefit from LHTH protocol, some criteria should be followed: i) sufficient long stay (not < 2 weeks), ii) sufficient altitude (around 1800–2500 m), iii) iron profile should be proper and supply sufficient iron if needed, iv) design training meticulously and after initial physiological acclimatization.

Live high train high (LHTH) refers to athletes living and training at a natural high altitude for a period of time (usually 2–4 weeks) majorly aimed to improve sea-level performance. Coaches and athletes generally consensus about the LHTH improvements on physiological capacities and according to the meta-analysis of Bonetti and Hopkins (2009), LHTH leads to 1.6 ±2.7% improvement in performance for elite athletes. The LHTH has the potential benefit over Live High Train Low (LHTL) in terms of additional hypoxic training stress, which may accelerate erythropoiesis to increase the relative training intensity even more (Pugliese et al., 2014; Sharma et al., 2018). Factors that might explain these equivocal findings include the altitude at which athletes lived and trained, the relative intensity of training sessions, athlete iron status or supplementation protocol, and a reduction in training quality mediated by lower oxygen availability (Sharma et al., 2018).

There are studies that support both the ergogenic effect and those that disprove the hypothesis of the beneficial effect of LHTH on performance (Vargas-Pinilla, 2014; Saunders et al., 2009; Bonetti & Hopkins, 2009; Pugliese et al., 2014; Sharma et al., 2018). Although many physiological and biochemical factors parallelly worked in sync with the hypoxic stress at higher altitudes to finally improve endurance performance. A limitation of many LHTH studies is that no clear training regimen was depicted, and many do not clarify the physiological percent changes to finally convert into performance changes in an individual manner. The present study sought to ascertain the impact of LHTH on the physiological and performance changes for a cohort of trained middle-long distance runners.

Methods

Subject selection

Seven (n = 7 including 3 girls and 4 boys; age = 16.4 ±1.51 years) middle-long-distance (MLD) runners of events 800m and 1500m with a minimum of four years of official training experience who are members of the Sports Authority of India (SAI) under the National Center of Excellence scheme participated willingly in the current study. The respondents were regarded as homogenous since they share the same socioeconomic status, dietary preferences, geographic location, meteorological circumstances, and similar exercise routines. Each individual involved in the study gave their informed consent, and the study procedure complies with the Declaration of Helsinki ethical principles.

Only athletes who were medically in good health, had no known genetic or cardio-respiratory disorders, and had undergone a clinical examination by a sports medicine professional prior to the start of the test were ultimately chosen for the current study. On a treadmill, each subject completed a graded exercise routine (T170 DE Sport Med., Cosmed srl, Italy). Assessments were carried out in two phases: 'before LHTH camps' and 'after LHTH camp' at sea level (at 123 m; Lucknow, Uttar Pradesh, India). Although none of the athletes had any previous experience of high-altitude camping.

Altitude training program

High altitude training camp was conducted at 8000ft (2400 m) above sea level at Shilaroo, Himachal Pradesh, India. The camp was continued for 6 weeks duration with an initial 7 days of the physiological adaptation phase. During the initial adaptation phase, daily monitoring of blood pressure, SpO_2, etc was followed to avoid excess physical stress. The training slowly started from the 8th day and the LHTH training load gradually increased in a weekly manner till the end. Total load was considered with the scoring of Rating of perceived exertion (RPE) following a modified Borg scale and weekly training volume (total kilometers covered in the week). The training was individualized for each athlete based on 'before camp' testing assessment, physical characteristics, performance target as per the current altitude training strategies, race pace, and some $\dot{V}O_{2max}$ intensity sessions were modified to include additional recoveries between intervals to maintain running speed, whereas threshold and low-intensity training were not modified (i.e., same interval/recovery length), but performed at a reduced speed and/or higher perceived effort compared to sea-level. A detailed morning session training plan was represented in Table 1. Where Monday was low-intensity running (6–10 km), Wednesday and Sunday were moderate-intensity running (10–20 km and 16–30 km respectively), Tuesday was interval running + hills ($\dot{V}O_{2max}$ intensity), Thursday was race pace, Friday was strength training and Saturday was threshold training. In the evening sessions, strength training was implemented on Monday, 30–40 min run at low intensity was implemented on Tuesday and Thursday, and a 6–8 km run at low intensity was implemented on Saturday. The overall volume of the evening sessions is moderately light in comparison to moderately higher loads in the morning sessions (Sharma et al., 2018).

Table 1. General structure of training week along with the initial week training plan (after 7 days of initial acclimatization) at high altitude

	MON	TUES	WED	THURS	FRI	SAT	SUN
The general structure of Altitude training (Mainly morning session)	6–10 km @ low intensity	Intervals + hills @ $\dot{V}O_{2max}$ intensity	10–20 km @ low to moderate intensity	Race pace	Strength training	Threshold	16–30 km @ low to moderate intensity
	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Initial week of training starts (after initial acclimatization)	Easy run	2 easy runs	2 runs or 1 medium length run	Threshold to $\dot{V}O_{2max}$	Strength training	Race pace	Long run

Anthropometric measurement

A digital Seca Alpha stadiometer was used to measure the standing height (cm), and a calibrated digital Seca Alpha weighing scale (model 770) was used to measure body weight (kg), also to the nearest 0.1 kg (Sarkar et al., 2020).

Iron profile and Erythropoietin measurement

Blood was drawn into a serum separator tube from the antecubital fossa vein, inverted 5–8 times, and left to coagulate for 30 minutes while the tube was upright. Serum separation was accomplished by centrifuging the blood for 15 minutes at 2200–2500 rpm. Iron, TIBC (Spinreact, Spain), and ferritin were measured from the separated serum (DrGdiagnostics, Germany). The cyanmethemoglobin method was used to measure hemoglobin, the enzyme immunoassay method for ferritin, and the Persjin method for TIBC. The following calculation was used to determine transferrin saturation: Iron/TIbc * 100. The HITACHI U-2000 Double beam UV/Vis spectrophotometer (Japan), ELx800 Absorbance Microplate Reader (Biotek, USA), and ERBA Smart wash-III, small automated ELISA washer (ERBA Diagnostics Mannheim GmbH, Germany) were the instruments utilized for these estimations (Srividhya et al., 2017). The Human EPO Quantikine™ IVD ELISA Kit (R&D Systems, Minneapolis, MN, USA) was used to measure the serum levels of EPO (Man et al., 2021).

Cardiorespiratory variables

The following cardio-respiratory variables were measured using a breath-by-breath metabolic gas analyzer (COSMED K5, Codmed Inc., Italy): oxygen pulse (VO_2/HR, ml/beats), pulmonary ventilation, or minute ventilation (VE, l/min), ventilatory equivalent for oxygen production (VE/VO_2), ventilatory equivalent for carbon dioxide excretion (VE/VCO_2), and $\dot{V}O_{2max}$, among others (Dhawan et al., 2014). Throughout the entire workout routine, a Polar heart rate monitor (Polar RS800CX, Polar Electro OY, Kempele, Finland) was utilized to track heart rate.

Prior to the commencement of the exercise test, each participant was briefed about the incremental exercise test protocol on the treadmill. The graded exercise test (GXT) was done by using the COSMED K5 (metabolic analyzer) on the treadmill by following the standard procedure of GXT to exhaustion, starting with 2min 6km/hr of warm-up on the treadmill followed by 2km/hr increment in every 2min with 2° inclination increase till exhaustion to reach the $\dot{V}O_{2max}$ limit (Marini et al., 2022). A built-in computer system connected to the COSMED K5 was used to record breath-by-breath data throughout the gradual exercise test. The 30-second sample period's average of the breath-by-breath cardio-respiratory data was then recorded (Sarkar et al., 2019).

Utilizing the COSMED K5, a direct method of metabolic gas analysis with the following standards, the maximum oxygen consumption ($\dot{V}O_{2max}$) was estimated. The following criteria must be met: i) VO_2 plateau (leveling off)

(2 $ml.kg^{-1}.min^{-1}$), (ii) respiratory exchange ratio (RER) 1.0, (iii) HR_{max}> 90% of age-predicted HR_{max} ±5% (Sarkar et al., 2019; Debray & Dey, 2007).

Following the ventilatory equivalent approach, AT was calculated (visual inspection). It occurs at a specific point when the VE/VO_2 increases but not simultaneously with the VE/VCO_2 (Sarkar et al., 2019; Nogueira & Pompeu, 2010). Two seasoned investigators visually assessed each person's AT determination process. The entire experiment was conducted in a space that ranged in temperature from 23°C to 25°C and relative humidity from 50 to 60%.

Spirometry measurement

Pneumotach Pony FX (COSMED pulmonary function equipment, Italy) was used to measure lung function. The test was administered to subjects in standing posture by using an inhaling mouthpiece and a nasal clip in accordance with the COSMED pulmonary testing protocol. FVC, FEV1, PEF, and VC were all directly measured. The conventional formula [(FEV1/FVC)x100] was also used to determine the forced expiratory ratio (percent). Before being extrapolated to a value for 1min stated in liters/minute, MVV was conducted over a 15s time period. A minimum of three tests were conducted, or until the two highest values were noted. Theophylline or ß-agonist inhalers were also forbidden before the test, as were exercise, alcohol, caffeine, smoking, and caffeine-containing beverages. A similar test was run twice: once before camp and once after camp, both times under the same climatic conditions: comfortable temperature (between 18 and 22 degrees Celsius), 760 millimeters of mercury, and 30 to 60 percent relative atmospheric humidity (Durmic et al., 2017).

Statistical analysis

The Statistical Program for the Social Sciences (SPSS) version 16.0 for Windows was used to conduct the statistical analysis (SPSS Inc., Chicago, Il, USA). The means and standard deviation of all values were used to express them (SD). The Levene's test and Shapiro-Wilk test were done to confirm the assumption of homogeneity and normality respectively. Effect size calculation was done via Cohen's d which was measured at 0.864, which falls under large (as Cohen's conventions 1988, >0.8 large). Using a paired sample t-test with two-tailed significance and a 95% confidence interval, differences between the means of the pre- and post-training groups for all variables were ascertained.

Results

Figure 1 depicted the mean comparative analysis of iron profile variables between the pre-and post-camp conditions of MLD players with individual player differences (shown as line graphs) from pre- to post-condition. Significant improvement was observed against some of the iron profile variables i.e., hemoglobin (7.8%) ($p = 0.031$, $t = -2.805$), serum iron (36.7%) ($p = 0.002$, $t = -5.168$), and TIBC (8.2%) ($p = 0.022$, $t = -3.072$) after camp conditions in comparison to pre-camp data. Whereas transferrin saturation (11.3%), and EPO (16.4%) were found to be increased and UIBC (1.3%), and ferritin (7.9%) were found to be decreased after camp but in a statistically insignificant manner.

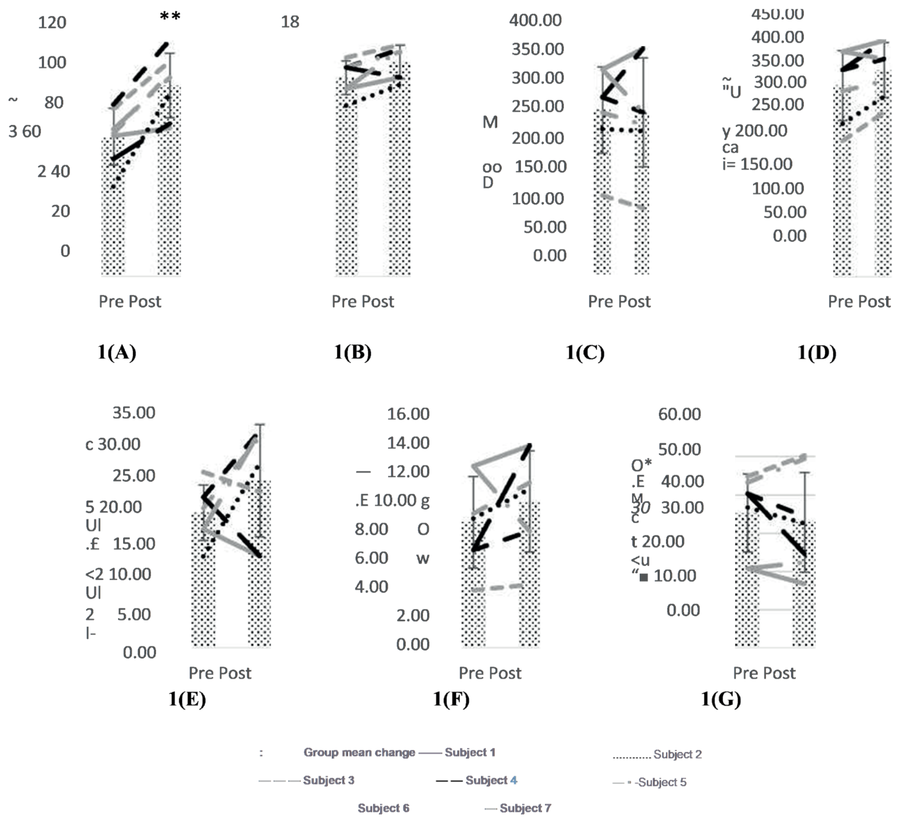

Figure 1. Comparative analysis of iron variables [1(A) iron, 1(B) hemoglobin, 1(C) UIBC, 1(D) TIBC, 1(E) transferrin saturation, 1(F) EPO, 1(G) ferritin] between the pre- and post-camp condition of MLD players with individual differences from pre to post condition.

[* = p < 0.05, ** = p < 0.01, UIBC= unsaturated iron-binding capacity, TIBC = total iron-binding capacity, EPO = erythropoietin]

Comparative profiling of cardio-respiratory variables between pre- and post-camp phases was represented in Table 2. Significant improvement was observed against some of the important physiological variables i.e., $\dot{V}O_{2max}$ (6.4%) ($p = 0.042$, $t = -2.572$) after camp conditions in comparison to pre-camp data. Whereas other cardiorespiratory variables i.e., HR at anaerobic threshold (1.0%), VO_2/HR (7.6%) were found to be increased and VE/VO_2(4.8%), VE_{max} (6.5%) was found to be decreased after camp but statistically insignificant manner.

Table 2. Comparative profiling of cardio-respiratory variables between the pre-and post-camp condition of athletes

Parameters	Pre-camp (n = 7)	Post-camp (n = 7)	t value	Sig.	% change
$\dot{V}O_{2max}$ (ml/kg/min)	64.50 ±4.31	68.66 ±5.26	–2.572	0.042*	6.4
HR at AT (bpm)	179.29 ±5.59	181.14 ±4.67	–0.734	0.491 ^{NS}	1.0
VO_2/HR (ml/beat)	19.40 ±4.40	20.87 ±5.34	–2.345	0.057 ^{NS}	7.6
VE/VO_2	31.16 ±4.12	29.67 ±2.27	1.109	0.310 ^{NS}	–4.8
VE_{max} (L/min)	114.41 ±15.83	106.94 ±27.66	1.306	0.239 ^{NS}	–6.5

*Values were expressed as mean ±SD, * = p < 0.05, NS= not significant, Sig. = level of significance, $\dot{V}O_2$max = maximal oxygen consumption, HR at AT = heart rate at anaerobic threshold point, VEmax = maximal ventilatory equivalent.*

Figure 2 represented the HR and lactate response (resting, peak, recovery 5 min, recovery 10 min) for both pre- and post-training conditions. Where, HR_{rest}, HR_{rec} (both 5 min and 10 min) were found to be significantly ($p < 0.05$, $p < 0.001$ respectively) decreased (1.8%, 2.3%, 4.7% respectively) in the post-training phase in comparison to pre-camp data. Whereas, only lactate peak was found to be significantly ($p < 0.001$) decreased (7.9%) in post-camp conditions.

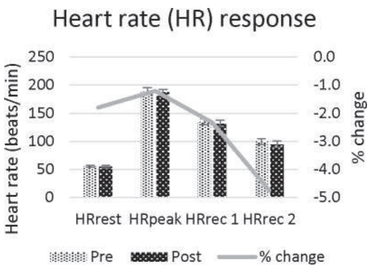

Figure 2(A)

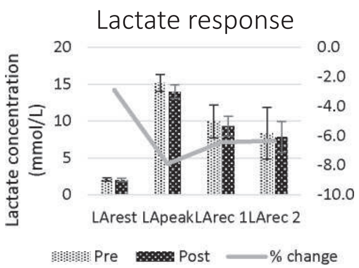

Figure 2(B)

Figure 2. Representing the (A) heart rate response and (B) lactate response. [HRrest = heart rate resting, $HR_{ma}x$ = heart rate maximum, $HR_{rec}1$ = heart rate recovery (5 min), $HR_{rec}2$ = heart rate recovery (10 min), LA_{rest} = lactate resting, LA_{peak} = lactatepeak, $LA_{rec}1$ = lactate recovery (5 min), $LA_{rec}2$ = lactate recovery (10 min)].

Table 3 depicts the comparative profiling of lung function variables between the pre-and post-camp phases of athletics players. Significant improvement ($p < 0.05$) was observed against PEF (10.7%) with $p = 0.013$ and $t = –3.496$ in the post-camp condition. Whereas all other lung function variables were found to depict statistically insignificant increase values in the post-camp phase in comparison to pre-camp data.

Table 3. Comparative profiling of lung function variables between the pre-and post-camp condition of athletics players

Parameters	Pre-camp (n = 7)	Post-camp (n = 7)	T value	Sig.	% change
FVC (L)	3.96 ±0.86	4.22 ±0.79	–0.684	0.519 ^{NS}	6.6
FEV1 (L/s)	3.48 ±0.69	3.63 ±0.73	–0.540	0.609 ^{NS}	4.4
FEV1/FVC (%)	85.57 ±4.93	87.86 ±4.91	–0.745	0.484 ^{NS}	2.7
PEF (L/s)	6.41 ±1.50	7.09 ±1.04	–3.496	0.013*	10.7
MVV (L/min)	134.00 ±23.79	138.63 ±19.93	–0.473	0.653 ^{NS}	3.5
VC (L)	4.55 ±1.17	4.70 ±1.01	–0.317	0.762 ^{NS}	3.4

Values were expressed as mean±SD, * = $p < 0.05$, NS = not significant, Sig. = level of significance, FVC = forced vital capacity, FEV1 = forced expiratory volume in 1sec, PEF = peak expiratory flow, MVV = maximum voluntary ventilation, VC = vital capacity.

Figure 3 depicted the percentage change in selected variables (i.e., $\dot{V}O_{2max}$, haemoglobin, iron content, and PEF) which were found significantly altered following LHTH training. Where, the group means change depicted 6.4%, 7.8%, 36.7%, and 10.7% changes by $\dot{V}O_{2max}$, haemoglobin, iron content, and PEF respectively.

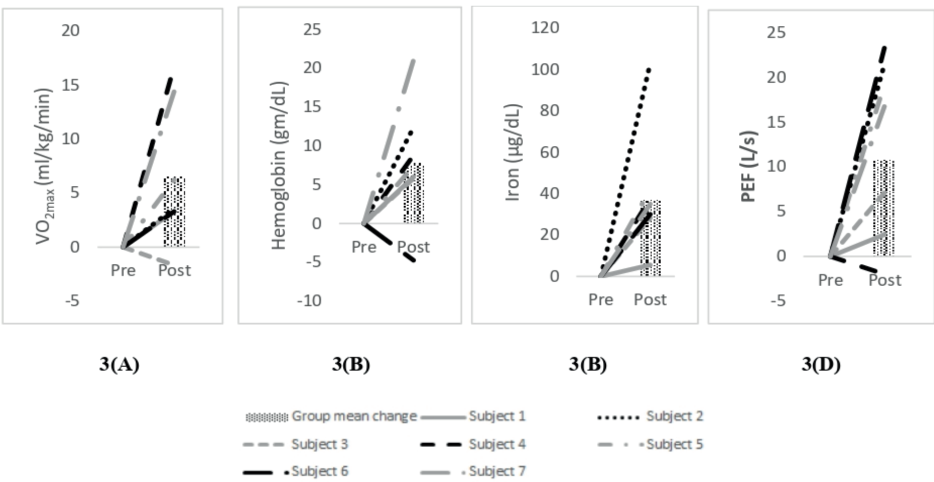

Figure 3. Represents the percentage change in some selected variables [(A) $\dot{V}O_{2max}$ (B) haemoglobin, (C) iron concentration, and (D) PEF values] following the LHTH training camp. Individual colored lines indicated individualizal plyers with histogram showing the group mean response. *[$\dot{V}O_2$max = maximal oxygen consumption, PEF = peak expiratory flow*]

Table 4 depicted the changes in performance timing at the individual level after the LHTH training camp where both pre- and post-performance timings were taken at sea level, where the performance change ranges from 0.3–2.6% at an individual level.

Table 4. Individual performance changes following LHTH at high altitude

Athlete	Gender	Event	Performance time PRE (mm: ss)	Performance time POST (mm: ss)	% change
Subject 1	F	1500 m	4:39.19	4:34.45	–1.7
Subject 2	F	1500 m	4:41.08	4:37.21	–1.7
Subject 3	M	800 m	1:54.38	1:54.06	–0.3
Subject 4	M	1500 m	4:23.06	4:16.31	–2.6
Subject 5	M	800 m	1:55.46	1:53.28	–1.9
Subject 6	F	1500 m	4:39.60	4:33.39	–2.2
Subject 7	M	800 m	1:51.52	1:49.79	–1.6

Discussion

The objective of the current study was to assess the alteration in cardiorespiratory variables, iron profile characteristics, after LHTH camping, and its relation with time trials of performance for trained MLD athletes. A significant improvement in VO_2max, HR response (resting, recovery), lactate response (peak), and PEF value was observed after 6 weeks of the LHTH camp in the current study, which was conducted at an altitude of 8000 feet (2400 m) above sea level. These improvements were also significantly correlated with improvements in haemoglobin mass and iron concentration.

The present study result was consistent with those of earlier studies where the result showed a significant increase in plasma EPO concentration (16.8%), hemoglobin (7.8%), transferrin receptor concentration (10.1%), plasma iron concentration (43.1%), and a significant decrease in serum ferritin concentration (10.7%) (Man et al., 2021; Chen et al., 2014; Stray-Gundersen et al., 2001; Muraoka & Gando, 2012; Płoszczyca et al., 2018). The hypoxic state at high altitude, which stimulates erythropoiesis and results in an apparent increase in oxygen supply to peripheral tissue, may be the cause of the current observation of better iron profile variables (Stray-Gundersen et al., 2001; Muraoka & Gando, 2012; Płoszczyca et al., 2018). A minimum exposure duration of 18 to 22 hours over the course of three to four weeks at an altitude of >2100 m to 2500 m is sufficient to stimulate erythropoiesis by lowering PaO2 and raising EPO levels (Ge et al., 2002; Wilber et al., 2007). Improvements in hematological variables, particularly those related to iron profile, hemoglobin concentration, and controlling erythropoiesis by stimulating erythropoietin (EPO), will all help to improve endurance capacity, running economy, and overall running timings in middle-long runners (Man et al., 2021; Płoszczyca et al., 2018).

Vargas-Pinilla (2014) found that after 3–4 weeks of residing and training at an altitude > 2000 m, overall Hbmass and/or RBC mass significantly increased by 6–9%. Total hemoglobin and RBC mass are increased as a result of increased erythropoiesis and reticulocytes brought on by hypoxia, which in turn results in decreased plasma volume as a result of water loss, associated with hyperventilation and fluid shifting to interstitial and intracellular areas. Even after returning to sea level, improved hemoglobin and red blood cell mass are closely correlated with excellent endurance performance (Vargas-Pinilla, 2014; Muraoka & Gando, 2012; Heinicke et al., 2005). Endurance capacity is increased by activating EPO, transferrin for iron metabolism and RBC synthesis, and the glycolytic enzymes (phosphofructokinase, hexokinase, and lactate dehydrogenase) with enhanced glucose uptake and pH regulation. Another crucial element is the hypoxia-induced factor (HIF-1) (Man et al., 2021; Semenza, 2004; Vogt et al., 2001).

According to the current study, high-altitude training results in a substantial ($p < 0.05$) improvement in $\dot{V}O_{2max}$ (6.4%) in the post-LHTH camp condition compared to the pre-camp result. The findings of the current study concur with those of Sinex and Chapman (2015) and Vargas-Pinilla (2014). According to the current research, high-altitude training will improve long-term adaptation and the ability of the cardio-respiratory system to function more effectively in aerobic conditions even when the workload is increased (Vargas-Pinilla, 2014). According to Ploszczyca et al. (2018), Heinicke et al. (2005), Vargas-Pinilla (2014), Muraoka and Gando (2012), and Stray-Gundersen et al. (2001), high-altitude induced hypoxia stimulates erythropoiesis and specifically improves the iron profile (EPO, Hb mass, etc.), which further stimulates and improves the anaerobic threshold, running economy, and ultimately the endurance capacity. Runners reported a considerable increase in muscle buffering capacity following two weeks of altitude training (between 2000 m and 2700 m), along with enhanced muscle myoglobin and oxidative capacity, which may also be the cause of the increase in $\dot{V}O_{2max}$. In order to boost endurance capacity, hypoxia-induced factor (HIF-1) persuaded adaptive regulation in skeletal muscle to activate EPO, iron metabolism, RBC synthesis, a glycolytic enzyme with improved glucose absorption, and pH regulation (Semenza, 2004; Vogt et al., 2001).

The present study depicted significant improvement in cardio-respiratory fitness as evidenced by i) an increase in maximal oxygen consumption ($\dot{V}O_{2max}$), ii) an increase in HR at anaerobic threshold, and iii) an increase in HR_{max} in post-camp condition after comparison with the pre-camp data. Heart rate response variables i.e., HR_{rest} ($p < 0.05$), recovery HR 5 min and 10 min (both $p<0.001$) were found to be significantly decreased with 1.8%, 2.3%, and 4.7% respectively in post-LHTH camp conditions which reflect the significant improvement although the HR_{peak} was not significantly improved. Cardiorespiratory factors from the current study are consistent with findings from earlier research by Sinex and Chapman (2015) and Vargas-Pinilla (2014). An increase in heart rate (HR) at the anaerobic threshold (AT) point, when comparing pre- and post-camp conditions, demonstrates a clear improvement in the anaerobic zone with stronger threshold response under increasing cardiac load (Vargas-Pinilla, 2014; Stray-Gundersen et al., 2001). A significant decrease in recovery HR and an insignificant decrease in resting HR was reported by Bhattari et al. (2018) and suggested that recovery completed within 5 min from step test cessation due to the parasympathetic reactivation as an effect of high-altitude training (Bhattari et al., 2018).

Along with the cardiorespiratory improvement the lactate rest, peak, recovery (5 min), and recovery (10 min) were found to be lower in the post-LHTH camp group with 2.8%, 7.9%, 6.5%, and 6.3% respectively in comparison to pre-camp data. The current finding is consistent with Vargas-Pinilla (2014), who observed that with increased endurance capacity, the total lactate response, including the peak and recovery, should decrease, indicating enhanced cardiorespiratory function. For a given workload, acute exposure to altitude causes an exacerbated lactate response characterized by increased lactate buildup in muscle and blood as well as lactate release from contracting muscle (Mazzeo, 2008). The levels of peak and submaximal blood lactate concentration do, however, decline relative to sea level (SL) with concurrent absolute exercise intensity as a result of acclimation. The drop in lactate happens despite the fact that VO_2 does not vary, and this phenomenon is known as the lactate paradox (Vargas-Pinilla, 2014; Mazzeo, 2008).

In the current study, under the lung function variables, only the PEF value showed a significant ($p < 0.05$) increase in the post-camp condition (10.7%). While all other lung function measures, including FVC, FEV1, FEV1/FVC, MVV, and VC, were shown to have improved after LHTH camp conditions by 6.6%, 4.4%, 2.7%, 3.5%, and 3.4%, respectively, the improvement was not statistically significant. The current study's findings are consistent with those of earlier research (Faramoushi et al., 2012; Valizadeh et al., 2012; Mason et al., 2000). According to the current research, decreased airway resistance and decreased air density at high altitudes lead to increased PEF (Faramoushi et al., 2012). The strengthening of ventilatory muscles during high-altitude training, where the air density and resistance, as well as atmospheric pressure at greater altitudes, were lowered, maybe the cause of the overall increase in lung function capacity (Faramoushi et al., 2012). High altitude induces a rise in the hormone adrenalin, which reduces the reversibility of the lungs and dilates blood vessels. As a result, airway resistance decreases, which may be another factor contributing to improved ventilation (Valizadeh et al., 2012).

The present study resulted in performance time trial improvement ranging from 0.3–2.6% (varies individually) following 6 weeks of LHTH camp at 2400m altitude which was supported by the significant $\dot{V}O_{2max}$ at an individual level. Although as per the literature, the LHTH-induced improvement in maximal aerobic power may be further supported by an increase in serum erythropoietin (EPO) levels and a series of accompanying physiological responses, e.g., increases in red cell volume (RCV) and hemoglobin (Hb) concentration, an increased erythrocyte concentration of 2,3-diphosphoglycerate (2,3-DPG) causing a rightward shift of the oxyhemoglobin dissociation curve, development of capillary density; an increase in mitochondrial capacity, oxidative enzyme activity, glycogen sparing, FFA mobilization, lactate threshold, muscle buffering capacity, and muscle myoglobin content have taken place after LHTH protocol as a result of the performance time decreased significantly with a sufficient stay at moderate-high altitude for 3–4 weeks' time (Muraoka & Gando, 2012). Some of these were measured in the present study and Hb concentration, iron content, HR response (resting, recovery), lactate response (peak), and PEF value were found to be improved significantly and support the fact of performance time trials improvement at the individual level.

One of the main drawbacks of the study is that there were only seven middle-long distance runners who took part. As a result, the study is regarded as a pilot study in a particular population cohort.

Conclusions

In order to improve running economy and maximum aerobic capacity, the LHTH protocol is especially helpful for endurance athletes (MLD runners). Significant improvements in cardio-respiratory variables, i.e., endurance capacity, running economy with a faster heart rate, and lactate recovery, were induced by high-altitude training. Decreased PaO_2 and a hypoxic stimulation at higher altitudes may trigger a number of transcription factors that control iron profile improvement added with improved lactate response. All participating athletes achieved lifetime personal best performances after the LHTH (overall group time trials improvement of 1.7%, individual improvement up to 2.6%). Individual improvements in maximal aerobic capacity (improved group mean of 6.4%, up to 16.7%) may be supported by improved erythropoiesis, iron metabolism, Hb concentrations, peak expiratory flow, glycolytic enzyme activity, improved oxidative capacity, and improved resting metabolic rate in response to a specific periodized training load with hypoxic stress at 2400 m altitude. The present study also suggests that periodization should be individualized and summative load volume response (addition of hypoxic physiological load and weekly training load) should be maintained during training. Also, the iron profile of those players before camp condition should be normal/optimal to get the LHTH benefits and they should get sufficient 6–7 days of initial physiological adaptation before starting training at high altitude. Altitude sickness and overtraining are two common concerns at LHTH training that should be closely monitored.

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