Research Paper:
Effects of Hypoxic and Normoxic Training in Altitude on HIF-1α and PGC-1α Levels in Elite Endurance Runners

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ABSTRACT

Introduction: Training at altitude or in a hypoxic environment has gained attention among athletes, coaches, and scientists to enhance sea-level performance. However, the efficacy of "Living-Low, Training-High, Training-Low" (LLTHTL) strategy to escalate the mechanisms associated with enhancing performance in the human athletes is still unknown. The present study aimed to investigate the effect of the LLTHTL on Hypoxia-Inducible Factor-1 alpha (HIF-1α) and peroxisome Proliferator-activated Receptor-Gamma Coactivator-1alpha (PGC-1α) levels in elite endurance runners.

Methods: The study has a crossover design in University laboratory. Eight elite male runners (Mean±SD age: 24.50±3.96 years; Mean±SD height: 179.75±4.62 cm; Mean±SD body mass: 67.37±3.42 kg; Mean±SD body mass index: 20.85±1.11 kg/m²) took part in the research. After 4 weeks of Living-Low (LL), the athletes performed 4 weeks Training-High (TH) and then, 3 weeks training-low (TL). Main Outcome Measures: Anthropometric parameters, 1500m running performance (1500 m), PGC-1α, and HIF-1α levels were measured in four different time points: pre-LL, post-LL, post-TH, and post-TL.

Results: There were no significant differences between the 4 time points for body mass and body mass index (P>0.05). The 1500m running performance was improved significantly (P<0.001) at post-TH as well as post-TL compared with the pre-LL and post-LL. TH decreased HIF-1α level but did not affect PGC-1α. Besides, TL increased both HIF-1α and PGC-1α.

Conclusion: Training at altitude reduces HIF-1α and training at sea-level increased PGC-1α and HIF-1α levels. Both types of training induced an improvement in the 1500m running performance . Athletes and coaches seek advice on the effective training strategy to enhance performance at different altitudes.

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Introduction

T raining at altitude or in a hypoxic environment to enhance sea-level performance has gained attention among athletes, coaches, and scientists [1]. The ability to tolerate high levels of altitude after living at a low altitude without disease is greatly desirable for endurance athletes [2]. Besides it is a practical model to get insight into physiological responses to hypoxia in humans [3]. Athletic endurance such as middle- and long-distance running often uses altitude training as an integral component of modern athletic preparation to enhance sea-level performance and to acclimatize to competition at altitude [4]. The rationale for training in hypoxia is to increase the metabolic stress on skeletal muscle beyond that achieved in normoxia [5].

Endurance performance can be improved through different altitude/hypoxic training methods: The Living High-Training High (LHTH), the living high-training low (LHTL), and the Living Low-Training High (LLTH) [1]. Although LHTH, LHTL, and LLTH approaches have shown usefulness in enhancing aerobic exercise performance and physiological adaptation in human athletes [1, 6, 7], the efficacy of "Living-low, Training-high, Training-low" (LLTHTL) strategy to escalate the mechanisms associated with enhancing performance in the human athlete is still unknown.

Intermittent or sustained exposure to hypoxic conditions is based on the hypothesis that short exposure to hypoxia is sufficient to induce positive muscular adaptation [8]. Short-term exposure to altitude has a positive acclimation in skeletal muscle [9-11] and improves muscle buffering capacity as well as glycolytic enzyme activities [12, 13]. However, sustained exposure to severe hypoxia results in negative effects on skeletal muscle function with a reduction in muscle oxidative capacity and loss of muscle mass [5]. The time course and exact mechanism of metabolic adaptation to the skeletal muscle with long-term altitude exposure need to be completely understood.

Hypoxia-inducible Factor-1 alpha (HIF-1α) and peroxisome Proliferator-activated Receptor-gamma Coactivator-1 alpha (PGC-1α) play a major role in the cellular response to hypoxia. HIF-1α is a transcription factor and the central mediator of hypoxic response, which regulates the expression of target genes important in angiogenesis, erythropoiesis, energy metabolism, and cell survival [14]. The activation of PGC-1α can be induced by hypoxia [15]. It stimulates mitochondrial bio-

genesis and improves exercise performance [16]. PGC-1α plays a key role in the skeletal muscle response to exercise [17] and is induced in response to exercise [18, 19]. HIF-1α protein levels increased in skeletal muscle in response to a single bout of exercise [20], and the effect of PGC-1α on HIF-1 may prolong the activity of HIF-1 allowing for a sustained adaptive response to exercise [21]. The Intermittent Hypoxic Exposure (IHE) has shown to promote an up-regulation of HIF-1α in human athletes [10, 22]. However, harmful effects on skeletal muscle function were induced by sustained exposure to severe hypoxia [5].

Given the vital role of PGC-1α in creating endurance adaptations, the effect of different exercise training modalities has been taken into consideration. However, it is unclear whether training in hypoxic conditions could affect PGC-1α expression. It is worth mentioning that the effect of training in hypoxia condition on PGC-1α expression has not been proven in the previous studies. The possible physiological effects of PGC-1α and HIF-1α and their associations with different environmental strategies are not usually taken into consideration in high altitude studies. Thus, it seems necessary to use a new training strategy to appreciate the mechanisms associated with improving the athletes' performance.

Previous studies have investigated the effect of hypoxic training on sea-level performance; however, the results about its efficiency are controversial [23, 24]. Improvement of fitness and performance capabilities at sea-level for elite athletes is a major challenge. To reach the upper limits of athletic ability, athletes and coaches are regularly searching for new and innovative ways of improving performance to gain a competitive edge. Both LHTL and LLTH are popular training approaches for many athletes; however, the optimal protocols to enhance athletic performance are unclear. Because little is known about the physiological effects of LLTHTL, the purpose of the present study was to determine the effect of this new training approach on the level of HIF-1α and PGC-1α in elite endurance runners, in an endeavor to provide more insight into the potential impact of this strategy on athletes' performance.

Participants and Methods

Study participants

In a repeated measures design, eight elite male runners (Mean±SD age: 24.50±3.96 y; Mean±SD height: 179.75±4.62 cm; Mean±SD body mass: 67.37±3.42 kg; Mean±SD body mass index: 20.85±1.11 kg/m^2) were recruited from the Iranian national athletic team run-

ners, including 2 runners for a 3000-m run, 2 runners for a 5000-m run, and 4 runners for a 10000-m race in the form of track and field. The volunteer subjects attended the Asian Championship of the last three years. In addition, they were busy with naitonal training camp for Asian Championships in Bahrain 2019 when the research was conducted. Moreover, none of the subjects were acclimatized or recently exposed to altitude.

Study participants completed a medical questionnaire before the study to provide information about the participants' health condition and ensure a lack of history of cardiovascular, neuromuscular, hematological, or musculoskeletal disease or using pharmacological treatments during the study period. Before their participation, all subjects were informed of the procedures, risks, and expected benefits, and signed an informed consent form.

The study was approved by the Institutional Ethics Committee (Olympic committee letter No. 2634) and performed per the ethical standards of the Declaration of Helsinki. After the purpose and risks of the protocol were explained, oral and written informed consent was obtained from the study participants.

Study design

The present study was conducted as follows (Figure 1, Table 1). athletes were residents at normoxia condition (LL+1200m) and trained for 4 weeks, afterward they trained for 4 weeks at high altitude (TH+2500m). afterward they trained for 4 weeks at normoxia condition (LL+1200m). afterward they trained for 3 weeks at see level (TH+0). In the test sessions, we assessed body composition, blood samples collection, and 1500m running performance test at four moments: Pre-LL, post-LL, post-TH, and post-TL.

The body composition and blood test measurements were begun two days before LL, and one day after LL, TH, and TL. The 1500m running performance test was measured one day before LL, and two days after LL, TH, and TL. All tests were performed in the normoxia environment. The athletes experienced the hypoxia training at 2500 m altitude in the Zagros Mountains, Zalayan defile, 25 km away from Borujerd, Lorestan.

Exercise training

The athletes were required to follow the training program of the R2M method (Table 1). Training sessions were performed in hypoxic (TH; FiO_2=16%; equivalent to 2500 m above sea level) and normoxic (TL; FiO_2=20.9%; 1200 m above sea level) environment. R2M stands for Running at Middle distance to Marathon. Table 1 and Figure 1 present the description of R2M training at the hypoxic and normoxic conditions. The training included a combination of endurance, speed, strength, interval, and plyometric training with different intensity and volume [25]. The runners performed 16 training sessions per week within 4 weeks in high altitude and 3 weeks at sea level (2 or 3 sessions per day at 6 AM, 10 AM, and 4 PM). Further details can be found in this study [26].

A physician accompanied the athletes at all times during the study. All runners enjoyed the same controlled conditions in terms of accommodation, nutrition, rest, and train-
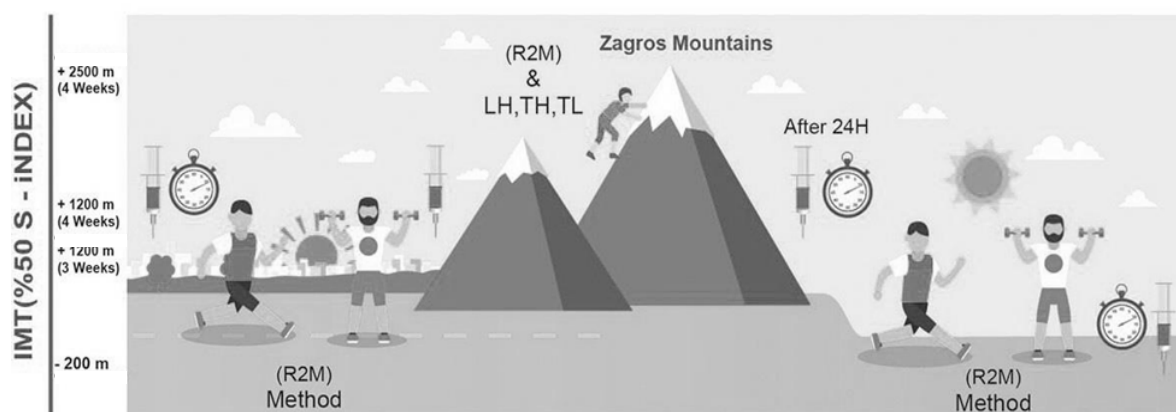

Figure 1. Schematic view of the training protocol

R2M method: Acronym for running at Middle Distance to Marathon.

LH: Live High; TH: Train High; TL: Train Low

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ing. A complete description of the protocol and is available elsewhere [26].

Anthropometry measurements

Height was measured to the nearest 1.0 cm while the participants were barefoot. Body weight was measured to the nearest 0.1 kg, with the participants wearing light clothes and no shoes. BMI was calculated as weight (kg) divided by height (m) squared. The same researcher took all the body weight and height measurements in triplicate and the mean values were calculated [27].

1500m running performance

An individual 1500-m 1500m running performance on a 400 m synthetic athletic track was completed at pre-LL, post-LL, post-TH, and post-TL, with split times and final time recorded on a stopwatch (Seiko, S120-4020, Tokyo, Japan). After a 30-min warm-up, the 1500m running performance was performed. The endurance run-

Table 1. Experimental design

Phases	Week	Day	Sessions/Day	Days/Week	Training Protocol
Pre-LL		1-3			Normoxic Level: Body composition, time trial, blood sample
LL Altitude: 1200 m Duration: 4 weeks FiO_2: 20.9%	1		2	2	Endurance running; tempo endurance
	2		3	3	Endurance running; strength of strength: tempo endurance
	3		2	2	Endurance; speed; strength of strength; tempo endurance; two endurance speeds; plyometric strength
	4		3	2	Endurance speed; strength of strength; tempo endurance; two-stroke speed; plyometric power speed; isodynamic
Post-LL		32-33			Hypoxic altitude: Body composition, time trial, blood sample
TH Altitude: 2500 m Duration: 4 weeks FiO_2: 16%	5		3	2	Endurance speed; strength of strength; tempo endurance; two-stroke speed; plyometric power speed; isodynamic
	6		3	3	Endurance speed; strength of strength; tempo endurance; two-stroke speed; racing strategy; plyometric power speed; isodynamic
	7		2	2	Endurance speed; strength of strength; tempo endurance; two-stroke speed; racing strategy; plyometric power speed; isodynamic
	8		3	2	Endurance speed; strength of strength; tempo endurance; two-stroke speed; racing strategy; plyometric power speed; isodynamic
Post-TH		62-63			Hypoxic altitude: Body composition, time trial, blood sample
TL Altitude: 1200 m Duration: 3 weeks FiO_2: 20.9%	9		2	2	Endurance speed; strength of strength; tempo endurance; two-stroke speed; racing strategy; plyometric power speed; isodynamic
	10		3	3	Endurance speed; strength of strength; tempo endurance; two-stroke speed; racing strategy; plyometric power speed; isodynamic
	11		2	2	Strength of strength; tempo endurance; two stamina; power speed; plyometric strength
Post-TL		84-85			Normoxic Level: Body composition, time trial, blood sample

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LL: Living-low; TH: Training-high; TL: Training-low; Pre-LL: Before living-low; Post-LL: After living-low; Post-TH: After training-high; Post-TL: After training-low

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ners were required to avoid intensive exercise on the day before the trial. The 1500m running performance was performed at 16:00 – 17:00 local time. The running time was expressed rounded to two decimal places.

Blood sampling

Each participant reported to the laboratory at the same time of day (from 8:30 AM to 9:30 AM) after a 12-h overnight fast. Blood samples (5 mL) were drawn from an antecubital vein by a trained phlebotomist, while the runners were in a sitting position in a fasting state to determine the changes in HIF-1α and PGC-1α using the ELISA kit according to the manufacturer's protocol. HIF-1α and PGC-1α levels were measured using a kit with 0.1 ng/mL sensitivity (ZellBio GmbH, Ulm, Germany).

Statistical analysis

The obtained data are presented as Mean±SD. The normal distribution of data was evaluated with the Shapiro–Wilk test. Analysis of Variance (ANOVA) with repeated measurements was used to determine the mean difference of quantitative parameters measured in 4 time points (pre-LL, post-LL, post-TH, post-TL). Afterward, if the ANOVA showed a significant main effect, we would determine the significance between each measurement by using the LSD post hoc test. Differences were considered significant at $P<0.05$.

Results

Body composition

Table 2 showed no significant differences between the 4 time points for body mass and BMI ($P<0.05$).

1500m running performance

Repeated ANOVA showed a significant main effect of time for running performance test ($F=20.82$; $P=0.000$). Figure 2 showed that the running performance test was significantly lower ($P<0.001$) at post-TH and post-TL compared with pre-LL and post-LL. No significant difference was detected between post-TH and post-TL ($P>0.05$).

Table 2. Anthropometric data of the participants

Levels	Pre-LL	Post-LL	Post-TH	Post-TL
Age (y)	24.5±3.96	-	-	-
Height (cm)	179.75±4.62	-	-	-
Body mass (kg)	67.37±3.42	67.3±3.4	66.32±3.22	67.11±3.39
BMI (kg/m²)	20.85±1.11	20.85±1.1	20.71±1.08	20.79±1.09

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Values are presented as means±SD (n=8). Abbreviations: BMI, body mass index; Pre-LL, before living-low; Post-LL, after living-low; Post-TH, after training-high; Post-TL, after training low.
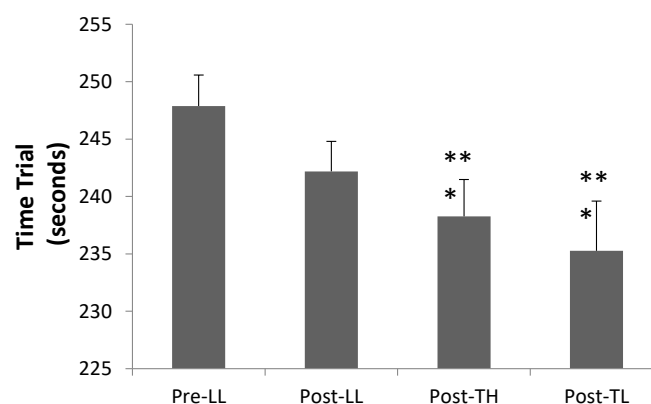

Figure 2. Time trial (seconds) during four time points of pre-LL, post-LL, post-TH, and post-TL

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Values are presented as Mean±SD (n=8); *** Significant difference in comparison with pre-LL and post-LL ($P<0.001$).

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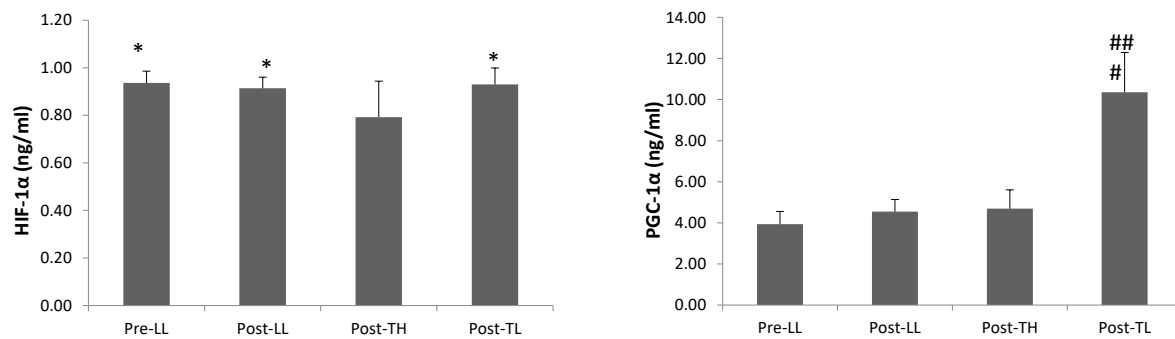
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Figure 3. HIF-1α (a) and PGC-1α (b) during four time points of pre-LL, post-LL, post-TH, and post-TL

Values are presented as Mean±SD (n=8).

* and ** Significant difference in comparison with post-TH (P<0.05; P<0.001); ### Significant difference in comparison with pre-LL, post-LL and post-TH (P<0.001).

HIF-1α

Repeated ANOVA showed a significant main effect of HIF-1α level (F=4.3, P=0.02). HIF-1α decreased significantly at post-TH compared too pre-LL (P=0.009) and post-LL (P=0.022) and then increased significantly (P=0.011) at post-TL compared with post-TH (Figure 3A).

PGC-1α

Repeated ANOVA showed a significant main effect of level (F=66.14, P=0.000) for PGC-1α. PGC-1α showed no significant effect after altitude training (post-TH). However, PGC-1α increased significantly (P<0.001) at post-TL than pre-LL, post-LL, and post-TH (Figure 3B).

Discussion

The present study verified the effect of a new training strategy, i.e. "Living-low, Training-high, Training-low" (LLTHTL) on HIF-1α and PGC-1α levels in elite Iranian endurance runners. This new training protocol under different environments included residing at sea-level, training at natural altitude conditions, and then training at or near sea-level.

Given the wide range of training intensities and frequencies that have been completed under different levels of hypoxia (altitude and duration of exposure), the interpretation of the findings from LLTHTL experimental work is problematic. Thus, the physiological and performance responses to the present new training require further investigation. Two major findings of our study are novel. First, 4 weeks of hypoxic training

(altitude=2500 m) decreased HIF-1α but did not affect PGC-1α. Second, 3 weeks of normoxic training (altitude =1200 m) increased both HIF-1α and PGC-1α. Since the present study was performed in a very small number of participants, the results should be considered cautiously until they are confirmed by further investigation. However, to our knowledge, this is the first study addressing the potential effect of the LLTHTL approach on the physiological values. This is an area of great interest given the performance relevance of the potential effects of this kind of exercise training in endurance athletes.

HIF-1α protein is a major hypoxia sensor and transcriptional regulator of oxygen-dependent gene expression [22, 28] during which it is stabilized and functioned in regulating angiogenesis through targeted activation of VEGF in human skeletal muscle [20, 29]. The present result agrees with previous studies' results. The amount of HIF-1α mRNA in muscle decreased after 3 weeks of intermittent hypoxic exposure in male endurance athletes [8]. On the other hand, the present result is not in line with the previous result. Six weeks of training program regulated HIF-1α gene expression in vastus lateralis by 104% [22]. Six weeks of training under hypoxic conditions (simulated altitude of 3850 m, 30 min, 5 d/wk) with both low and high-intensity training increased HIF-1α mRNA and mitochondrial density [30]. HIF-1α mRNA concentrations were augmented significantly in repeated sprint sessions in the hypoxia group [31]. Differences in HIF-1α expression across these studies may be explained, at least in part, by the differences in the number of hypoxic exercise bouts over a different time course and level of altitude training.

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The present study showed that HIF-1α increased significantly after 3-week normoxic training (R2M) at sea-level (training-low). In agreement with the present finding, a transient increase in HIF-1α/HIF-2α mRNA has been previously observed after an acute 3 h exercise about at approximately 50% of maximum capacity exercise in normoxic conditions [32]. LHTL increases HIF-1α mRNA responses to the acute hypoxic test and induces a higher sensitivity of HIF-1α mRNA to acute hypoxia in elite athletes [33]. Otherwise, a normoxic one-leg training program (45 min/d, 7 times in 10 days) did not affect HIF-1 α mRNA in vastus lateralis muscle [34]. Faiss et al. detected no change in HIF-1α after repeated sprint sessions in normoxia [31].

These discrepancies of results in normoxic training (training-low) may due to the different methodologies used such as intensity, volume, as well as duration of normoxic training. In the present study, the protocol of the sustained periods of hypoxic training (4 weeks) and normoxic training (3 weeks) was sufficient for inducing a significant downregulation and upregulation of HIF-1 α, respectively, suggesting that the augmentation in HIF-1 α mRNA leads to downstream activation of HIF-1 dependent pathway [35].

The present study is one of the first works that assesses the HIF-1α response after a chronic hypoxic and normoxic exercise in humans. When the current work is taken together with previous investigations, it suggests that the duration, temporal nature, or intensity of hypoxia and normoxic exposure could be a critical factor in human HIF-1α mRNA expression and subsequent adaptations that may prove beneficial to performance at altitude as well as at sea-level.

Some evidence suggests that a more pronounced transcriptional response of genes is observed after LLTH interventions, which could be partly related to changed levels of the regulatory HIF-1α. HIF-1α is continuously degraded under normoxic conditions but stabilized under hypoxia, which blocks its ubiquitin/proteasome-dependent degradation through the inhibition of prolyl hydroxylase activity [36-38]. HIF-1α protein levels in normoxia through mechanisms that generally differ from those used under low oxygen concentration is up-regulated by several growth factors, hormones, and cytokines. These agents act by increasing HIF-1α gene transcription and or mRNA translation without affecting protein stability [39, 40], and, thus, can cooperate with hypoxia to induce HIF-1α accumulation in an additive manner [41].

The present study did not show any effect on PGC-1α after 4 weeks of hypoxic training. However, PGC-1α level was at higher values at sea-level after 3 weeks of normoxic training. Similarly, PGC-1α decreased after 4 weeks of repeated sprint cycling in the hypoxic training group compared with the normoxic group, following a cycle performance test [31].

Taking into consideration that the present study was the first study performed with athletes and conducted in the hypoxic and normoxic environments, a positive outcome revealed. That is the R2M method utilized in the normoxic and hypoxic environment was well tolerated by the athletes. Also, the hypoxic training strategy appeared to be insufficient to cause further changes in the PGC-1α response. Whether PGC-1α assessment is a sensitive indicator of the changes resulting from hypoxia training exposure, need to be further examined, with the consideration of the contradictory results from the literature.

Our present findings are not in line with previous studies' results. Several human studies were conducted to investigate the physiological and cellular responses to hypoxic and normoxic training that occur due to repeated or chronic exposure. However, the results were contradictory and inconsistent. In a recent animal model study, PGC-1α and mitochondrial transcription factor A were higher in the skeletal muscle of the highland than the lowland deer mice [42]. The addition of two training sessions per week under hypoxic conditions (FiO_2=0.145) to the normal training over 6 weeks reported significant increases in mRNA levels of PGC-1α, PFK, COX1, and COX4, but no significant change in VEGF after training [22]. PGC-1α is the most critical angiogenic growth factor, which increases the VEGF mRNA expression observed after exercise protocols given the regulation of PGC-1α by post-translational modifications [43, 44]. These conflicting results may be due to methodological differences, including the dose of hypoxic stimulus, type, and intensity of training, participant training status, and time-point to maximize the effectiveness of hypoxic and normoxic training.

The present study revealed that 4 weeks of training at moderate altitude (2500 m) and training at low altitude (1200 m) improved the 1500m running performance more than 4 weeks living low (1200 m). These findings denote the beneficial effect of training at altitude and sea level in improving the running time. 5000-m run1500m running performance time was significantly improved only over the initial sea-level value in the LHTL group and was similar 7, 14, and 21 days post-altitude,

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suggesting that the beneficial effects of LHTL may last for up to 3 weeks [45]. A combined approach of live high/train low plus train high (LH/TL+TH) significantly increased 3-km 1500m running performance performance time immediately after altitude in well-trained endurance runners [46]. However, several previous studies did not confirm our findings [47-49].

In summary, we can explain our findings by the persistent effect of altitude training on sea-level training. It was hypothesized that the return to sea level after an altitude training camp has three phases [50]: The first phase is positive and is observed during the first 2–4 days, the second phase is a phase of the progressive re-establishment of sea-level training volume and intensity where the probability of good performance is reduced, and the third phase is the optimal delay for competition and may last 15–21 days after return to sea level which is characterized by a plateau in fitness [50]. Therefore, the post-training effect needs further scientific investigation.

Enhancement of competition performance is an important goal and 1500m running performance or performance tests are arguably the most appropriate measures of progress during a season. The timing of performance after altitude training is an important factor to induce performance gains from physiological adaptations following altitude training. The dose-response to hypoxia seems to be a key issue, and differences in the methodology employed in the studies may be a limiting factor in establishing the most effective protocol. The optimal time to maximize performance gains at sea-level following altitude training is a key feature of the success of any altitude training intervention.

The present study has several limitations. The first limitation is the absence of a control group, to confirm whether any change in performance is a result of altitude or a training effect. Second, sample sizes are often low in studies of elite athletes, so conventional statistical approaches may not be able to detect small changes in performance that are important for athletes. Further investigations with a larger sample size are required to determine optimal training programs, e.g. the oxygen level, duration, and frequency to understand the biological mechanisms of the acclimation. Furthermore, the present investigation did not measure the ergo-spirometry and hemodynamic parameters. However, these assessments are complex and expensive. Besides, we could not measure VO2 before, during, and after hypoxic and normoxic training due to technical reasons.

Conclusion

The present study demonstrated that hypoxic training at 2500 m decreased HIF-1α, and normoxic training at 1200 m increased HIF-1α and PGC-1α levels in endurance elite runners. Besides, the 1.500-m trial time tended to improve after hypoxic training and normoxic training. These outcomes suggest that the enhanced running time resulting from long hypoxia and normoxic training could, in part, contribute to improved endurance performance in trained athletes.

In this study, we used LLTHTL as a new training approach for elite endurance runners, which may have the potential to cause additional changes in the physiological parameters as compared with other training methods. However, its efficacy and biological mechanisms of the acclimation to LLTHTL have not been thoroughly examined in the human field. Additionally, it may be speculated that LLTHTL could be enough to obtain similar adaptations. Nevertheless, further studies are needed to confirm these conclusions.

As for practical applications, athletes and coaches seek advice on the best effective protocol to gain a competitive edge. Therefore, this type of training could be helpful for already well-trained athletes to increase their training stimulus, and consequently, their training adaptation. Other combinations of altitude training and exposure are needed for substantial enhancements in 1500m running performance.

Ethical Considerations

Compliance with ethical guidelines

The study was approved by the Research Ethics Committee of the Sport Sciences Research Institute.

Funding

Authors' contributions

Conceptualization: Rahman Soori, Mahla Mohamad Zadeh, Siroos Choobineh; Methodology: Amine Ghram, Mahla Mohamad Zadeh, Roohallah Mohammadi Mirzaei; Investigation: Mahla Mohamad Zadeh and Roohallah Mohammadi Mirzaei; Writing – original draft: Rahman Soori, Amine Ghram; Writing – review & editing: Amine

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