รายงานวิจัยฉบับสมบูรณ์

โครงการ ผลของการฝึกออกกำลังกายบนที่สูงกว่าระดับน้ำทะเลต่อตัวแปรในเลือดและกลไกที่อยู่เบื้องหลังของกลุ่มที่ตอบสนองดี และไม่ดีต่อการฝึกบนที่สูงในนักกีฬาฟุตบอล

The effects of low altitude training on hematological variables and mechanisms behind responders and non-responders in soccer athletes

โดย ผศ.ดร. อภิวันท์ มนิมนากร

เดือน ปี ที่เสร็จโครงการ

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สัญญาเลขที่ MRG5680084

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**ผู้วิจัยสังกัด ภาควิชาสรีรวิทยา คณะแพทยศาสตร์
มหาวิทยาลัยขอนแก่น**

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย

(ความเห็นในรายงานนี้เป็นของผู้วิจัย สกว. ไม่จำเป็นต้องเห็นด้วยเสมอไป)

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(The effects of low altitude training on hematological variables and mechanisms behind responders and non responders in soccer athletes)

Investigator : ชื่อนักวิจัย และสถาบัน
ผศ.ดร. อภิวันท์ มนิมนากร ภาควิชาสรีรวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
E-mail Address : Apiwanta@yahoo.com, mapiwa@kku.ac.th
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บทคัดย่อ
การฝึกออกกำลังกายบนที่สูงคือการศึกษาเกี่ยวกับการนำนักกีฬาซึ่งมีที่อยู่อาศัยที่พื้นราบหรือระดับน้ำทะเลขึ้นไปฝึกซ้อมบนที่สูงทั้งระยะเวลาสั้นและยาวเพื่อผลในการปรับตัวของร่างกาย เช่น การเพิ่มขึ้นของเม็ดเลือดแดง (RBC) และฮีโมโกลบิน (Hb) ปัจจัยสำคัญต่อการปรับตัวเหล่านี้รวมทั้งการสร้างสารอีริทโทรพอยอีทิน (EPO) ขึ้นอยู่กับเวลาและความหนักของภาวะขาดออกซิเจนและการฝึกเมื่ออยู่บนที่สูง การศึกษาเพื่อเพิ่มสมรรถภาพในกีฬาฟุตบอลนั้นต้องการร่างกายที่มีความทนทาน ความแข็งแรงและความเร็ว การฝึกออกกำลังกายบนที่สูงอาจเป็นวิธีที่สามารถเพิ่มสมรรถภาพในกีฬาฟุตบอลดังกล่าวได้

วัตถุประสงค์
งานวิจัยนี้ต้องการศึกษาผลของการฝึกบนที่สูงจริงร่วมกับเครื่องจำลองบรรยากาศและการให้ธาตุเหล็กเสริมต่อการเปลี่ยนแปลงของตัวแปรในเลือด และสมรรถภาพทางกาย อีกทั้งต้องการศึกษาการเปลี่ยนแปลงทางสรีรวิทยาเช่น ความแปรปรวนของอัตราเต้นของหัวใจ (HRV) ส่วนประกอบของร่างกายและความเข้มข้นของแล็กเตทในเลือดในนักกีฬาฟุตบอล

วิธีการทดลอง
อาสาสมัครนักกีฬาฟุตบอล เพศชาย 40 คน ถูกแบ่งออกเป็น 4 กลุ่มๆละ 10 คน ได้แก่ 1. กลุ่มฝึกที่พื้นราบ (ST) 2. กลุ่มฝึกที่พื้นราบร่วมกับได้รับธาตุเหล็ก (SI) 3. กลุ่มฝึกบนที่สูง (AT) 4. กลุ่มฝึกบนที่สูงร่วมกับได้รับธาตุเหล็ก (AI) ได้มีการจัดให้นักกีฬาเข้ากลุ่มโดยมีทักษะทางกีฬาฟุตบอลโดยเฉลี่ยเท่ากันทุกกลุ่ม การฝึกใช้โปรแกรมการฝึกฟุตบอลนาน 8 สัปดาห์เหมือนกันทุกกลุ่ม ทั้งบนที่สูง 852 เมตร ร่วมกับเครื่องจำลองบรรยากาศเทียบเท่าความสูง 3,300 เมตร และฝึกที่พื้นราบ 123 เมตรเหนือกว่าระดับน้ำทะเล นักกีฬาถูกวัดค่าต่างๆทั้งก่อนการฝึกและภายหลังการฝึก 1 วันและ 14 วัน

ผลการทดลอง

สาร EPO เพิ่มขึ้นอย่างมีนัยสำคัญ ($p<0.05$) ใน AI (31.7±29.4), AT (14.8±11.0) มากกว่า กลุ่ม ST (4.8±14.2) ภายหลัง 8 สัปดาห์ของการฝึกบนที่สูง RBC เพิ่มขึ้นอย่างมีนัยสำคัญ ($p<0.05$) ใน AI (3.0±2.8), AT (2.8±2.8) เทียบกับกลุ่ม ST (-1.8±4.1) ภายหลัง 8 สัปดาห์ของการฝึกบนที่สูง การวัดภายหลังจากการฝึก 14 วัน พบว่า ค่า HRV (RMSSD and HF) เพิ่มขึ้นอย่างมีนัยสำคัญ ($p<0.05$) ในกลุ่ม AT (10.7± 55.6 ms และ 12.4 ± 96.5 ms^2) เทียบกับกลุ่ม ST (53.0 ± 53.0 ms และ -54.8 ± 106.3 ms^2), ± 90% CL ร้อยละของไขมันร่างกาย ลดลงอย่างมีนัยสำคัญ ($p<0.05$) ในทุกกลุ่มเมื่อเทียบกับค่าตั้งต้น (AT=18.4, 14.9%; AI=17.5, 14.8%; ST=18.9, 16.1%; SI=17.0, 14.3%) mean ±SD การวัดภายหลังจากการฝึก 1วัน พบว่า ค่าแล็กเตทในเลือดในกลุ่ม AT และ AI เมื่อเทียบกับค่าตั้งต้น อย่างไรก็ตามในกลุ่ม ST และ SI พบว่าลดลงอย่างมีนัยสำคัญ ($p<0.05$) ทั้งภายหลังจากการฝึก 1วันและ 14 วัน

สรุปผลการทดลอง

จากผลการทดลองที่ได้แสดงให้เห็นอย่างชัดเจนว่าการฝึกบนทีสูงร่วมกับการฝึกด้วยเครื่องจำลองบรรยากาศสามารถเพิ่มค่าตัวแปรในเลือดและค่าสมรรถภาพทางกายบางตัวในนักกีฬาฟุตบอล นอกจากนี้ยังความแปรปรวนของอัตราการเต้นของหัวใจเปลี่ยนแปลงดีขึ้นซึ่งมีความสำคัญกับการทำงานของระบบประสาทอัตโนวัติ การเปลี่ยนแปลงดังกล่าวข้างต้นอาจมีอิทธิพลต่อความสามารถในการฝึกซ้อมในนักกีฬาฟุตบอลและอาจส่งผลต่อการแพ้ชนะในการแข่งขัน

คำสำคัญ : red blood cells, erythropoietin, root mean square, body fat mass, blood lactate, live high train high

Abstract

Training at altitude is the study that brings the athletes from the sea level to a higher altitude for short or long training periods in order to study the effects of adaptation of their bodies such as hemoglobin production and red blood cell content. As the hypoxic dose emerges as a key factor of erythropoietin adaptations, selection of the appropriate altitude and duration of the stay or training period are important factors. Studies to improve soccer performance have often focused on technique and tactics at the expense of physical resources such as endurance, strength, and speed. The altitude training is the potent method that may use to improve the performance and ability of athletics.

Objectives: For these reasons, in our research, we mainly focused on studying and comparing the results of training at altitudes that affect hematological variables and physical fitness. This research also aimed to investigate the effects of natural altitude training supplemented with intermittent hypoxic training (IHT) and iron supplementation on heart rate variability (RMSSD, LF, HF LF/HF ratio), body composition in and blood lactate concentration in soccer players.

Methods: The forty volunteer soccer players were divided into four groups to investigate the effects of iron supplementation in both altitude and sea level training athletes. The first group, the sea-level training (ST;N=10), the second group, Sea-level training with iron supplementation (SI;N=10), group, the third group altitude training (AT;N=10) and lastly the fourth group. Altitude training with iron supplementation (AI;N=10). All athletes were matched on their soccer ability. Training soccer program in eight weeks similarly all group. This study was conducted at the two stations; the altitude group was trained Khonkaen University observatory on Chulaporn Dam, Chaiyaphum province which is 852 m high above the sea level plus simulate altitude which is about 3,300 meters above the sea level, sea level group was trained at Nakhonratchasima Ratchabhat University Stadium, Nakhonratchasima province which is about 123 meters above the sea level.

Results: There was a significantly increased ($p<0.05$) in EPO in AI (31.7±29.4), AT (14.8±11.0) greater than ST (4.8±14.2) groups following 8 week altitude training, Red blood cell significantly increased ($p<0.05$) in AI (3.0±2.8), AT (2.8±2.8) compared ST (-1.8±4.1) groups following 10 week altitude training. By day 14 post camp, RMSSD and HF increased substantially in the AT group (10.7± 55.6 ms and 12.4 ± 96.5 ms^2) compared with ST group (53.0 ± 53.0 ms and -54.8 ± 106.3 ms^2), ± 90% CL. Percent body fat were substantially reduced in all groups compared to their baselines (AT=18.4, 14.9%; AI=17.5, 14.8%; ST=18.9, 16.1%; SI=17.0, 14.3%) mean ±SD. By day 1 post camp, blood lactate concentration showed higher level in both altitude training groups (AT and AI) compared to baseline. However, sea-level groups (ST and SI), blood lactate concentration significantly decreased in all day 1 and day 14 post camp.

Conclusion: Our data strongly suggested that training at altitude significantly enhanced some hematological variables and physical performances in soccer players. The eight weeks of living and training at low altitude with 15 min daily of IHT improves heart rate variability important to autonomic nervous system especially parasympathetic function improvement. These changes may influence training ability for soccer players and may lead to win in match competition.

Keywords: red blood cells, erythropoietin, root mean square, body fat mass, blood lactate, live high train high

Executive Summary

Training and living at the low altitude (825meters above sea level) conjunction with both breath hypoxia gas (FiO_2 15%) and iron supplementation has been used in this study. This form altitude training showed substantial improvement in some hematological variables, as well as physical fitness in soccer players.

The major findings of the study were classified into many points. Firstly, the level of EPO significantly increased after the athletics were trained at the real altitude for eight weeks which resulted in the improvement of oxygen consumption. Secondly, the iron supplementation elevated the hematological markers such as EPO and RBC and improved both aerobic and anaerobic. Moreover, the iron treatment also enhanced the overall physical performance of soccer players. The combination treatment of altitude level and iron supplementation was the best treatment which strongly enhanced the broadly physical performance and blood parameters of the soccer players. In addition, this study reflected that altitude training more likely to induce autonomic nervous system via sympathetic outflow. The sympathetic and parasympathetic nervous systems are responsible for the regulations of several physiological responses including heart rate, respiratory rate and substrate utilization. The other important finding is blood lactate concentration which showed less reduction immediately after short distance running, in other word the athletes’ muscle exposed to acidic environment but gave efficiency work through physical fitness. This phenomenon indicated our protocol (altitude training with soccer training program in this study) effective to improve anaerobic based performance (100m and 400m run). Finally, the body composition did not alter with this 8-weeks altitude training or live high train high program.

In conclusion, this exercise strategy can be applied for soccer training program and other sports. These results may use for further study in particular health promotion in normal and patient subjects.

Introduction

Altitude training has been applied to develop to athlete's sea-level physical performance. In addition, several publications have been reported that altitude training improved aerobic base performance (Hamlin et al., 2013). Several approaches and modifications for altitude training have been used such as live and train high at altitude (LHTH) and live high train low at sea level (LHTL). However, there were no clearly conclusion that indicated the method and dose which strongly increased athlete capacity, neither any literature suggested the effected period of altitude training.

Training at altitude is the study that brings the athletes from the sea level to a higher altitude (more than 500 meters above sea level) in order to study the effects of adaptation of their bodies that has been interest since 1968. Several approaches and modifications for altitude training have been used such as live and train high at altitude (LHTH) and live high train low at sea level (LHTL). Theoretically, the body will adapt by breathing more frequently to bring more oxygen in to the cells then athletes should acquire the beneficial effects of altitude acclimatization, particularly stimulation of the oxygen delivery system increasing in the total body hemoglobin for maximizing oxygen transport and utilization, and enhancing ability to train without reducing training intensity (Zhang et al, 2007). For long term exposed to altitude, the deleterious effects of low partial pressure of oxygen in blood force the human body to adapt and change, such as increasing the number of red blood cells and subsequently performance gain (West et al., 1983). Moreover, the body will be adapted by increasing erythrocyte to carry with oxygen.

The adaptation at the physiological level was indicated by hematological parameters such as erythropoietin (EPO), red blood cell content (RBC) and total iron-binding capacity (TIBC). The increase of blood parameters after altitude training leaded to the increase of physical performance in athletes included speed, strength, endurance, jumping, oxygen consumption and also skill (Bailey et. al., 1988, Ekblom, 1991). On the other hand, some literature suggested that altitude training was no effect on hematological parameters (Bangsbo et al, 2001)

Soccer is one of the most widely played and complex sports in the world, where players need technical, tactical, and physical skills to succeed. However, studies to improve soccer performance have often focused on technique and tactics at the expense of physical resources such as endurance, strength, and speed (Helgerud et al, 2001). Soccer is a game of strength, endurance, flexibility, agility, skill and teamwork (Chaiyong, 1975). Soccer players need to have physical fitness such as strength, endurance, speed, agility, flexibility and power perfectly (Gramer et al, 1996). Physiological, technical, and tactical skills are all important to soccer performance. Factors such as acceleration, running velocity, vertical jump, and capacity to release energy are major importance (Helgerud, 2002). The main energy source (approximately 90%) of 90 minutes soccer match is from aerobic metabolism which players run about 10 km (Bangsbo et al, 2001) at an intensity close to anaerobic threshold or 80-90% of maximal heart rate (Hoff et al, 2001). It has been estimated that elite soccer players cover between 10 and 13 km over the duration of a competitive match, suggesting that elite level performance may, in part, be determined by a large aerobic capacity (Edwards, 2003).

The prevalence of iron depletion in top level soccer players based on low serum ferritin levels seems to be poorly analyzed although the hematologic parameters can be crucial for predicting optimal physical performance (Schumacher et al, 2002). In addition, a need for iron supplementation was proposed on the basis of observed changes in blood count and serum iron parameters during periods of intense training. A recent review has reported that the iron stores were lower in athletes than in sedentary subjects (Resina, 1990). The iron cost seems to be mainly related to theload of exercise (Helberg, 1984), although the type of the exercise may also be of importance. Soccer is very popular sport in several countries, and movement is an essential role in soccer training (Ekblom, 1986). However, little is known about changes in iron status of soccer players. Although iron deficiency seems to compromise work capacity, it is still controversial whether the performance may be impaired in athletes who have low iron stores but are not yet anemic.

Altitude training directly affected to various blood parameters such as hemoglobin, red blood cell content and VO_2max (Levine and Stray-Gundersen 1997). Moreover, various researches revealed altitude also affected on heart rate variability (HRV). Perini, Milesi et al. (1996) reported the effect of altitude acclimation on HRV.

They found that the altitude group significantly reduced HRV when compared to the sea-level group.

Based on the electrical activity of the heart, the interval heart beat (R to R peak) was indicated as heart rate variability (HRV) which represented the adaptive capacity of cardiovascular system under various conditions. HRV relied on personal activities, diseases, gender as well as age (Aubert, Seps et al. 2003, Acharya, Joseph et al. 2006). Because HRV corresponded to the heart beat interval which regulated by automatic nervous system (ANS), it strongly reflected the activities of ANS (Acharya, Joseph et al. 2006). The ANS referred to the nervous system that works without consciousness which was divided into two categories; sympathetic and parasympathetic nervous system. Generally, heart rate tightly regulated by both sympathetic and parasympathetic nervous system in the contrast manners. When considered cardiovascular system, the parasympathetic trends to decreased heart rate while sympathetic system increased heart rate activity (Aubert, Seps et al. 2003)(Saito, Tanobe et al. 2005) reported the positive correlation between arterial oxygen saturation and heart rate variability at high altitudes and suggested that automatic response was reduced under hyperbaric environment. Many studies have reported that the sympathetic nervous system is up - regulated at rest and during exercise in response to acute and chronic exposure to altitude as evidenced by increasing in epinephrine and norepinephrine in plasma (Mazzeo et al., 1998, 2001). Other study reported that the increase in blood lactate concentration during submaximal and maximal exercise during altitude exposure has been related with the hypoxia – induced increase in epinephrine (Kayser 1996; Lundby et al., 2000). From the previous data, it did not clearly indicate the correlation between altitude training on HRV and blood lactate response. For this reason, in this work, we performed a series of physiological test that may lead us better understanding about this issue.

Summarily, the aims of this study were mainly focus on the effect of altitude training with and without iron supplementation on the hematological parameters, heart rate variability (HRV), body composition, blood lactate concentration and physical fitness in soccer players. The outcome of this study will bring us to the better understanding of the effects of LHTH plus simulated altitude, iron supplementation combined with soccer program training in term of acute and chronic effects on hematological, physiological adaptation as well as the response mechanism under hypoxic condition that may practically apply to the soccer training program in the future.

Methods

Forty male subjects of Nakhonratchasima Ratchabhat University (age range between 18-22 year olds) were purposive selected (based on math group method) and allocated into four groups: group 1. Sea-level training group (ST;N=10), group 2. Sea–level training with iron supplementation group (SI;N=10), group 3. Altitude training group (AT;N=10), group 4. Altitude training with iron supplementation group (AI;N=10). All athletes were matched according to their soccer ability. Before the experiment, all athletes had high physical performance, free from injury. Moreover, they all lived at sea level and had not been residents at attitude within the past 6 months. Ethically, the participants were fully informed about the aim and experimental protocol of this study. Likewise, their written informed consent was obtained.

Experimental Design

The athletes in altitude groups (AT and AI groups) stayed and also were trained at altitude while sea level groups slept stayed and were trained at or near sea level (123 meters). All tests were conducted at sea level including; 1) physical performance test: 50, 100, 400 and 2,800 meters time trial runs, speed, agility, jump power and repeated high intensity endurance test 2) hematological tests: red blood cell (RBC) count, erythropoietin (EPO), hematocrit (Hct), hemoglobin (Hb) concentration, and Iron study 3) physiological tests: heart rate (HR), oxygen saturation (SpO2), 4) subjective score on fatigue, sleep, muscle soreness, training performance rate of perceive exertion, acute mountain sickness. During the experimental period, athletes in group 2 and 4 were received iron supplementation (Ferrogrand C, Abbott laboratories Ltd.). Blood parameters and performance were measured before and after the 8 weeks of experiment.

Soccer Program Training

All athletes were asked to train in the soccer program 3 hours a day, 5 days a week for 8 weeks. Participants were taken 5 steps for training each day; 1) warm up and stretching (15 minutes) 2) physical fitness: strength, endurance, agility speed (25minutes) 3) skill (25minutes), 4) Team strategy (40 minutes), 5) Cool down (15 minutes). All athletes strictly followed the same instructions of soccer training program in each week from the coaches during the experimental period.

Heart Rate and SpO2 Monitoring

Heart rate and SpO_2 were monitored during resistance training every group by a pulse oximeter and record intermediately after finish of each exercise session.

Simulated Altitude Program

All athletes were performed exercise on a cycling ergometer for 15 minute 3 day per weeks at 100-120 watt for 8 weeks, whereas the athletes in altitude groups were added with simulated altitude (hypoxicator, FiO2 0.15) during exercise.

Measurement of Hematological Parameters

All athletes were ethically asked to have blood tests. Blood samples were taken from median cubital vein or cephalic vein to measure red blood cell count (RBC), hemoglobin concentration, erythropoietin (EPO), serum iron (Si), white blood cell count (WBC), hematocrit (HCT) and hemoglobin. Blood samples were taken 3 times, before, during and after experimental period for baseline, POST1 and POST2. Blood samples were transported with ice in ice chest within one day to Srinagarind Hospital, KhonKaen University for the hematological analysis.

Altitude Training Time line

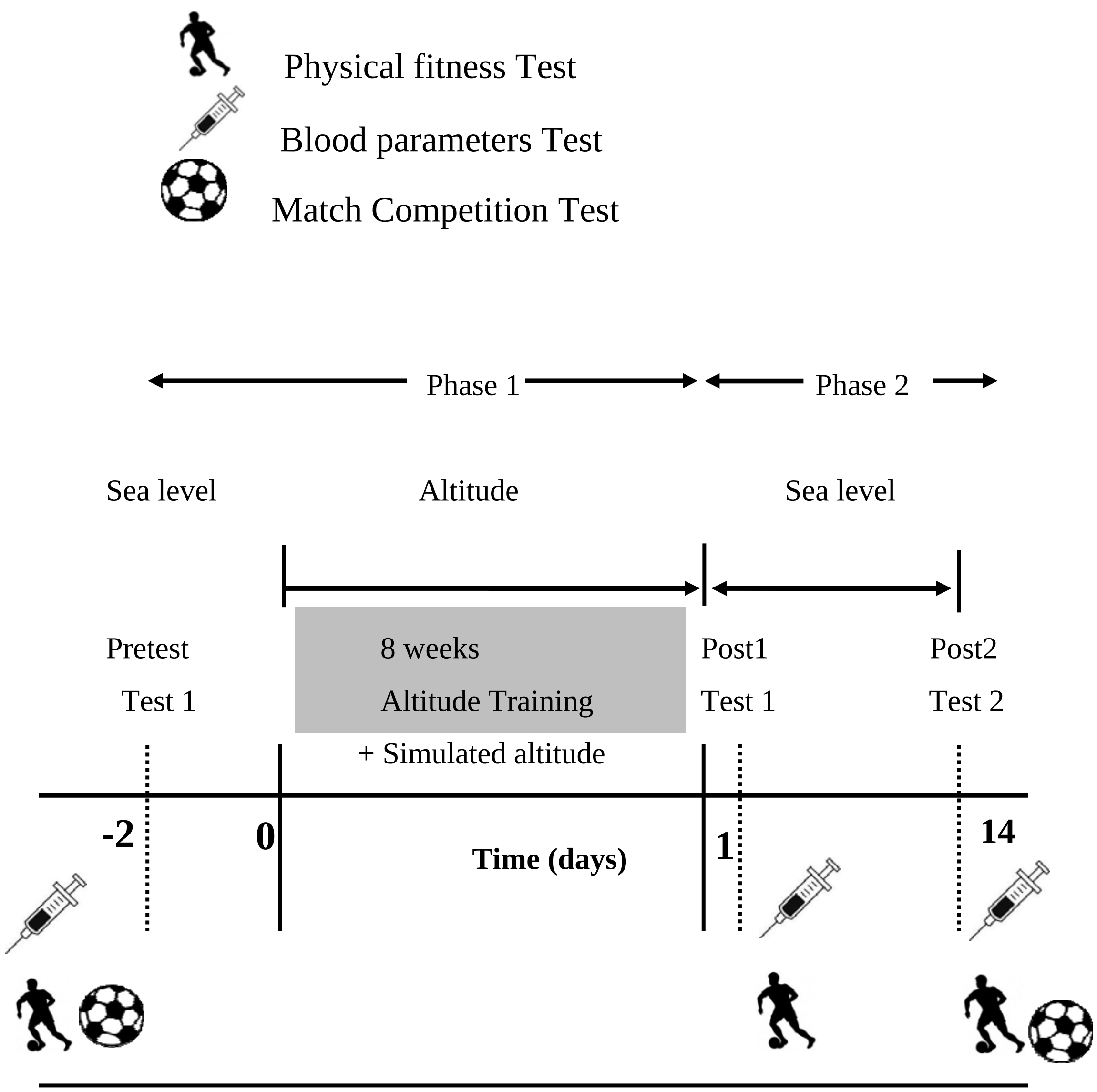


Figure 1 Timeline of altitude training program

Figure1. The overall timeline of altitude training program, briefly all athlete groups were measured hematological parameters and performance test 3 time points; 1. Two days before training camp (PRE), 2. The end of training camp (POST1) and 3, Two weeks after training camp (POST2). Moreover, the simulated altitude was performed under 15%O_2 condition by using hypoxic high altitude commercial systems.

Measurement of Physical Performance Parameters

Outcome variables were measured pre-and post-training program within 1-2 days before and after training programs. The primary outcome was measured of this study was by skill-related physical fitness test which it consist by;

Running Tests

Performance assessed by individual 100 meters and 2,800 meters timed run trials at a standard field near sea level. Time trial runs were performed at the same time. Athletes completed a standardized 10-15 minutes warm-up with stretching prior to the runs, but were notprovide any feedback on time pace. Rate of perceive exertion ratio was measured immediately after the tests.

Physical Performance Tests

Seven to two days before the eight weeks training period all athletes completed a series of physical performance fitness tests for soccer players including: The explosive power was evaluated by the maximum effort countermovement jump using standard procedures (Contact-Mat Kinemetic timing system, Australia). The best of three attempts were recorded. The sprint tests involve running distances, 5 and 10 m for which athletes were asked to perform maximum effort sprints. The better of two trials was recorded. The agility 505 test was designed to tests agility by minimizing the influence of running speed while measuring acceleration before and after change of direction. The test begins at walking speed and increases progressively until exhaustion following the standard procedure from Lambert (1982) (Leger & Lambert, 1982). The maximum oxygen consumption (VO_2max) and maximum attained speed (MAS) were predicted based on beep tests data before and the after training period.

Heart Rate Variability Test

The RR interval or time between two R waves of the recorded cardiac electrical activity was measured 3 minutes in supine and 3 minutes in standing position by using a Polar RX800CX heart rate monitor (Polar, Kemplel, Finland). All athletes were asked to measure 2 times for before and after training program.

Measurement of Blood Lactate Concentration

Blood samples were collected three times; before experiment (Pre test), 1-2 days after training (Post test1) and 14 days after training (Post test2). Approximately 5 ml of blood was taken from the fingertip via Accutrend® Plus system (Roche, USA). The blood sample was immediately collected before and after 400 meters running test to analyze blood lactate concentration by using lactate analyser.

The Maximum Oxygen Consumption (VO_2max)

All athletes were subjected for maximal oxygen uptake (VO_2max) on the cycle ergometer protocol based on Astrand-rhyming cycle ergometer test (Astand, 1960). Firstly, subjects walked on a cycle ergometer for 5 minutes. Heart rate was measured every minute, and the steady state heart rate is determined. Secondly, the steady state heart rate was monitored to determine an estimation of VO_2max.

Repeated High Intensity Endurance Test (RHIET)

The test was conducted on a non-slip surface in order to provide good traction for the athletes. A corridor or track of 60 meters lengths provides for a 40 meters test track and 20 meters in deceleration zones. Measure a distance of 40 meters with pylons was placed at 0 and 40 meters. Timing lights were place at 5 and 35 meters respectively. Procedure: 1. Warm up of 5-10 minutes, which included some short 20-25 meters all out sprinting. 2. A 3-5 minutes rest interval should separate the warm-up from the test. 3. The test involved a 40meters sprint (0-40meters) followed by a stop (turn) with a return to the start line. The subject touched the end line with one foot before returning to the start. 4. The test were measured for each interval first begins as the athlete crosses the 5 meters line and stopping at the 35 meters line, second began as the athlete crosses the 35meters line and stops as the athlete crosses the 5meters line. 5. Total time was recorded for each interval.

Body composition test

All subjected were recorded the body composition such as percentage of fat, body weight, body mass index (BMI), lean body mass (LBM), percentage of body fat (P.B.F), soft lean mass (S.L.M) and size lower body by using X-scan body composition analyzer (Jawon Medical, Korea). We recorded 2 times; before and after altitude training.

Statistical analysis

Data in the text and figures and presented as means with 90% CL and CI, respectively were presented as mean±SD. The data for descriptive statistics were calculated. The statistical significance of differences in the red blood cell count (RBC), erythropoietin (EPO), hematocrit (Hct), hemoglobin (Hb) concentration, heart rate (HR), oxygen saturation (SpO2), serum iron, serum ferritin, 100meters, 2,800meters, agility and VO_{2max} of AI, AT, SI and ST groups. The magnitude of the correlations was rated using Hopkins scale. For the magnitude inference test, baseline was calculated and used as a covariate in the analysis to adjust for any differences between groups at baseline. Because the small performance changes can be beneficial for elite athletes which can not detected by the conventional method, contemporary statistical method was used and applied to test our data (Hopkins, Hawley, & Burke, 1999). Specifically, we used magnitude-based inferences about effect sizes, and then to make inferences about true (population) values of the effect, the uncertainty in the effect was expressed as 90% confidence limits (CL).

Results

Male athletes (age between 18-22 year olds) in all groups were subjected to test the general data including body weight, height, body mass index (BMI) and also the basic performance including agility, VO_2max and training intensity. All parameters were measured and compared by using one-way analysis of variance. The basic physiological parameters and physical performance revealed no significantly difference. In other word, the comparison clearly showed the homogeneity of subjects in all groups (table 1).

Table 1 General characteristic of subjects in the four training groups.

	AI	AT	SI	ST
Age (yr.)	20.3±1.3	20.9±0.7	20.4±0.7	20.3±0.7
Body weight (kg)	68.0±7.5	67.5±9.5	62.3±6.3	69.2±9.8
Height (cm)	174.8±3.5	173.0±5.8	168.4±5.3	174.1±6.1
BMI ($kg \cdot m^{-2}$)	22.2±2.0	22.5±2.8	21.9±1.5	23.0±2.7
Agility (sec.)	14.1±0.2	14.6±0.1	14.1±0.3	14.7±0.1
V02max	43.42±5.22	42.37±0.9	43.62±8.5	41.70±2.7
Training Intensity(hr/wk)	9.22±2.55	9.51±2.33	9.43±2.17	9.17±2.74
Warm-up Stretching	1.12±0.13	1.17±0.14	1.17±0.12	1.18±0.14
Physical Fitness	2.08±0.15	2.18±0.11	2.18±0.12	2.19±0.13
Skill	2.03±0.16	2.08±0.15	2.17±0.12	2.17±0.10
Team Stagiest	3.15±0.14	3.19±0.15	3.18±0.12	3.17±0.10
Cool Down	1.07±0.11	1.11±0.11	1.12±0.10	1.10±0.09

Values represented mean ±SD. The altitude training with iron supplementation group (AI; N=10), altitude training group (AT; N=10), Sea-level training with iron supplementation group (SI; N=10), Sea-level training group (ST; N=10). No significant differences were found between groups for any variable.

Figure 2. SpO_2 during 15minutes cycling test recovered in 8-weeks experimental period in all groups

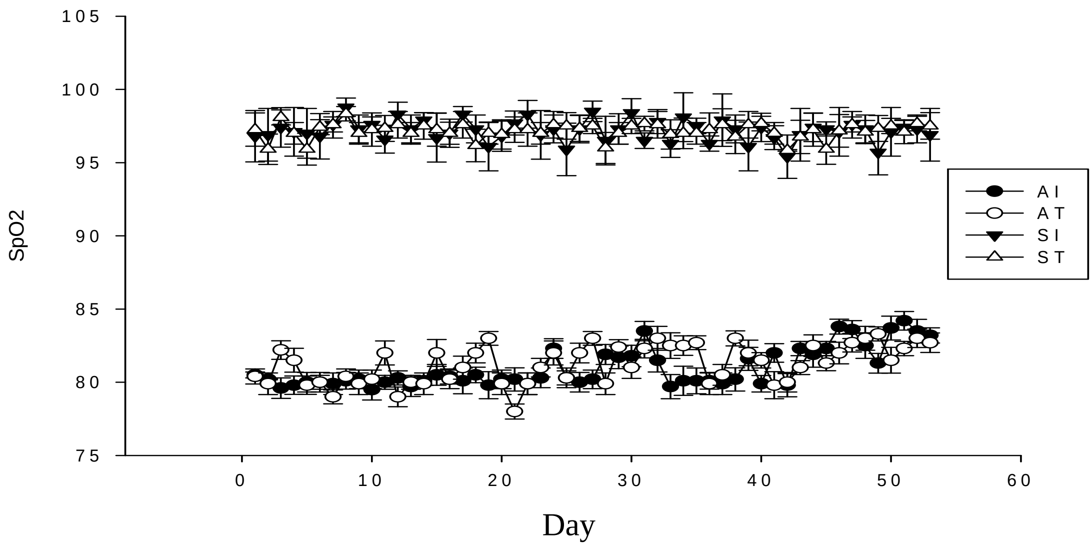


Figure 2 indicated that SpO_2 level in the sea-level groups (SI and ST) showed the dramatically higher when compared those in the altitude groups (AI and AT). However, the averages SpO2 in sea-level groups were 96-98% while the averages of SpO_2 in altitude subjects were 80-85%. Moreover, when trained at the sea-level, it showed no significantly difference among the subject group with and without iron application. On the other hand, in the altitude subject, they revealed the contrast response. After treated with ferrous sulfate for 30 days, the SpO_2 value showed the positively response to iron application.

Table 2. The mean±SD changes in hematological variables measured before (PRE) and after altitude training day1 (POST1) and day14 (POST2)

		ST N=10	SI N=10	AT N=12	AI N=10
EPO					
	PRE	9.33±4.90	13.90±12.51	13.05±3.85	10.80±3.53
	POST1	9.31±3.45	14.33±12.63	14.12±2.76	14.56±6.62*
	POST2	9.46±2.54	15.57±9.02	10.30±3.08	12.53±6.91
RBC					
	PRE	5.25±0.66	4.96±0.31	5.48±0.33	5.15±0.22
	POST1	5.38±0.60	5.22±0.29	5.69±0.30*	5.30±0.20*
	POST2	5.11±0.37	5.21±0.33	5.61±0.31*	5.30±0.25*
WBC					
	PRE	7.24±0.97	6.84±0.58	8.14±1.91	7.41±1.21
	POST1	7.05±1.07	6.67±0.68	7.61±1.58	7.57±1.55
	POST2	6.65±2.02	5.93±0.95	6.36±1.57	6.44±0.92
HCT					
	PRE	43.15±1.85	43.16±3.57	42.70±2.44	44.02±2.50
	POST1	44.63±1.87	44.56±3.19	44.25±2.25	44.93±2.88
	POST2	44.72±4.63	47.27±3.43	45.10±6.62	47.17±4.20
HGB					
	PRE	14.22±0.73	14.36±1.16	13.97±0.91	14.78±0.93
	POST1	14.38±0.83	14.45±1.13	14.30±0.85	14.81±0.85
	POST2	14.05±1.28	14.80±1.16	14.33±1.02	15.08±1.02
Si					
	PRE	103.21±29.08	106.00±43.76	86.57±23.77	79.28±16.08
	POST1	124.71±38.94	141.00±44.00	94.14±16.02	97.71±27.89
	POST2	136.42±43.77	156.28±49.88	139.21±32.73*	144.67±16.80*
TIBC					
	PRE	3.5±16.10	7.4±13.32	5.1±9.43	18.25±21.31
	POST1	-15.5±39.55	-15.7±41.42	-24.8±71.50	23.3±12.42
	POST2	-18.4±48.92	-21.5±28.35	-28.4±71.33	-35.1±26.43

The values represented mean±SD. *$p<0.05$

Table 2, it revealed the response of hematological indices of the seal-level groups (SI and ST) and altitude groups (AI and AT) measured before (PRE) and after training (POST1 and POST2). It clearly showed that the RBC level dominantly responded to altitude training. The RBC level significantly increased in both with and without iron supplemented groups after 8 weeks of altitude training (AI and AT respectively). Moreover, the RBC level remain the almost the same level at day 14 after training (POST2) in both groups (AI and AT). When considered Si level, it significantly increased in AI and AT group at day 14 after training (POST2). Furthermore, the EPO level, only the altitude group that treated with ferrous sulfate (AI) displayed significantly higher in EPO level (14.56±6.62mIU/ml) after 8 weeks of training.

Table 3 Comparative mean changes of performance and the change that the true difference represents a substantial improvement or impairment in HRV parameters in stand and supine position in AT/ST, AI/SI, AI/AT and AI/ST group. The percentage change of each HRV parameters were calculated by [POST-PRE]/PRE *100%.

	Change in performance %			Percentage chance of HRV parameters (%)	
			Difference90%CL	%	Qualitative
AT vs ST group	AT	ST			
RMSSD stand (ms)	-2.9±78.0	-13.3±51.8	11.0±67.1	22	unclear
RMSSD supine (ms)	6.7±47.7	2.9±27.3	-26.5±44.8	24	unclear
LF stand (ms^2)	4.5±86.1	-42.1±51.8	3.8±43.1	4	unclear
LF supine (ms^2)	11.7±104.9	-5.5±101.1	6.4±115.8	28	unclear
HF stand (ms^2)	8.8±102.5	0.2±135.0	-8.4±150.5	39	unclear
HF supine (ms^2)	19.9±108.6	19.9±77.7	0.0±107.1	30	unclear
LF/HF Ratio stand(ms^2)	3.0±108.1	-67.4±63.7	102.3±108.6	2	unclear
LF/HF Ratio supine(ms^2)	-0.1±81.9	-8.4±90.7	8.7±96.2	30	unclear
AI vs SI group	AI	SI			
RMSSD stand (ms)	25.7±63.6	0.6±58.4	28.6±61.6	11	unclear
RMSSD supine (ms)	-7.7±52.7	22.9±40.3	-26.5±44.8	81	likely
LF stand (ms^2)	20.8±111.9	-53.2±92.6	109.7±137.7	4	unclear
LF supine (ms^2)	-76.6±122.1	-31.0±89.5	-36.6±136.8	71	unclear
HF stand (ms^2)	33.6±128.5	-5.0±143.9	47.2±192.3	18	unclear
HF supine (ms^2)	-21.4±123.4	58.7±92.1	-55.2±135.9	87	likely
LF/HF Ratio stand (ms^2)	-28.3±103.5	-16.3±116.1	-14.8±140.9	52	unclear
LF/HF Ratio supine(ms^2)	-50.3±80.3	-88.5±67.7	46.6±82.6	7	unclear
AI vs AT group	AI	AT			
RMSSD stand (ms)	25.7±63.6	-2.9±78.0	19.1±74.8	19	unclear
RMSSD supine (ms)	-7.7±52.7	6.7±47.7	-11.1±48.6	50	unclear
LF stand (ms^2)	20.8±111.9	4.5±86.1	24.5±118.8	19	unclear
LF supine (ms^2)	-76.6±122.1	11.7±104.9	-58.2±145.7	89	likely
HF stand (ms^2)	33.6±128.5	8.8±102.5	-5.1±184.0	31	unclear
HF supine (ms^2)	-21.4±123.4	19.9±108.6	-31.7±149.2	61	unclear
LF/HF Ratio stand (ms^2)	-28.3±103.5	3.0±108.1	-50.0±161.5	83	unclear
LF/HF Ratio supine(ms^2)	-50.3±80.3	-0.1±81.9	-21.8±90.8	61	unclear
AI vs ST	AI	ST			

RMSSD stand (ms)	25.7±63.6	-13.3±51.8	26.1±56.0	10	unclear
RMSSD supine (ms)	-7.7±52.7	2.9±27.3	3.8±4.31	24	unclear
LF stand (ms^2)	20.8±111.9	-42.1±51.8	59.5±77.2	4	unclear
LF supine (ms^2)	-76.6±122.1	-5.5±101.1	6.4±115.8	28	unclear
HF stand (ms^2)	33.6±128.5	0.2±135.0	-8.4±150.5	39	unclear
HF supine (ms^2)	-21.4±123.4	19.9±77.7	0.0±107.1	30	unclear
LF/HF Ratio stand (ms^2)	-28.3±103.5	-67.4±63.7	102.3±108.6	2	unclear
LF/HF Ratio supine(ms^2)	-50.3±80.3	-8.4±90.7	8.7±96.2	30	unclear

±90% confidence limits; add and subtract this number to the mean effect to obtain the 90% confidence limits for the true difference. LF, low frequency reflecting sympathetic predominance; HF, high frequency reflecting parasympathetic predominance; LF/HF, sympathetic-parasympathetic neurovegetative balance; RMSSD, root mean square of the standard deviation of the R-R intervals.; ST, sea level training group; SI, sea level training with iron supplementation group; AT, altitude training group; AI, altitude training with iron supplementation group.

Table 4 Comparative mean changes of performance and the change that the true difference represents a substantial improvement or impairment in HRV parameters in stand and supine position in AT/ST, AI/SI, AI/AT and AI/ST group. The percentage change of each HRV parameters were calculated by [POST-PRE]/PRE *100%.

	Change in performance %			Percentage chance of HRV parameters (%)	
			Difference90%CL	%	Qualitative
AT vs ST group	AT	ST			
RMSSD stand (ms)	10.7±55.6	53.0±53.0	-34.5±50.5	89	likely
RMSSD supine (ms)	36.7±47.2	46.4±38.0	-9.2±38.7	47	unclear
LF stand (ms^2)	4.8±65.1	17.3±81.5	-11.8±77.0	49	unclear
LF supine (ms^2)	51.6±121.5	34.7±109.6	18.4±138.6	24	unclear
HF stand (ms^2)	12.4±96.5	91.8±96.9	-54.8±106.3	90	likely
HF supine (ms^2)	77.9±103.8	91.0±98.3	-12.2±104.4	41	unclear
LF/HF Ratio stand (ms^2)	5.9±64.8	-77.4±103.1	130.1±96.3	1	unclear
LF/HF Ratio supine(ms^2)	-21.3±51.4	-63.2±67.2	52.1±59.1	3	unclear
AI vs SI group	AI	SI			
RMSSD stand (ms)	24.3±69.0	33.8±47.1	-9.1±59.4	50	unclear
RMSSD supine (ms)	-31.1±91.5	29.0±64.5	-45.2±86.6	90	likely
LF stand (ms^2)	52.0±107.4	-1.3±84.7	70.6±125.3	8	unclear
LF supine (ms^2)	-84.6±118.6	-38.6±122.9	-36.8±164.4	71	unclear
HF stand (ms^2)	46.5±146.8	36.5±102.6	10.5±172.0	32	unclear
HF supine (ms^2)	-72.6±196.5	67.8±142.3	-75.5±286.3	93	likely
LF/HF Ratio stand (ms^2)	-8.7±80.7	3.4±86.0	-11.5±94.9	49	unclear
LF/HF Ratio supine(ms^2)	-78.4±96.5	-94.7±77.7	138.6±103.4	1	unclear
AI vs AT	AI	AT			
RMSSD stand (ms)	24.3±69.0	10.7±55.6	5.4±63.5	28	unclear
RMSSD supine (ms)	-31.1±91.5	36.7±47.2	-48.3±79.9	94	likely
LF stand (ms^2)	52.0±107.4	4.8±65.1	45.7±102.1	8	unclear

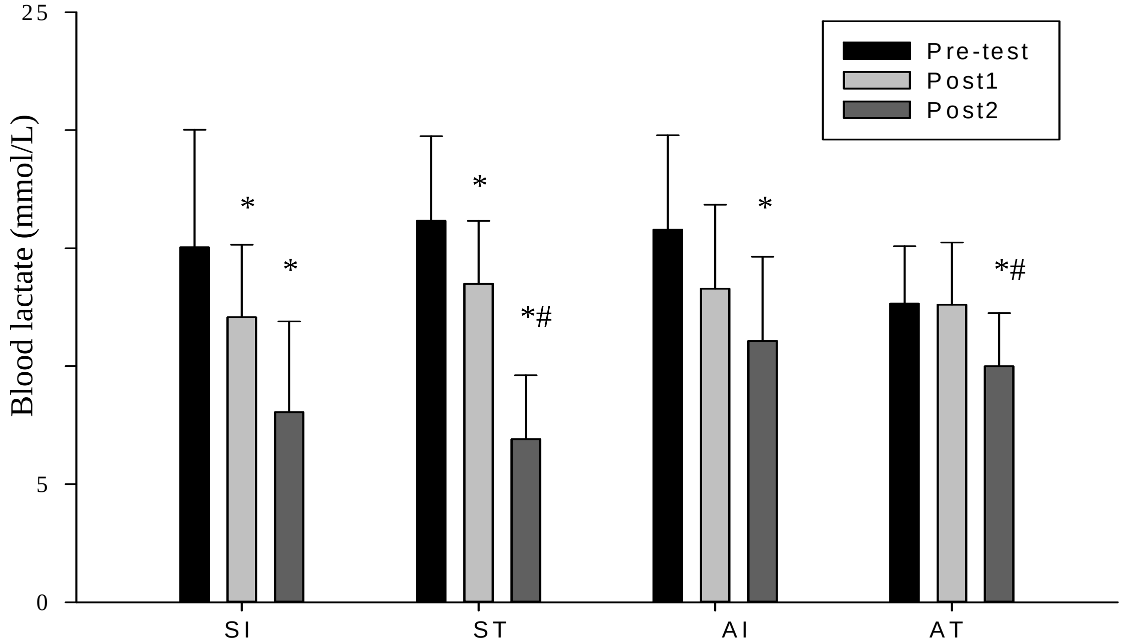
LF supine (ms^2)	-84.6±118.6	51.6±121.5	-72.0±158.1	97	likely
HF stand (ms^2)	46.5±146.8	12.4±96.5	6.0±171.2	39	unclear
HF supine (ms^2)	-72.6±196.5	77.9±103.8	-77.6±250.6	95	likely
LF/HF Ratio stand (ms^2)	-8.7±80.7	5.9±64.8	-35.5±98.0	76	unclear
LF/HF Ratio supine(ms^2)	-78.4±96.5	-21.3±51.4	51.0±86.8	7	unclear
AI vs ST	AI	ST			
RMSSD stand (ms)	24.3±69.0	53.0±53.0	-31.5±60.8	83	likely
RMSSD supine (ms)	-31.1±91.5	46.4±38.0	-9.2±38.7	47	unclear
LF stand (ms^2)	52.0±107.4	17.3±81.5	-11.8 ±76.0	49	unclear
LF supine (ms^2)	-84.6±118.6	34.7±109.6	18.4±138.6	24	unclear
HF stand (ms^2)	46.5±146.8	91.8±96.9	-54.8±106.3	90	likely
HF supine (ms^2)	-72.6±196.5	91.0±98.3	-12.2±114.4	41	unclear
LF/HF Ratio stand (ms^2)	-8.7±80.7	-77.4±103.1	130.1±96.3	1	unclear
LF/HF Ratio supine(ms^2)	-78.4±96.5	-63.2±67.2	52.1±59.1	3	unclear

±90% confidence limits; add and subtract this number to the mean effect to obtain the 90% confidence limits for the true difference. LF, low frequency reflecting sympathetic predominance; HF, high frequency reflecting parasympathetic predominance; LF/HF, sympathetic-parasympathetic neurovegetative balance; RMSSD, root mean square of the standard deviation of the R-R intervals.; ST, sea level training group; SI, sea level training with iron supplementation group; AT, altitude training group; AI, altitude training with iron supplementation group

For the results, when compared the changes of performance and the change that the true difference represents a substantial improvement or impairment in HRV parameters in stand and supine position at PRE and POST1 time point, we found that the $LF_{suprine}$ was likely to increase in altitude training with iron supplement (AI) group when compared to the altitude training (AT). Likewise, $RMSSD_{suprine}$ and $HF_{suprine}$ revealed the decrease in AI group when compared to sea-level with iron supplement group (SI) (Table2).

Furthermore, when considered changes of performance and the change that the true difference represents a substantial improvement or impairment in HRV parameters in stand and supine position, we found that $RMSSD_{stand}$ and HF_{stand} trended to reduce in AT group when compared to ST group. Besides, $RMSSD_{stand}$ and HF_{stand} decreased in AI group when compared to ST group. Likewise, $RMSSD_{suprine}$ and $HF_{suprine}$ also decreased in AI group when compare to SI group. Similarly, $RMSSD_{suprine}$, $LF_{suprine}$ and $HF_{suprine}$ declined in AI group when compared to AT group. (Table3)

Figure 3. Blood lactate content among the experiment groups (SI, ST, AI and AT) at the 3 time points; pre-test (PRE), 1st post test (POST1) and 2nd post test (POST2).



*= significantly difference when compared to PRE ($p<0.05$)
#=significantly difference when compare POST1 and POST2 ($p<0.05$)

To elucidate the response of blood lactate among the experiment groups (SI, ST, AI and AT), we measured blood lactate at the 3 time points; pre-test (PRE), 1st post test (POST1) and 2nd post test (POST2) and compared mean by using one-way analysis of variance. Overall, blood lactate content was slightly decreased after training in all groups.

The blood lactate in both sea-level training group (ST and SI) significantly dropped after 8 weeks of training (POST1) and after 2 weeks of POST1 timing (POST2). In addition, in ST group, blood lactate content was dramatically decreased at POST2 time point.

In altitude training (AT and AI), blood lactate was showed differently response. The AT group maintained the blood lactate level at PRE and POST1 time point. However, blood lactate content significantly reduced at POST2. In the other hand, blood lactate content in AI group was showed significantly reduced only at POST1 time point.

Table 5 The average of Physical fitness content among the experiment groups (SI, ST, AI and AT) at the 3 time points; pre-test (PRE), 1st post test (POST1) and 2nd post test (POST2)

Physical Fitness Test	**AT**			**AI**			**ST**			**SI**		
	Pre	Post1	Post2	Pre	Post1	Post2	Pre	Post1	Post2	Pre	Post1	Post2
Runing(m)												
50m(sec)	7.24	6.46*	6.39*	6.99	6.30*	6.24*	7.17	6.60*	6.48*	6.69	6.25	6.20*
400m(min)	1.20	1.25	1.25	1.14	1.20	1.18	1.20	1.31	1.32	1.17	1.26	1.24
Jump												
Power(w)	313.3	900.8*	955.5*#	540.4	990.1*	1021.*#	314.2	937.8*	948.8*	280.7	838.0*	897.7*
Height(cm)	29.9	32.9*	35.7*#	30.4	32.0*	33.8*#	29.1	30.60	30.70	28.10	32.2*	33.10*
RHIET test (sec)	17.29	15.97*	15.81*	16.67	15.95*	15.7*#	16.95	16.47*	16.24*	16.86	16.20*	16.11*

*= significantly difference when compared to baseline (p<0.05)
#=significantly difference between POST1 and POST2 (p<0.05)

Table 5 revealed the average of physical fitness among the experimental groups (SI, ST, AI and AT) measured before (Pre) and after training (Post1 and Post2). For the AT group, the physical fitness test revealed that the altitude training significantly improved the running ability in all distance (50m, 100m, 400m and 2800m) when compared to the beginning of the test. However, this result clearly showed the difference after 10 weeks of training (Post2). Besides, the jump power and jump height also displayed the similar trend. The AT group increased the jump power and height after training. In addition, the agility in AT group also increased after training (Post1 and post2). Interestingly, running ability, jump ability and RHIET test values in Post2 significantly increased than those in Post1. These data indicated that they progressively increased after training.

For the AI group, the physical fitness of AI group dramatically improved the running ability when compared to the beginning of training. The running test in AI group clearly showed that running ability in Post2 higher than Post1 of all distance tested. Furthermore, when considered the jump ability in AI group, it showed that AI treatment significantly increased jump performance when compared to before training period. While, the agility and RHIET test also revealed the similar trend that the AI treatment significantly elevated these parameters.

For the ST group, the physical fitness test revealed that the sea-level training significantly improved the running ability in all distance (50m, 100m, 400m and 2800m) when compared to the beginning of the test. Moreover, the jump power, agility and RHIET test results displayed that the ST group significantly enhanced RHIET capacity of those two parameters after training in both Post1 and Post2 time point.

For the SI group, the running ability of SI group significantly improved after 8 weeks training. Besides, the SI group still improved their running ability at the Post2 time point. For the jump power parameters, jump height and RHIET test displayed the similar response to physical fitness test by which the SI athletics continuously elevated their jump ability and RHIET results. In addition, the agility test of SI subject also showed the significantly improved only Post2 time point.

Table 6 The average of Body composition and anthropometry among the experiment groups (SI, ST, AI and AT) at the 2 time points; pre-test (PRE) and 1st post test (POST1)

Body Composition	**AT**		**AI**		**ST**		**SI**	
	Pre	Post1	Pre	Post1	Pre	Post1	Pre	Post1
Body Composition								
BMI	22.5	21.9*	22.2	21.9	23.0	22.6	21.9	21.7
P.B.F.	18.4	14.9*	17.5	14.8*	18.9	16.1*	17.0	14.3*
L.B.M.	54.6	55.4	52.0	52.4	55.8	56.7*	51.6	53.0*
S.L.M.	50.7	51.6	55.9	56.2	51.8	52.7*	48.0	49.4*
Anthropometry								
Thigh.R(cm)	54.4	53.7	54.8	54.9	53.5	53.7	50.5	49.8
Thigh.L(cm)	54.7	53.2*	54.5	54.2	52.8	53.0	50.0	50.1
Waistline(cm)	80.7	78.0	78.3	77.6	82.3	81.0	76.9	75.9
Hip(cm)	99.5	92.2	92.3	92.8	90.8	93.8	88.8	90.5
Calf.R(cm)	35.6	37.8*	36.3	37.7*	36.1	37.3	36.9	37.9
Calf.L(cm)	35.6	37.2*	36.4	37.6*	36.5	37.1	36.32	37.6

*= significantly difference when compared to baseline ($p<0.05$)

To elucidate the effect altitude and iron supplementation on body composition and size lower body content among the experiment groups, we measured the parameters included body mass index (BMI)body fat percentage (P.B.F), lean body mass (L.B.M.), soft lean mass (S.L.M.), thigh, waistline, hip and calf diameter at 2 time point which were pre-test (PRE) and 1st post test (POST1). Then we compared the average of each parameter between PRE and POST1 by using paired sample t-test analysis to determine the significant factors.

The results showed that altitude training (AI and AT) significantly improved BMI, P.B.F., both left and right calf after training. However, the average of left thigh was significantly decreased when compared to PRE. On the other hand, the sea-level training (SI and ST) revealed the similar response among the group. SI and ST group significantly reduced P.B.F. after training. While, L.B.M. and S.L.M. significantly increased in POST1 time post.

Table 7. Match competition

Match competition	Before Camp				7 Day After Camp			
	AT Vs ST		AI Vs SI		AT Vs ST		AI Vs SI	
Goal	1	1	3	1	3	1	5	1
Shoot	6	7	9	6	8	4	11	6
Shoot on Target	4	4	5	3	5	2	8	4
Possession	54%	46%	63%	37%	61%	39%	67%	33%

AT & ST before camp

For the match competition of AT and ST group before the training, AT drew against ST. For the statistical of AT group, they had 6 total shoots with 4 shoot on targets. Moreover, they had 54% of ball possession. On the other side, the ST group had 7 total shoots with 4 shoot on targets. Likewise, they had 46% of ball possession. From the statistics of both team, they showed the very similar in term of soccer performance such as ball possession, shoot on target and goal.

AT & ST 7 after camp

However, after 8 weeks of training, AT beat ST with score 3-1. The AT group had 8 total shoots with 5 shoot on targets and had 61% of ball possession. While, the ST group had 4 total shoots with 2 shoot on targets and they had 59% of ball possession. From this result, it clearly showed that the AT team dramatically improve in soccer performance that included ball possession percentage, shoot on target and gold which lead to the team success.

AI & SI before camp

Before the training and iron treatment, the AI beat SI team with score 3-1. For the soccer performance, the AI team had 9 total shoots with 5 shoot on targets and they had 63% of ball possession. On the other hand, the SI team had 6 total shoots with 3 shoot on targets. However, they had only 31% of ball possession. From this analysis, it suggested that the AI team showed the significantly higher in soccer performance over the SI team.

AT & ST 7 after camp

After training and iron treatment, the AI team still beat SI team with score 5-1. The statistical analysis showed that the AT group had 11 total shoots with 8 shoot on targets and they had 67% of ball possession. While, the SI group had 6 total shoots with 4 shoot on targets and they had 33% of ball possession. These data clearly showed that the AI team significantly enhanced in the soccer performance that lead them to the match success.

Discussion

Training and living at the low altitude (825meters above sea level) conjunction with both breath hypoxia gas (FiO_2 15%) and iron supplementation has been used in this study. This form altitude training showed substantial improvement in some hematological variables as well as physical fitness in soccer players.

The major findings of this study were classified into five points: hematological parameters, physical fitness, heart rate variability, blood lactate concentration and body composition. Firstly, hematological parameters; the level of EPO significantly increased after the athletics were trained at the real altitude for eight weeks which resulted in the improvement of oxygen consumption. The iron supplementation elevated the hematological markers such as EPO and RBC and improved both aerobic and anaerobic. Moreover, the iron treatment also enhanced the overall physical performance of soccer players. The combination treatment of altitude level and iron supplementation was the best treatment which strongly enhanced the broadly physical performance and blood parameters of the soccer players.

The overall variables, we monitored the blood parameters and physical performance of all subjects in two phases which were after 1 day and 14 days of training indicated as POST1, POST2 respectively. Based on our investigation, the altitude group (AI and AT) significantly higher in the EPO level when compare to the sea-level training group (SI and ST) when measured after a day of training period. Although, no significant difference in EPO level was found 14 days later, RBC and HGB level were significantly higher in this stage (Table2.). The EPO level is the very sensitive parameter that indicates the response of athletics to hypoxia condition. In other word, EPO is the hematological marker that response to low oxygen condition. EPO is the renal hormone that induced the red blood cell production through the induction of hypoxic condition. At the molecular level, EPO gene contains hypoxia response element within the promoter region of gene which regulated the expression of EPO gene during hypoxic environment (Jelkmann, 2011). Training increases RBC and stimulates erythropoiesis, adaptive changes that are regulated by different mechanisms that show different temporal responses to the onset of physical activity. Increased red cell production is also associated with an expansion of red bone marrow. The increase of EPO directly regulated hemoglobin concentration through the control of red blood cell production. These hematological changes may improve an athlete's VO_2 by enhancing blood circulation to deliver oxygen to the exercising muscles. That resulted in the enhancement of aerobic performance (Hall and Guyton, 2010, Wilber, 2004). In support of this, the studies of Levienand Stray-Gundersen (1997) found that a significant 5% in RBC mass and 9% hemoglobin improvement. Stray-Gundersen (2001) has also found erythropoietin increased 92% in LHTL subjects (Gundersen, 2001). Likewise, the body has hemoglobin as 13.4 grams per 100 cubic centimeters of blood at sea level. After the body exposed to higher altitude, hemoglobin increases to 17 g per 100 cubic centimeters of blood (Thoranin, 1979). Performance measures in these studies included sport specific ergometers and endurance time during an exhaustive incremental exercise test. All of these investigations showed decrements in aerobic performance upon acute exposure to altitude. Decrements in aerobic performance at altitude have been associated with hypoxia-induced reductions in VO_2max secondary to reductions in SaO_2. In sports such as cycling and speed skating in which aerodynamics play an important role, the decrement in VO_2max may be offset by aerodynamic advantages up to elevations of approximately 4,000 m (Wilber, 2004).The deduction in FiO_2 (oxygen in air) and PaO_2 (oxygen in lungs) result in decrements of kidney PaO_2 and low oxygenation in kidney, there by stimulates the synthesis and release of EPO.

The hematological changes may significantly improve aerobic performance in endurance athletes by enhancing the delivery of oxygen to working muscles. In short, the increased of RBC that was induced by low oxygen environment played the key role of adaptive mechanism to altitude. Iron supplementation is a common strategy to increase the ability of red blood cells to deliver oxygen to exercising muscles. In our experiment, when apply the iron supplementation to the soccer player both in altitude training and sea-level training, we found that the iron-treated group significantly increased the EPO content in both altitude and sea-level training. However, only the high altitude group clearly showed the possibly higher in RBC (Table 2). In addition, when monitored the hematological parameters in the long term, the iron treatment significantly prolonged the EPO level of both altitude and sea-level group.

At the physiological level, iron supplementation slightly modulated the level of ferritin in blood (Nielsen and Nachtigall, 1998). Ferritin is the storage form of iron in blood and also plays an important role for endurance athlete in maintaining amount of iron, especially during the increase of training, especially for the athlete at altitude because it is requires increasing the erythropoiesis process that would occur when the oxygen decreases. For this reason, ferritin is very important for endurance athlete in maintaining amount of iron, especially during the increase of training, especially for the athlete at altitude because it is requires increasing the erythropoiesis process that would occur when the oxygen decreases.

Secondly, table 5 revealed the average of physical fitness among the experimental groups (SI, ST, AI and AT) measured before (Pre) and after training (Post1 and Post2). For the AT group, the physical fitness test revealed that the altitude training significantly improved the running ability in all distance (50m, 100m, 400m and 2800m) when compared to the beginning of the test. However, this result clearly showed the difference after 10 weeks of training (Post2). Besides, the jump power and jump height also displayed the similar trend. The AT group increased the jump power and height after training. In addition, the agility in AT group also increased after training (Post1 and post2). Interestingly, running ability, jump ability and RHIET test values in Post2 significantly increased than those in Post1. These data indicated that they progressively increased after training.

Training at altitude elevated some physical fitness such as agility, jump power and also running speed at 2800 meters. When applied iron supplement, it increased the maximum aerobic capacity and prolonged the endurance time to fatigue that was resulted from the positively induction of serum ferritin and hemoglobin (Magazanik et al, 1991). When compared the match result before and after training, we discovered that the altitude training improved the soccer performance when compare to the sea-level training. The altitude training altered some hematological factors that contribute to the increasing in physical fitness of soccer players. In term of psychological sense, the long-term group training that they ate, slept and lived together for the long period of time, it improve the relationship among the soccer players. Moreover, the training at the good environment was also the important factor that induced the subjects to concentrate and focus on training activities.

The iron compensation increased RBC in AI and AT and still increased during training period and after 14days of training while Si showed significantly increased during experimental period (Table2). Moreover, to elucidate the effect of iron compensation by compared AI and ST, it clearly showed that AI increased EPO level and TIBC during experimental period (Table3). However, after 14days of experimental period, EPO level and RBC still increased in AI (Table3)

Thirdly, the heart rate variability (HRV) measured the interval time of heartbeat which reflected the balance of nervous system function. Moreover, in altitude training, HRV was used to monitor physical activity in athlete. In order to study the correlation altitude training and the HRV on athlete's performance, we conducted the series of physiological experiments and measured HRV parameters to elucidate the effects of altitude training. We performed the experiment in two differences level, the sea-level training (123 meters above sea-level) and altitude training (825 meters above sea-level). In addition, we also test the effect of iron supplementation by using ferric oxide supplementation.

Heart rate variability (HRV) is a monitoring tool used to detect changes in autonomic nervous control that may indicate slow or impaired adaptation to altitude. In our research, altitude training significantly reduced some of HRV parameters such as RMSSD stand and HF stand (table2). The effects of hypoxia when trained at altitude (altitude groups) and exercise training (sea level groups) on HRV showed difference results. An increase training load or stressor by hypoxia at altitude may negatively influence vagally-mediated HRV indices (Sandercock et al. 2005) as RMSSD stand and HF stand in both altitude groups (AT and AI) showed substantially decreased compared with sea-level group (ST group). The athletes in hypoxic environment that did not respond positively to the 8-weeks live high train high showed a withdrawal of vagally-mediated HRV parameters similar to Mourot and colleages (2004) observations, which suggests that HRV may be a useful tool at identifying athletes under too much stress, whether it is from training, or a combination of training and hypoxia. It seems likely that the effect of high training loads with the addition of hypoxic stress has a cumulative effect, such that the normal adaptation to such stressors is overwhelmed and sympathetic activity predominates. If recovery is not sufficient and training with hypoxia continue, the athlete has little chance of recovery and gradually shifts into a sympathetically innervated overstress-type syndrome causing a reduction in performance potential (Hamlin et al. 2011). However, the athletes' physical fitness (run 50,100,400m, jump height) did not correspond to these HRV results; our results may indicate the stressor from either hypoxic environment or training load not influence to muscle activity via somatic nervous system but HRV via autonomic nervous system. This is speculative and further research is required to elucidate these changes.

Fourthly, cellular lactic acid was generated from anaerobic respiration, so blood lactate content reflected the hypoxia condition at the cellular level. When blood lactate content increased, it induced exhaustion in athletes. Moreover, the blood lactate level indicated exercise intensity. In our experiment, sea-level group (ST and SI) significantly reduced blood lactate content after training and still decreased after experiment period (figure 3). However, compared to the sea-level groups, altitude training groups (AT and AI), blood lactate level presented slowly decreased immediately after 400m run at both day 1 and day 14 (figure 3). These findings indicated the anaerobic metabolism involved in the athletes in altitude groups. It more likely that altitude groups worked harder, although get the same training program. Lactic acid is known as the chemical substance that activates growth hormone release and muscle hypertrophy later all (Manimmanakorn et al. 2013). Our results showed the thigh and calf muscles cross-sectional area increased significantly after training in AT and AI groups (Table 6). This may indicate the mechanism involve as described above. Theoretically, a professional soccer player should ideally be able to maintain a high level of intensity throughout the whole game. This study may suggest that the athletes had improve buffering capacity in their bodies as a results of RHIET trend to improve than sea level training although no significant were found (Table 5). Some studies, however, have shown a reduction in distance covered, a lower fractional work intensity, reduced blood sugar levels and reduced lactate levels in the second half of games compared with the first half (Douglas, 1993). Other study reported that the increase in blood lactate concentration during submaximal and maximal exercise during altitude exposure has been related with the hypoxia – induced increase in epinephrine (Kayser 1996; Lundby et al., 2000). Furthermore, Spengler, Roos et al. (1999) observed the relation between blood lactate level and oxygen consumption in athletes during endurance exercise. They suggested that the two main reasons of decrease of blood lactate concentration were 1). The muscle itself reduced the production of lactic acid due to the decrease of energy demand and 2). Muscle uptakes lactate and catabolized to server as energy. In determining aerobic endurance is considered the most important element as well as for soccer game.

Finally, the body composition: BMI was only finding that changed after altitude training in AT groups (Table 6). This may due to water loss during hypoxic environment training not fat mass, because of percent body fat were decreased significantly in all four groups.

In conclusion, our data reflected that altitude training more likely to induce red blood cell mass, erythropoietin, autonomic nervous system via sympathetic outflow. The sympathetic and parasympathetic nervous systems are responsible for the regulations of several physiological responses including heart rate, respiratory rate and substrate utilization. The other important finding is blood lactate concentration which showed less reduction immediately after short distance running, in other word the athletes' muscle exposed to acidic environment but gave efficiency work through physical fitness. This phenomenon indicated our protocol (altitude training with soccer training program in this study) effective to improve anaerobic based performance or physical fitness (100m and 400m run). Finally, the body composition did not alter with this 8-weeks altitude training or live high train high program.

Future researches

Further study need to identify whether simulated altitude or live low train high improves other sports performance. Moreover, altitude training or hypoxia increases red blood cell, erythropoietin, physical fitness and muscular strength in such patients, such as thalassemia, disability people and non-communicating diseases (NCDs) need to be investigated.

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Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. การนำผลงานวิจัยไปใช้ประโยชน์ เชิงวิชาการ (มีการพัฒนาการเรียนการสอน/สร้างนักวิจัยใหม่) จากการศึกษาเรื่อง “ผลของการฝึกออกกำลังกายบนที่สูงกว่าระดับน้ำทะเลต่อตัวแปรในเลือดและ กลไกที่อยู่ เบื้องหลังของกลุ่มที่ตอบสนองดี และไม่ดีต่อการฝึกบนที่สูงในนักกีฬาฟุตบอล” ได้นำความรู้ที่ได้ไปสอนให้กับนักศึกษาสาขาวิทยาศาสตร์การกีฬาปริญญาโทและเอก ซึ่งสามารถนำความรู้ที่ได้จากงานวิจัยนี้ ไปใช้ประโยชน์ได้ โดยสามารถนำรูปแบบวิธีการฝึกบนที่สูงไปใช้เพื่อเพิ่มสมรรถภาพร่างกายในนักกีฬาฟุตบอล ตลอดทั้งนักกีฬาประเภทอื่นๆ อีกทั้งนำความรู้ที่ได้ไปพัฒนาต่อยอดหรือนำไปประยุกต์ใช้กับคนทั่วไปและในผู้ป่วยในอนาคตได้
2. **ส่วนหนึ่งของงานวิจัยนี้ได้นำเสนอ แบบ poster ที่งานประชุม Medical Science Congress 2015 ณ เมือง Queenstown ประเทศ New Zealand**
   Wonnabussapawich P, Tussaneeyakul W, Thuwakum, W, Hamlin MJ, Manimmanakorn N, **Manimmanakorn A.*** Living and training at 825m incorporated with simulated altitude improves blood parameters in soccer players. Medical Science Congress 2015, Queenstown, New Zealand, p81-82.

   (ภาคผนวก ดังเอกสารแนบหมายเลข 3)

หมายเหตุ

งานวิจัยนี้ได้ดำเนินการแล้วเสร็จ โดยใช้เงินทุนวิจัยของ สกว. เป็นระยะเวลา 2 ปี ผู้วิจัยได้เก็บข้อมูลโดยใช้อาสาสมัครที่เป็นนักกีฬาฟุตบอล 40 คน เนื้อหางานวิจัยมีมากเพียงพอจึงได้แบ่งออกเป็น 2 ส่วน เพื่อการตีพิมพ์ 2 เรื่องในวารสารวิจัยนานาชาติ ซึ่งอยู่ในระหว่างรอการตีพิมพ์ ทั้งสองเรื่องมีเนื้อหาของทุกส่วนครบสมบูรณ์ในตัวเอง ได้แก่ บทคัดย่อ คำสำคัญ วัตถุประสงค์ วิธีการทดลอง ผลการทดลอง สรุปและวิจารณ์ผลการทดลอง และเอกสารอ้างอิง ดังนี้ (เอกสารแนบหมายเลข 1 และ 2)

ภาคผนวก

<u>เอกสารแนบ หมายเลข 1</u>

Manuscript เรื่องที่ 1 (ฉบับร่าง)

Title: Acute and chronic effects of altitude training with iron supplementation on blood parameters and physical performance in soccer players

<u>เอกสารแนบ หมายเลข 2</u>

Manuscript เรื่องที่ 2 (ฉบับร่าง)

Title: Acute and chronic effects of altitude training with iron supplementation on heart rate variability and body composition in soccer players

<u>เอกสารแนบ หมายเลข 3</u>

นำเสนอผลงานวิจัยแบบ poster ที่งานประชุม Medical Science Congress 2015 ณ เมือง Queenstown ประเทศ New Zealand

Manuscript เรื่องที่ 1 (ฉบับร่าง)

Title: Acute and chronic effects of altitude training with iron supplementation on blood parameters and physical performance in soccer players

Manimmanakorn A.[1], Wonnabussapawich P.[2], Tussaneeyakul W.[3], Thuwakum, W. [2] Hamlin M.J.[4], Manimmanakorn N.[5]

[1] Department of Physiology, Faculty of Medicine, Khon Kean University, Thailand,

[2] Department of Graduate School, Khon Kean University, Thailand,

[3] Department of Pharmacology, Faculty of Medicine, Khon Kean University, Thailand,

[4] Department of Tourism Sport and Social Science, Faculty of environmental Science, Lincoln University, New Zealand,

[5] Department of Rehabilitation Medicine, Faculty of Medicine, Khon Kean University, Thailand.

Abstract

Training at altitude is the study that brings the athletes from the sea level to a higher altitude for short or long training periods in order to study the effects of adaptation of their bodies such as hemoglobin production and red blood cell content. As the hypoxic dose emerges as a key factor of erythropoietin adaptations, selection of the appropriate altitude and duration of the stay or training period are important factors. Studies to improve soccer performance have often focused on technique and tactics at the expense of physical resources such as endurance, strength, and speed. The altitude training is the potent method that may use to improve the performance and ability of athletics.

Objective: For these reasons, in our research, we mainly focused on studying and comparing the results of training at altitudes that affect hematological variables and physical fitness.

Methods: The forty volunteer soccer players were divided into four groups to investigate the effects of iron supplementation in both altitude and sea level training athletes. The first group, the sea-level training (ST;N=10), the second group, Sea-level training with iron supplementation (SI;N=10), group, the third group altitude training (AT;N=10) and lastly the fourth group. Altitude training with iron supplementation (AI;N=10). All athletes were matched on their soccer ability. Training soccer program in eight weeks similarly all group. This study was conducted at the two stations; the altitude group was trained Khonkaen University observatory on Chulaporn Dam, Chaiyaphum province which is 852 m high above the sea level plus simulate altitude which is about 3,300 meters above the sea level, sea level group was trained at Nakhonratchasima Ratchabhat University Stadium, Nakhonratchasima province which is about 123 meters above the sea level.

Result: There was a significantly increased ($p<0.05$) in EPO in AI (31.7±29.4), AT (14.8±11.0) greater than ST (4.8±14.2) groups following 8 week altitude training, Red blood cell significantly increased ($p<0.05$) in AI (3.0±2.8), AT (2.8±2.8) compared ST (-1.8±4.1) groups following 10 week altitude training. Improve sea level performance. The VO_2max and RHIET test significantly increased ($p<0.05$) also improved in the AI (151.0±17.6, -4.6±2.9), AT (177.6±20.5, -7.0±2.9), and SI 98.6±9.0, -3.6±1.9) compared ST(12.6±15.7, -2.4±3.1) groups respectively.

Conclusion: Our data strongly suggested that training at altitude significantly enhanced hematological variables and physical performance in soccer players.

Keywords: red blood cells, erythropoietin, hematocrit, hemoglobin, live high train high, simulated altitude

Introduction

Altitude training has been applied to develop to athlete's sea-level physical performance. In addition, several publications have been reported that altitude training improved aerobic base performance (Hamlin et al., 2013). Several approaches and modifications for altitude training have been used such as live and train high at altitude (LHTH) and live high train low at sea level (LHTL). However, there were no clearly conclusion that indicated the method and dose which strongly increased athlete capacity, neither any literature suggested the effected period of altitude training.

The adaptation at the physiological level was indicated by hematological parameters such as erythropoietin (EPO), red blood cell content (RBC) and total iron-binding capacity (TIBC). The increase of blood parameters after altitude training leaded to the increase of physical performance in athletes included speed, strength, endurance, jumping, oxygen consumption and also skill (Bailey et. al., 1988, Ekblom, 1991). On the other hand, some literature suggested that altitude training was no effect on hematological parameters (Bangsbo et al, 2001)

Soccer is one of the most widely played and complex sports in the world, where players need technical, tactical, and physical skills to succeed. However, studies to improve soccer performance have often focused on technique and tactics at the expense of physical resources such as endurance, strength, and speed (Helgerud et al, 2001). Soccer is a game of strength, endurance, flexibility, agility, skill and teamwork (Chaiyong, 1975). Soccer players need to have physical fitness such as strength, endurance, speed, agility, flexibility and power perfectly (Gramer et al, 1996). Physiological, technical, and tactical skills are all important to soccer performance. Factors such as acceleration, running velocity, vertical jump, and capacity to release energy are major importance (Helgerud, 2002). The main energy source (approximately 90%) of 90 minutes soccer match is from aerobic metabolism which players run about 10 km (Bangsbo et al, 2001) at an intensity close to anaerobic threshold or 80-90% of maximal heart rate (Hoff et al, 2001). It has been estimated that elite soccer players cover between 10 and 13 km over the duration of a competitive match, suggesting that elite level performance may, in part, be determined by a large aerobic capacity (Edwards, 2003).

The prevalence of iron depletion in top level soccer players based on low serum ferritin levels seems to be poorly analyzed although the hematologic parameters can be crucial for predicting optimal physical performance (Schumacher et al, 2002). In addition, a need for iron supplementation was proposed on the basis of observed changes in blood count and serum iron parameters during periods of intense training. A recent review has reported that the iron stores were lower in athletes than in sedentary subjects (Resina, 1990). The iron cost seems to be mainly related to theload of exercise (Helberg, 1984), although the type of the exercise may also be of importance. Soccer is very popular sport in several countries, and movement is an essential role in soccer training (Ekblom, 1986). However, little is known about changes in iron status of soccer players. Although iron deficiency seems to compromise work capacity, it is still controversial whether the performance may be impaired in athletes who have low iron stores but are not yet anemic.

For these reasons, we would like to investigate the effect of altitude training with and without iron supplementation on the hematological factors in soccer players. The outcome of this study will bring us to the better understanding of the effects of LHTH plus simulated altitude, iron supplementation combined with soccer program training in term of acute and chronic effects on hematological factors, physical fitness variables as well as the response mechanism under hypoxia condition that may practically apply to the soccer training in the future.

Methods

Forty male subjects of Nakhonratchasima Ratchabhat University (age range between 18-22 year olds) were purposive selected (based on math group method) and allocated into four groups: group 1. Sea-level training group (ST;N=10), group 2. Sea–level training with iron supplementation group (SI;N=10), group 3. Altitude training group (AT;N=10), group 4. Altitude training with iron supplementation group (AI;N=10). All athletes were matched according to their soccer ability. Before the experiment, all athletes had high physical performance, free from injury. Moreover, they all lived at sea level and had not been residents at attitude within the past 6 months. Ethically, the participants were fully informed about the aim and experimental protocol of this study. Likewise, their written informed consent was obtained.

Experimental Design

The athletes in altitude groups (AT and AI groups) slept, stayed and also were trained at altitude while sea level groups slept stayed and were trained at or near sea level (123 meters). All tests were conducted at sea level including; 1) physical performance test: 50, 100, 400 and 2,800 meters time trial runs, speed, agility, jump power and repeated high intensity endurance test 2) hematological tests: red blood cell (RBC) count, erythropoietin (EPO), hematocrit (Hct), hemoglobin (Hb) concentration, and Iron study 3) physiological tests: heart rate (HR), oxygen saturation (SpO2), 4) subjective score on fatigue, sleep, muscle soreness, training performance rate of perceive exertion, acute mountain sickness. During the experimental period, athletes in group 2 and 4 were received iron supplementation (Ferrogrand C, Abbott laboratories Ltd.). Blood parameters and performance were measured before and after the 8 weeks of experiment.

Soccer program training

All athletes were asked to train in the soccer program 3 hours a day, 5 days a week for 8 weeks. Participants were taken 5 steps for training each day; 1) warm up and stretching (15 minutes) 2) physical fitness: strength, endurance, agility speed (25minutes) 3) skill (25minutes), 4) Team strategy (40 minutes), 5) Cool down (15 minutes). All athletes strictly followed the same instructions of soccer training program in each week from the coaches during the experimental period.

Heart rate and SpO2 monitoring

Heart rate and SpO_2 were monitored during resistance training every group by a pulse oximeter and record intermediately after finish of each exercise session.

Rating of perceived exertion

Rating of perceived exertion (RPE) was measured using Borg scale and pain assessment was recorded at the end of each session every group. All participants reported on stress, fatigue, and muscle soreness, sleep and training performance on every day of the experiment.

Altitude Training Time line

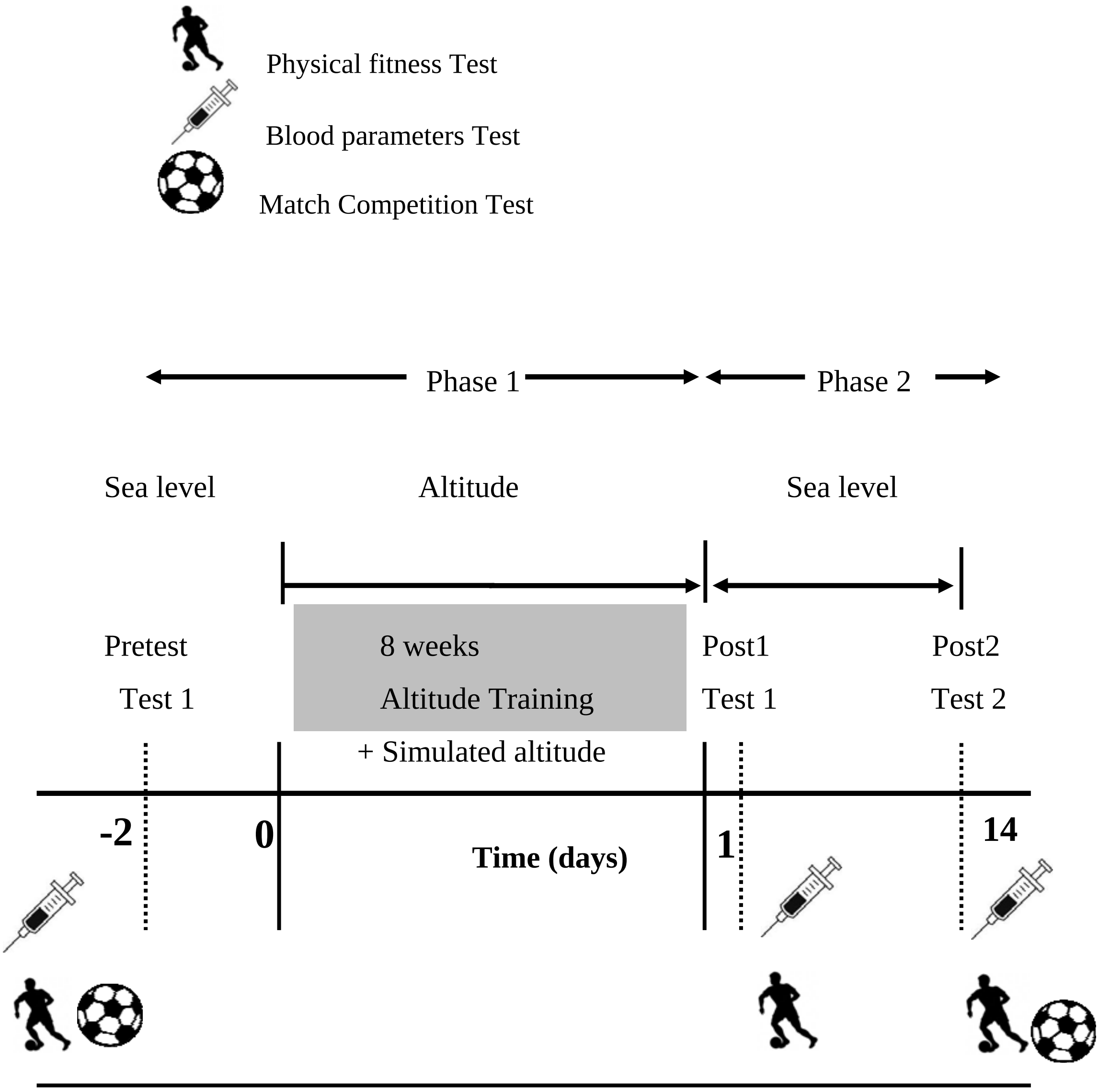

Figure 1 Timeline of altitude training program

Figure1. The overall timeline of altitude training program, briefly all athlete groups were measured hematological parameters and performance test 3 time points; 1. Two days before training camp (PRE), 2. The end of training camp (POST1) and 3, Two weeks after training camp (POST2). Moreover, the simulated altitude was performed under 15%O_2 condition by using hypoxic high altitude commercial systems.

Simulated Altitude program

All athletes were performed exercise on a cycling ergometer for 15 minute 3 day per weeks at 100-120 watt for 8 weeks, whereas the athletes in altitude groups were added with simulated altitude (hypoxicator, FiO2 0.15) during exercise.

Measurement of hematological parameters

All athletes were ethically asked to have blood tests. Blood samples were taken from median cubital vein or cephalic vein to measure red blood cell count (RBC), hemoglobin concentration, erythropoietin (EPO), serum iron (Si), white blood cell count (WBC), hematocrit (HCT) and hemoglobin. Blood samples were taken 3 times, before, during and after experimental period for baseline, POST1 and POST2. Blood samples were transported with ice in ice chest within one day to Srinagarind Hospital, KhonKaen University for the hematological analysis.

Measurement of physical performance parameters

Outcome variables were measured pre-and post-training program within 1-2 days before and after training programs. The primary outcome was measured of this study was by skill-related physical fitness test which it consist by;

Running tests

Performance assessed by individual 100 meters and 2,800 meters timed run trials at a standard field near sea level. Time trial runs were performed at the same time. Athletes completed a standardized 10-15 minutes warm-up with stretching prior to the runs, but were notprovide any feedback on time pace. Rate of perceive exertion ratio was measured immediately after the tests.

Physical Performance Tests

Seven to two days before the eight weeks training period all athletes completed a series of physical performance fitness tests for soccer players including: The explosive power was evaluated by the maximum effort countermovement jump using standard procedures (Contact-Mat Kinemetic timing system, Australia). The best of three attempts were recorded. The sprint tests involve running distances, 5 and 10 m for which athletes were asked to perform maximum effort sprints. The better of two trials was recorded. The agility 505 test was designed to tests agility by minimizing the influence of running speed while measuring acceleration before and after change of direction. The test begins at walking speed and increases progressively until exhaustion following the standard procedure from Lambert (1982) (Leger & Lambert, 1982). The maximum oxygen consumption (VO_2max) and maximum attained speed (MAS) were predicted based on beep tests data before and the after training period.

Statistical analysis

Data in the text and figures and presented as means with 90% CL and CI, respectively were presented as mean±SD. The data for descriptive statistics were calculated. The statistical significance of differences in the red blood cell count (RBC), erythropoietin (EPO), hematocrit (Hct), hemoglobin (Hb) concentration, heart rate (HR), oxygen saturation (SpO2), serum iron, serum ferritin, 100meters, 2,800meters, agility and VO_{2max} of AI, AT, SI and ST groups. The magnitude of the correlations was rated using Hopkins scale. For the magnitude inference test, baseline was calculated and used as a covariate in the analysis to adjust for any differences between groups at baseline. Because the small performance changes can be beneficial for elite athletes which can not detected by the conventional method, contemporary statistical method was used and applied to test our data (Hopkins, Hawley, & Burke, 1999). Specifically, we used magnitude-based inferences about effect sizes, and then to make inferences about true (population) values of the effect, the uncertainty in the effect was expressed as 90% confidence limits (CL).

Results

Table 1. Subjects characteristics in the four training groups

	AI	AT	SI	ST
Age (yr.)	20.3±1.3	20.9±0.7	20.4±0.7	20.3±0.7
Body weight (kg)	68.0±7.5	67.5±9.5	62.3±6.3	69.2±9.8
Height (cm)	174.8±3.5	173.0±5.8	168.4±5.3	174.1±6.1
BMI (kg·m^{-2})	22.2±2.0	22.5±2.8	21.9±1.5	23.0±2.7
Agility (sec.)	14.1±0.2	14.6±0.1	14.1±0.3	14.7±0.1
$V0_2$max	43.42±5.22	42.37±0.9	43.62±8.5	41.70±2.7
Training Intensity(hr/wk)	9.22±2.55	9.51±2.33	9.43±2.17	9.17±2.74
Warm-up Stretching	1.12±0.13	1.17±0.14	1.17±0.12	1.18±0.14
Physical Fitness	2.08±0.15	2.18±0.11	2.18±0.12	2.19±0.13
Skill	2.03±0.16	2.08±0.15	2.17±0.12	2.17±0.10
Team Stagiest	3.15±0.14	3.19±0.15	3.18±0.12	3.17±0.10
Cool Down	1.07±0.11	1.11±0.11	1.12±0.10	1.10±0.09

Values represented mean±SD. The altitude training with iron supplementation group (AI;N=10), altitude training group (AT;N=10), Sea-level training with iron supplementation group (SI;N=10), Sea-level training group (ST;N=10). No significant differences were found between groups for any variable.

Figure 2. SpO_2 during 15minutes cycling test recovered in 8-weeks experimental period in all groups

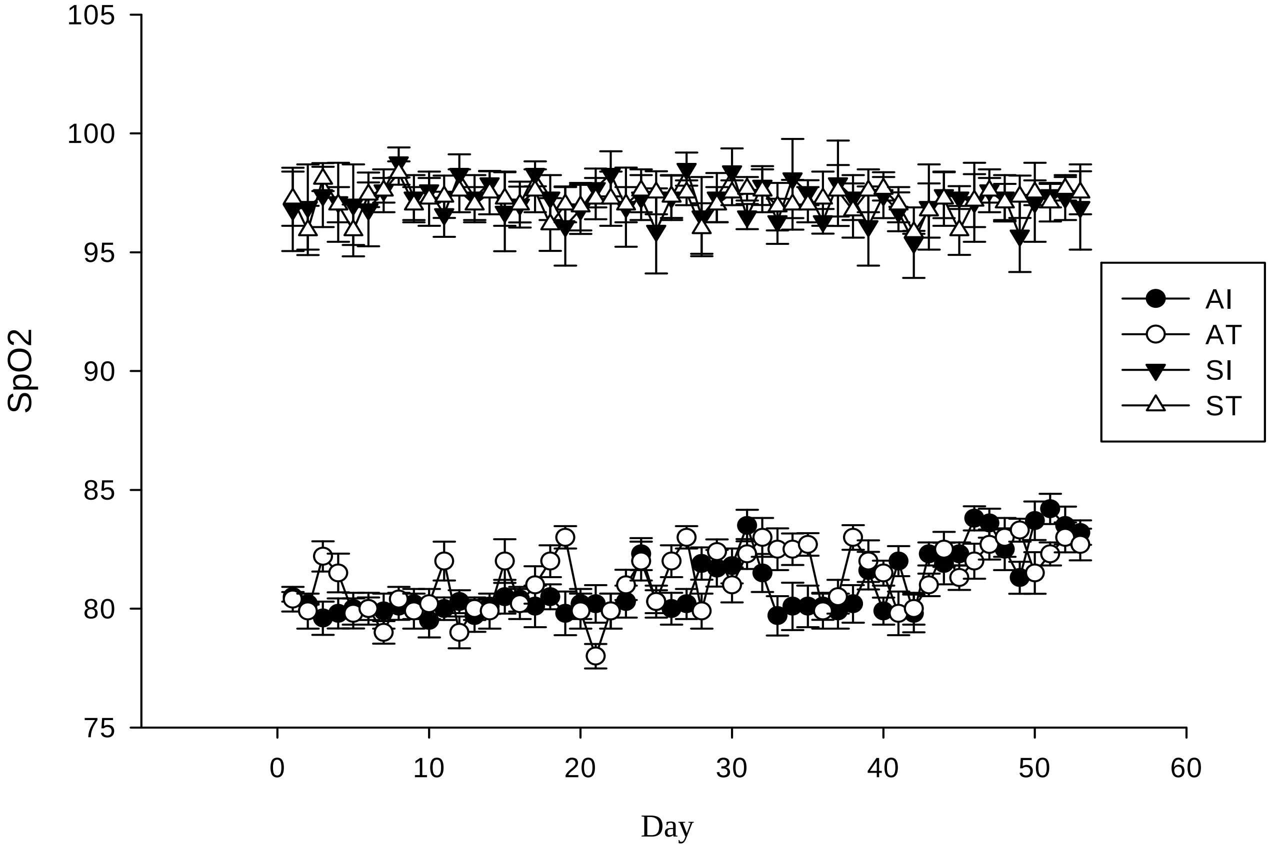

Figure 2 indicated that SpO_2 level in the sea-level groups (SI and ST) showed the dramatically higher when compared those in the altitude groups (AI and AT). However, the averages SpO2 in sea-level groups were 96-98% while the averages of SpO_2 in altitude subjects were 80-85%. Moreover, when trained at the sea-level, it showed no significantly difference among the subject group with and without iron application. On the other hand, in the altitude subject, they revealed the contrast response. After treated with ferrous sulfate for 30 days, the SpO_2 value showed the positively response to iron application.

Table 2. The mean±SD changes in hematological variables measured before (PRE) and after altitude training day1 (POST1) and day14 (POST2)

		ST N=10	SI N=10	AT N=12	AI N=10
EPO					
	PRE	9.33±4.90	13.90±12.51	13.05±3.85	10.80±3.53
	POST1	9.31±3.45	14.33±12.63	14.12±2.76	14.56±6.62*
	POST2	9.46±2.54	15.57±9.02	10.30±3.08	12.53±6.91
RBC					
	PRE	5.25±0.66	4.96±0.31	5.48±0.33	5.15±0.22
	POST1	5.38±0.60	5.22±0.29	5.69±0.30*	5.30±0.20*
	POST2	5.11±0.37	5.21±0.33	5.61±0.31*	5.30±0.25*
WBC					
	PRE	7.24±0.97	6.84±0.58	8.14±1.91	7.41±1.21
	POST1	7.05±1.07	6.67±0.68	7.61±1.58	7.57±1.55
	POST2	6.65±2.02	5.93±0.95	6.36±1.57	6.44±0.92
HCT					
	PRE	43.15±1.85	43.16±3.57	42.70±2.44	44.02±2.50
	POST1	44.63±1.87	44.56±3.19	44.25±2.25	44.93±2.88
	POST2	44.72±4.63	47.27±3.43	45.10±6.62	47.17±4.20
HGB					
	PRE	14.22±0.73	14.36±1.16	13.97±0.91	14.78±0.93
	POST1	14.38±0.83	14.45±1.13	14.30±0.85	14.81±0.85
	POST2	14.05±1.28	14.80±1.16	14.33±1.02	15.08±1.02
Si					
	PRE	103.21±29.08	106.00±43.76	86.57±23.77	79.28±16.08
	POST1	124.71±38.94	141.00±44.00	94.14±16.02	97.71±27.89
	POST2	136.42±43.77	156.28±49.88	139.21±32.73*	144.67±16.80*
TIBC					
	PRE	3.5±16.10	7.4±13.32	5.1±9.43	18.25±21.31
	POST1	-15.5±39.55	-15.7±41.42	-24.8±71.50	23.3±12.42
	POST2	-18.4±48.92	-21.5±28.35	-28.4±71.33	-35.1±26.43

The values represented mean±SD. *p<0.05

Table 2, it revealed the response of hematological indices of the seal-level groups (SI and ST) and altitude groups (AI and AT) measured before (PRE) and after training (POST1 and POST2). It clearly showed that the RBC level dominantly responded to altitude training. The RBC level significantly increased in both with and without iron supplemented groups after 8 weeks of altitude training (AI and AT respectively). Moreover, the RBC level remain the almost the same level at day 14 after training (POST2) in both groups (AI and AT). When considered Si level, it significantly increased in AI and AT group at day 14 after training (POST2). Furthermore, the EPO level, only the altitude group that treated with ferrous sulfate (AI) displayed significantly higher in EPO level (14.56±6.62mIU/ml) after 8 weeks of training.

Table 3. Comparative change in performance (X) on hematological variables measured before (PRE) and after altitude training (POST1) in all groups.

	AI Vs ST	AI Vs AT	AI Vs SI	AT Vs SI	AT Vs ST	SI Vs ST
EPO	25.6±24.2*	14.6±24.2	29.6± 27.4*	6.9±18.2	18.2±13.7*	52.5±46.1*
RBC	-0.1±3.4	2.2±3.1	-1.0±4.0	1.3±5.0	1.8±3.7	0.9±4.6
WBC	7.7±12.6	9.3±13.6	8.5±13.8*	1.5±14.0	-2.1±9.9	-1.1±9.8
HCT	-0.8±3.8	-1.3±3.1	-1.0±3.9	0.1±3.3	-0.2±3.0	-0.1±3.9
HGB	-0.1±4.6	-0.5±4.0	1.5±4.6	2.3±3.6	0.9±3.4	-1.3±4.2
SI	-9.4±37.7	6.2±27.7	-24.6±45.9	-30.4±36.7	-16.9±26.4	-15.9±37.6
TIBC	20.5±23.3*	12.5±19.2	26.3±21.8*	7.7±12.9	9.1±15.1	3.8±16.2

The values represented means ± sd with difference 90% CI (asterisk).

The RBC level measured before (PRE) and after altitude training (POST1) of all experimental groups and found the obvious results in EPO level, WBC and TIBC. The EPO level in all group, we found that the EPO in AI and AT significantly higher than SI and ST groups. Moreover, the WBC level significantly difference when compared AI and SI group. Furthermore, only the TIBC level of AI group showed the significantly dominant when compared to ST and SI groups (Table3)

Table 4. Comparative change in performance (X) on hematological variables measured before (PRE) and after altitude training (POST2) in all groups.

	AI Vs ST	AI Vs AT	AI Vs SI	AT Vs SI	AT Vs ST	SI Vs ST
EPO	-3.4±25.9	41.4±24.4*	-12.4±37.4	-38.1±33.9	-16.7±21.5	29.0±71.6*
RBC	4.8±3.9*	0.1±3.4	-0.9±6.0	0.7±7.8	5.2±3.9*	4.4±6.5
WBC	-2.7±21.9	9.1±17.3	2.4±14.5	-6.7±20.1	-11.6±28.5	-4.4±23.6
HCT	3.6±8.7	0.3±4.0	-2.6±4.7	-3.9±3.9	-2.1±8.4	6.2±8.4
HGB	3.5±6.7	0.1 ±4.4	0.7±5.1	0.2±4.1	4.2±5.9	3.7±6.3

SI	11.0±28.8	11.3±20.4	-5.3±43.7	-8.4±44.9	7.2±30.9	-18.3±45.1
TIBC	-9.7±43.4	1.9±72.9	5.4±47.0	8.2±67.9	3.3±66.9	0.2±45.0

The values represented means ± SD with difference 90% CI (asterisk).

The RBC level measured before (PRE) and after altitude training (POST2) of all experimental groups were significant in EPO and RBC. The EPO level showed significantly increased in AI and SI when compared to AT and ST respectively. In addition, the RBC content also showed the difference in the comparison of AI vs. ST and AT vs. ST (Table4)

Table 5. The average of physical fitness content among the experimental groups (at the 3 time points; before (PRE), 1day (POST1) and 14day (POST2) after training

Physical Fitness Test	**AT**			**AI**			**ST**			**SI**		
	PRE	POST1	POST2	PRE	POST1	POST2	PRE	POST1	POST2	PRE	POST1	POST2
Runing(m)												
100m(sec)	13.81	12.98*	12.86*	12.85	11.93*	11.84*	13.33	12.82	12.65*	13.01	12.26	12.21*
2800m(min)	16.08	15.02*	14.95*	14.10	13.40*	13.05*#	16.92	15.41*	14.98*	15.91	14.34	14.17*
Jump												
Power(w)	313.3	900.8*	955.5*#	540.4	990.1*	1021.*#	314.2	937.8*	948.8*	280.7	838.00*	897.7*
Height(cm)	29.9	32.9*	35.7*#	30.4	32.0*	33.8*#	29.1	30.60	30.70	28.10	32.2*	33.10*
Agility(sec)	14.64	14.22*	14.23*	14.11	13.86*	13.80*	14.64	14.24*	14.21*	14.14	13.80	13.77*
Vo_2max	42.37	50.25*	52.41*	43.42	57.24*	57.97*	41.70	46.30	50.92*	43.62	53.89*	54.98*

The values represented means. *p<0.05

For the AT group, the physical fitness test revealed that the altitude training significantly improved the running ability in all distance (50m, 100m, 400m and 2800m) when compared to their baseline. However, this result clearly showed the difference after 10 weeks of training (POST2). Besides, the jump power and jump height also displayed the similar trend. The AT group increased the jump power and height after training. In addition, the agility in AT group also increased after training (POST1 and POST2).

For the AI group, the physical fitness of AI group dramatically improved the running ability when compared to the beginning of training. The running test in AI group clearly showed that running ability in POST2 higher than POST1 of all distance tested. Furthermore, when considered the jump ability in AI group, it showed that AI treatment significantly increased when compared to before training time point. The agility also revealed the similar trend that the AI treatment significantly elevated these parameters.

For the ST group, the physical fitness test revealed that the sea-level training significantly improved the running ability in all distance (50m, 100m, 400m and 2800m) when compared to the beginning. Moreover, the jump power, agility and results displayed that the ST group had capacity those two parameters after training both POST1 and POST2.

For the SI group, the running ability of SI group significantly improved after 8 weeks training. Besides, the SI group still improved their running ability at the POST2 time point. The jump power, jump height and displayed the similar response to physical fitness test by which the SI athletics continuously elevated their jump ability. Finally, the agility test of SI subject showed the significantly improved only POST2 time point.

Table 6. Match competition

Match competition	Before Camp				7 Day After Camp			
	AT Vs ST		AI Vs SI		AT Vs ST		AI Vs SI	
Goal	1	1	3	1	3	1	5	1
Shoot	6	7	9	6	8	4	11	6
Shoot on Target	4	4	5	3	5	2	8	4
Possession	54%	46%	63%	37%	61%	39%	67%	33%

AT & ST before camp

For the match competition of AT and ST group before the training, AT drew against ST. For the statistical of AT group, they had 6 total shoots with 4 shoot on targets. Moreover, they had 54% of ball possession. On the other side, the ST group had 7 total shoots with 4 shoot on targets. Likewise, they had 46% of ball possession. From the statistics of both team, they showed the very similar in term of soccer performance such as ball possession, shoot on target and goal.

AT & ST 7 after camp

However, after 8 weeks of training, AT beat ST with score 3-1. The AT group had 8 total shoots with 5 shoot on targets and had 61% of ball possession. While, the ST group had 4 total shoots with 2 shoot on targets and they had 59% of ball possession. From this result, it clearly showed that the AT team dramatically improve in soccer performance that included ball possession percentage, shoot on target and gold which lead to the team success.

AI & SI before camp

Before the training and iron treatment, the AI beat SI team with score 3-1. For the soccer performance, the AI team had 9 total shoots with 5 shoot on targets and they had 63% of ball possession. On the other hand, the SI team had 6 total shoots with 3 shoot on targets. However, they had only 31% of ball possession. From this analysis, it suggested that the AI team showed the significantly higher in soccer performance over the SI team.

AT & ST 7 after camp

After training and iron treatment, the AI team still beat SI team with score 5-1. The statistical analysis showed that the AT group had 11 total shoots with 8 shoot on targets and they had 67% of ball possession. While, the SI group had 6 total shoots with 4 shoot on targets and they had 33% of ball possession. These data clearly showed that the AI team significantly enhanced in the soccer performance that lead them to the match success.

Discussion

Training and living at the low altitude (825meters above sea level) conjunction with both breath hypoxia gas (FiO_2 15%) and iron supplementation has been used in this study. This form altitude training showed substantial improvement in some hematological variables as well as physical fitness in soccer players.

The major findings of this study were classified into three points. Firstly, the level of EPO significantly increased after the athletics were trained at the real altitude for eight weeks which resulted in the improvement of oxygen consumption. Secondly, the iron supplementation elevated the hematological markers such as EPO and RBC and improved both aerobic and anaerobic. Moreover, the iron treatment also enhanced the overall physical performance of soccer players. The combination treatment of altitude level and iron supplementation was the best treatment which strongly enhanced the broadly physical performance and blood parameters of the soccer players.

The overall variables, we monitored the blood parameters and physical performance of all subjects in two phases which were after 1 day and 14 days of training indicated as POST1, POST2 respectively. Based on our investigation, the altitude group (AI and AT) significantly higher in the EPO level when compare to the sea-level training group (SI and ST) when measured after a day of training period. Although, no significant difference in EPO level was found 14 days later, RBC and HGB level were significantly higher in this stage (Table2.). The EPO level is the very sensitive parameter that indicates the response of athletics to hypoxia condition. In other word, EPO is the hematological marker that response to low oxygen condition. EPO is the renal hormone that induced the red blood cell production through the induction of hypoxic condition. At the molecular level, EPO gene contains hypoxia response element within the promoter region of gene which regulated the expression of EPO gene during hypoxic environment (Jelkmann, 2011). Training increases RBC and stimulates erythropoiesis, adaptive changes that are regulated by different mechanisms that show different temporal responses to the onset of physical activity. Increased red cell production is also associated with an expansion of red bone marrow. The increase of EPO directly regulated hemoglobin concentration through the control of red blood cell production. These hematological changes may improve an athlete's VO_2 by enhancing blood circulation to deliver oxygen to the exercising muscles. That resulted in the enhancement of aerobic performance (Hall and Guyton, 2010, Wilber, 2004). In support of this, the studies of Levienand Stray-Gundersen (1997) found that a significant 5% in RBC mass and 9%

hemoglobin improvement. Stray-Gundersen (2001) has also found erythropoietin increased 92% in LHTL subjects (Gundersen, 2001). Likewise, the body has hemoglobin as 13.4 grams per 100 cubic centimeters of blood at sea level. After the body exposed to higher altitude, hemoglobin increases to 17 g per 100 cubic centimeters of blood (Thoranin, 1979). Performance measures in these studies included sport specific ergometers and endurance time during an exhaustive incremental exercise test. All of these investigations showed decrements in aerobic performance upon acute exposure to altitude. Decrements in aerobic performance at altitude have been associated with hypoxia-induced reductions in VO_2max secondary to reductions in SaO_2. In sports such as cycling and speed skating in which aerodynamics play an important role, the decrement in VO_2max may be offset by aerodynamic advantages up to elevations of approximately 4,000 m (Wilber, 2004).The deduction in FiO_2 (oxygen in air) and PaO_2 (oxygen in lungs) result in decrements of kidney PaO_2 and low oxygenation in kidney, there by stimulates the synthesis and release of EPO.

The hematological changes may significantly improve aerobic performance in endurance athletes by enhancing the delivery of oxygen to working muscles. In short, the increased of RBC that was induced by low oxygen environment played the key role of adaptive mechanism to altitude. Iron supplementation is a common strategy to increase the ability of red blood cells to deliver oxygen to exercising muscles. In our experiment, when apply the iron supplementation to the soccer player both in altitude training and sea-level training, we found that the iron-treated group significantly increased the EPO content in both altitude and sea-level training (Table3 and 4). However, only the high altitude group clearly showed the possibly higher in RBC (Table3). In addition, when monitored the hematological parameters in the long term, the iron treatment significantly prolonged the EPO level of both altitude and sea-level group (Table3 and 4).

At the physiological level, iron supplementation slightly modulated the level of ferritin in blood (Nielsen and Nachtigall, 1998). Ferritin is the storage form of iron in blood and also plays an important role for endurance athlete in maintaining amount of iron, especially during the increase of training, especially for the athlete at altitude because it is requires increasing the erythropoiesis process that would occur when the oxygen decreases. For this reason, ferritin is very important for endurance athlete in maintaining amount of iron, especially during the increase of training, especially for the athlete at altitude because it is requires increasing the erythropoiesis process that would occur when the oxygen decreases.

In term of physical performance, altitude training strongly improved endurance, anaerobic metabolism and VO_{2max} (Table2.). After training, all experimental groups increased in aerobic and anaerobic respiration which reflected by the running test in 50meters and 2800meters (Table5). In addition, The AI group reduced time of running in POST1 and POST2 indicated that the running test also reflected both the acute and chronic effects in AI group. Likewise, the agility test significantly improved in all groups where the AI was the best treatment that improved agility and it clearly showed the chronic effect after 14 days of training (Table5)

Training at altitude elevated some physical fitness such as agility, jump power and also running speed at 2800meters. When applied iron supplement, it increased the maximum aerobic capacity and prolonged the endurance time to fatigue that was resulted from the positively induction of serum ferritin and hemoglobin (Magazanik et al, 1991). When compared the match result before and after training, we discovered that the altitude training improved the soccer performance when compare to the sea-level training. The altitude training altered some hematological factors that contribute to the increasing in physical fitness of soccer players. In term of psychological sense, the long-term group training that they ate, slept and lived together for the long period of time, it improve the relationship among the soccer players. Moreover, the training at the good environment was also the important factor that induced the subjects to concentrate and focus on training activities.

The iron compensation increased RBC in AI and AT and still increased during training period and after 14days of training while Si showed significantly increased during experimental period (Table2). Moreover, to elucidate the effect of iron compensation by compared AI and ST, it clearly showed that AI increased EPO level and TIBC during experimental period (Table3). However, after 14days of experimental period, EPO level and RBC still increased in AI (Table3)

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Manuscript เรื่องที่ 2 (ฉบับร่าง)

Title: Acute and chronic effects of altitude training with iron supplementation on heart rate variability and body composition in soccer players

Manimmanakorn A.[1], Wonnabussapawich P.[2], Tussaneeyakul W.[3], Thuwakum, W. [2] Hamlin M.J.[4], Manimmanakorn N.[5]

[1] Department of Physiology, Faculty of Medicine, Khon Kean University, Thailand,

[2] Department of Graduate School, Khon Kean University, Thailand,

[3] Department of Pharmacology, Faculty of Medicine, Khon Kean University, Thailand,

[4] Department of Tourism Sport and Social Science, Faculty of environmental Science, Lincoln University, New Zealand,

[5] Department of Rehabilitation Medicine, Faculty of Medicine, Khon Kean University, Thailand.

Abstract

Much of the research on altitude training for athletes has focused on haematological variables; with inconclusive results. The lack of consistent findings may due to the dose and forms of altitude training and the iron status of the individual athletes.

Objective: This research aimed to investigate the effects of natural altitude training supplemented with intermittent hypoxic training (IHT) and iron supplementation on heart rate variability (RMSSD, LF, HF LF/HF ratio), body composition in and blood lactate concentration in soccer players.

Methods: Forty university soccer athletes were divided into 4 groups; Sea-level training (ST; N=10), sea-level training with iron supplementation (SI; N=10), supplemented altitude training (AT; N=10), supplemented altitude training with iron supplementation (AI; N=10). All athletes were matched on their soccer ability and followed the same soccer training program for 8 weeks. The altitude groups stayed and trained at 825 m with the addition of 15 min simulated altitude training daily on bike. All athletes performed a pre-test (1-2 days prior to training) and two post tests on day 1 and day 14 after the training camp.

Results: By day 14 post camp, RMSSD and HF increased substantially in the AT group (10.7± 55.6 ms and 12.4 ± 96.5 ms^2) compared with ST group (53.0 ± 53.0 ms and -54.8 ± 106.3 ms^2), ± 90% CL. Percent body fat were substantially reduced in all groups compared to their baselines (AT=18.4, 14.9%; AI=17.5, 14.8%; ST=18.9, 16.1%; SI=17.0, 14.3%) mean ±SD. By day 1 post camp, blood lactate concentration showed higher level in both altitude training groups (AT and AI) compared to baseline. However, sea-level groups (ST and SI), blood lactate concentration significantly decreased in all day 1 and day 14 post camp.

Conclusion: Supplementing 8 weeks of living and training at low altitude with 15 min daily of IHT improves heart rate variability important to autonomic nervous system especially parasympathetic function improvement. These changes may influence training ability for soccer players.

Keywords: root mean square, body fat mass, blood lactate, live high train high

Introduction

Soccer is one of the most widely played and complex sports in the world, where players need technical, tactical, and physical skills to succeed. However, studies to improve soccer performance have often focused on technique and tactics at the expense of physical resources such as endurance, strength, and speed (Helgerud et al, 2001). Soccer players need to have physical fitness such as strength, endurance, speed, agility, flexibility and power perfectly (Gramer et al, 1996). Moreover, factors such as acceleration, running velocity, vertical jump, and capacity to release energy are major importance.

Training at altitude is the study that brings the athletes from the sea level to a higher altitude (more than 500 meters above sea level) in order to study the effects of adaptation of their bodies that has been interest since 1968. Several approaches and modifications for altitude training have been used such as live and train high at altitude (LHTH) and live high train low at sea level (LHTL). Theoretically, the body will adapt by breathing more frequently to bring more oxygen in to the cells then athletes should acquire the beneficial effects of altitude acclimatization, particularly stimulation of the oxygen delivery system increasing in the total body hemoglobin for maximizing oxygen transport and utilization, and enhancing ability to train without reducing training intensity (Zhang et al, 2007). For long term exposed to altitude, the deleterious effects of low partial pressure of oxygen in blood force the human body to adapt and change, such as increasing the number of red blood cells and subsequently performance gain (West et al., 1983). Moreover, the body will be adapted by increasing erythrocyte to carry with oxygen.

Altitude training directly affected to various blood parameters such as hemoglobin, red blood cell content and VO_2max (Levine and Stray-Gundersen 1997). Moreover, various researches revealed altitude also affected on heart rate variability (HRV). Perini, Milesi et al. (1996) reported the effect of altitude acclimation on HRV. They found that the altitude group significantly reduced HRV when compared to the sea-level group.

Based on the electrical activity of the heart, the interval heart beat (R to R peak) was indicated as heart rate variability (HRV) which represented the adaptive capacity of cardiovascular system under various conditions. HRV relied on personal activities, diseases, gender as well as age (Aubert, Seps et al. 2003, Acharya, Joseph et al. 2006). Because HRV corresponded to the heart beat interval which regulated by automatic nervous system (ANS), it strongly reflected the activities of ANS (Acharya, Joseph et al. 2006). The ANS referred to the nervous system that works without consciousness which was divided into two categories; sympathetic and parasympathetic nervous system. Generally, heart rate tightly regulated by both sympathetic and parasympathetic nervous system in the contrast manners. When considered cardiovascular system, the parasympathetic trends to decreased heart rate while sympathetic system increased heart rate activity (Aubert, Seps et al. 2003)(Saito, Tanobe et al. 2005) reported the positive correlation between arterial oxygen saturation and heart rate variability at high altitudes and suggested that automatic response was reduced under hyperbaric environment. Many studies have reported that the sympathetic nervous system is up - regulated at rest and during exercise in response to acute and chronic exposure to altitude as evidenced by increasing in epinephrine and norepinephrine in plasma (Mazzeo et al., 1998, 2001). Other study reported that the increase in blood lactate concentration during submaximal and maximal exercise during altitude exposure has been related with the hypoxia – induced increase in epinephrine (Kayser 1996; Lundby et al., 2000). From the previous data, it did not clearly indicate the correlation between altitude training on HRV and blood lactate response. For this reason, in this work, we performed a series of physiological test that may lead us better understanding about this issue.

Summarily, the aims of this study were mainly focus on, firstly, the effect of altitude training on HRV. Secondly, the effect of live high, train high plus simulated altitude with iron supplementation on blood lactate concentration and body composition.

Methods

Based on matching match group method, forty male soccer players of Nakhonratchasima Ratchabhat University (age between 18-22 year olds) were purposive selected and allocated into four groups: group 1. Sea-level training group (ST;N=10), group 2. Sea–level training with iron supplementation group (SI;N=10), group 3. Altitude training group (AT;N=10), group 4. Altitude training with iron supplementation group (AI;N=10). All athletes were matched according to their soccer ability. Before the experiment, all athletes had high physical performance, free from injury. Moreover, they all lived at sea level and had not been residents at attitude within the past 6 months. Ethically, the participants were fully informed about the aim and experimental protocol of this study.

Experimental Design

The athletes in altitude groups (AT and AI groups) slept, stayed and also were trained at altitude while sea level groups slept stayed and were trained at or near sea level (123 meters). All tests were conducted at sea level including; 1) physiological tests: heart rate variability (HRV), heart rate (HR), oxygen saturation (SpO2), 2) physical performance test: 50, 100, and 400 meters time trial runs, speed, jump high, jump power and repeated high intensity endurance test. 3)blood lactate content, 4) subjective score on fatigue, sleep, muscle soreness, training performance rate of perceive exertion, acute mountain sickness. During the experimental period, athletes in group 2 and 4 were received iron supplementation (Ferrogrand C, Abbott laboratories Ltd.). Physiological and performance were measured before and after the 8 weeks of experiment.

Training: soccer program

All athletes were asked to train in the soccer program 2 hours a day, 5 days a week for 8 weeks. Participants were taken 5 steps for training each day; 1) warm up and stretching including passive and active stretching (15 minutes) 2) physical fitness including strength, endurance, agility and speed (25minutes) 3) skills including pass and receive, bass mastery, ball control and ball movement (25minutes), 4) Team strategy including small size game, defender game, counter attack and group tactic (40 minutes), 5) Cool down and body conditioning (15 minutes). All athletes strictly followed the same instructions of soccer training program in each week from the coaches during the experimental period.

Daily monitoring: Heart rate and SpO2

Heart rate and peripheral capillary oxygen saturation (SpO_2) were monitored during resistance training every group by a pulse oximeter and record intermediately after finish of each exercise session.

Subjective measurement;

Rating of perceived exertion

Rating of perceived exertion (RPE) was measured using Borg scale and pain assessment was recorded at the end of each session every group. All participants reported on stress, fatigue, muscle soreness, sleep and training performance on every day of the experiment. The Lake Louise Acute Mountain Sickness (AMS) score was monitor throughout the altitude camp by using a questionnaire. The subjective were score on fatigue, sleep, muscle soreness, training performance every day during experimental period.

Altitude Training Timeline

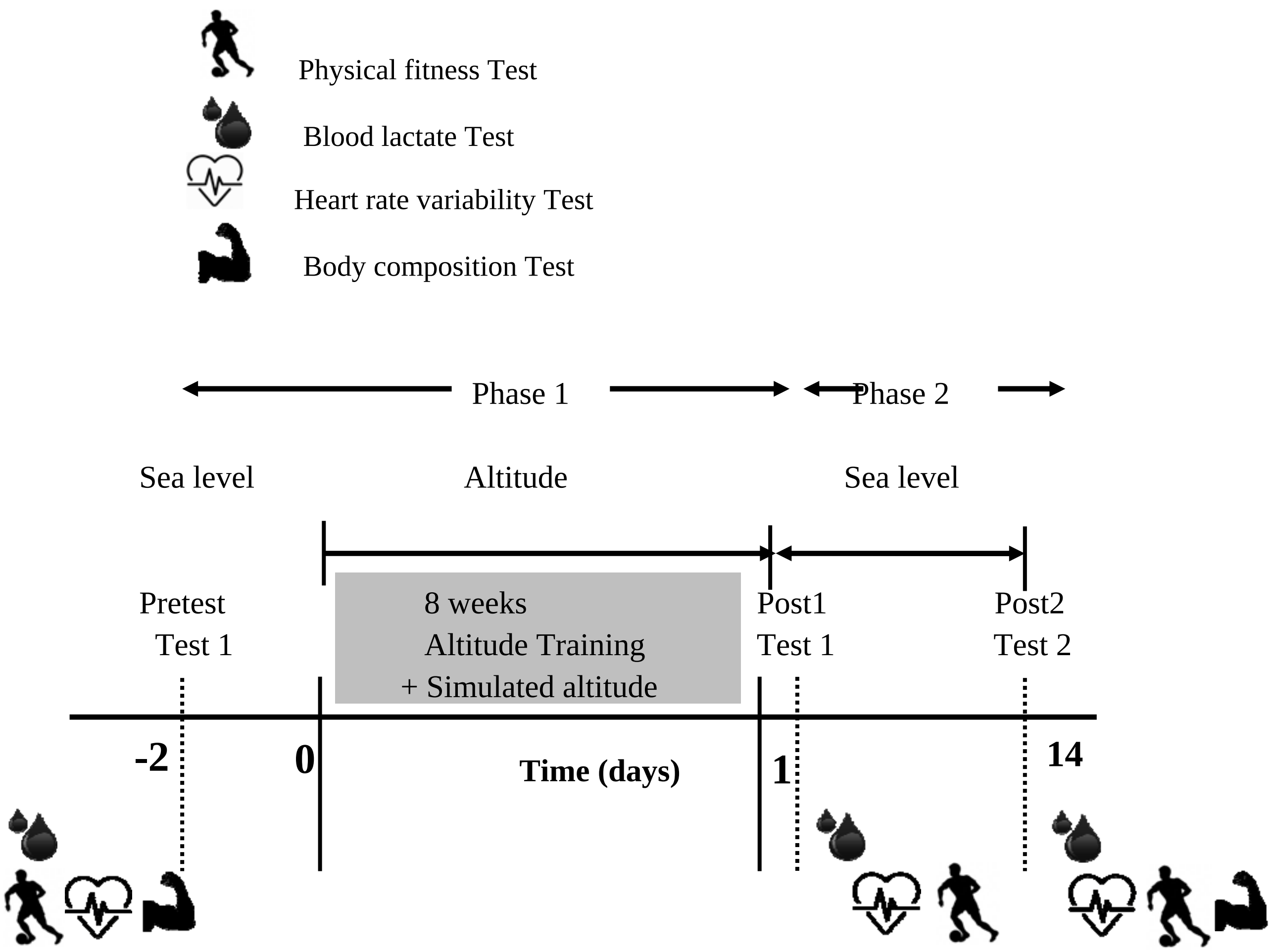


Figure 1 Timeline of altitude training program

Figure1. Overall timeline of altitude training program. Briefly, all athletes were measured hematological parameters and performance test 3 time points; 1. Two days before training camp (PRE), 2. The end of training camp (POST1) and 3, Two weeks after training camp (POST2). The simulated altitude was performed under 15%O_2 condition by using hypoxicator (beurer model:po30 made USA)

Simulated Altitude program

Simulated altitude program for 8 weeks of experiment, all athletes were performance exercise on cycling ergometer at 100-120 Watt for 15 minutes per day, 3 times per week, whereas the athletes in altitude groups were added with simulated altitude operated by hypoxicator (FiO_2=0.15) during exercise which equal to degree of hypoxia corresponding to an altitude 3,300 meters or 15% O_2 concentration condition.

Measurement of physical performance parameters

Outcome variables were measured pre-and post-training program within 1-2 days before and after training programs. The skill-related physical fitness test was measured in many activities including;

Running tests

Performance assessed by individual 50, 100 and 400 meters timed run trials at a standard field near sea level. Time trial runs were performed at the same time. Athletes completed a standardized 10-15 minutes warm-up with stretching prior to the runs, but were not provided any feedback on time pace.

Heart Rate Variability Test

The RR interval or time between two R waves of the recorded cardiac electrical activity was measured 3 minutes in supine and 3 minutes in standing position by using a Polar RX800CX heart rate monitor (Polar, Kemplel, Finland). All athletes were asked to measure 2 times for before and after training program.

Measurement of Blood lactate concentration

Blood samples were collected three times; before experiment (Pre test), 1-2 days after training (Post test1) and 14 days after training (Post test2). Approximately 5 ml of blood was taken from the fingertip via Accutrend® Plus system (Roche, USA). The blood sample was immediately collected before and after 400 meters running test to analyze blood lactate concentration by using lactate analyser.

Physical performance test

Before the eight weeks training, all athletes completed a series of physical performance fitness tests for soccer players including: The explosive power was evaluated by the maximum effort countermovement jump using standard procedures

(Contact-Mat Kinemetic timing system, Australia). The best of three attempts were recorded. Time for all running test were recorded using electronic speed timing lights (Speed light, Kinematic timing system, Australia).

The maximum oxygen consumption (VO_2max)

All athletes were subjected for maximal oxygen uptake (VO_2max) on the cycle ergometer protocol based on Astrand-rhyming cycle ergometer test (Astand, 1960). Firstly, subjects walked on a cycle ergometer for 5 minutes. Heart rate was measured every minute, and the steady state heart rate is determined. Secondly, the steady state heart rate was monitored to determine an estimation of VO_2max.

Repeated High Intensity Endurance Test (RHIET)

The test was conducted on a non-slip surface in order to provide good traction for the athletes. A corridor or track of 60 meters lengths provides for a 40 meters test track and 20 meters in deceleration zones. Measure a distance of 40 meters with pylons was placed at 0 and 40 meters. Timing lights were place at 5 and 35 meters respectively. Procedure: 1. Warm up of 5-10 minutes, which included some short 20-25 meters all out sprinting. 2. A 3-5 minutes rest interval should separate the warm-up from the test. 3. The test involved a 40meters sprint (0-40meters) followed by a stop (turn) with a return to the start line. The subject touched the end line with one foot before returning to the start. 4. The test were measured for each interval first begins as the athlete crosses the 5 meters line and stopping at the 35 meters line, second began as the athlete crosses the 35meters line and stops as the athlete crosses the 5meters line. 5. Total time was recorded for each interval.

Body composition test

All subjected were recorded the body composition such as percentage of fat, body weight, body mass index (BMI), lean body mass (LBM), percentage of body fat (P.B.F), soft lean mass (S.L.M) and size lower body by using X-scan body composition analyzer (Jawon Medical, Korea). We recorded 2 times; before and after altitude training.

Subjective score

Subjective score described the effect of altitude training on rate of perceive exertion score, pain rating scale and acute mountain sickness by using standard protocol.

Rate of perceive exertion score (RPE score)

RPE score reflected the exhaustion of athletes by which the athletes was asked to check for *RPE score* every days after training by using Borg's ratings of perceived exertion (RPE) method.

Pain rating scale

Pain rating scale described the level of pain after training by which the athletes was asked to check for *RPE score* every days after training (Scott and Huskisson, 1976)

Acute mountain sickness

After simulated altitude training (15%O2), all athletes were tested for acute mountain sickness by using Environmental Symptoms Questionnaire (ESQ) (Sampson et al., 1983)

Statistical analysis

The magnitude-based inference analysis was applied to compare the significantly difference among heart rate variability (HRV) of AI, AT, SI and ST groups. The magnitude of the correlations was rated using Hopkins scale. For the magnitude inference test, baseline was calculated and used as a covariate in the analysis to adjust for any differences between groups at baseline. Because the small performance changes can be beneficial for elite athletes whom cannot detected by the conventional method, contemporary statistical method was used and applied to test our data (Hopkins, Hawley, & Burke, 1999). Specifically, we used magnitude-based inferences about effect sizes, and then to make inferences about true (population) values of the effect, the uncertainty in the effect was expressed as 90% confidence limits (CL). According to magnitude-based inference analysis, the data in the text and figures and presented as means with 90% CL and CI, respectively. In addition, the t-test 50meters, 100meters, 400meters, repeated high intensity endurance test, agility and VO_{2max} , *RPE ratio* and blood lactate concentration among experimental group were statistically compare by using t-test analysis.

Results

Male athletes (age between 18-22 year olds) in all groups were subjected to test the general data including body weight, height, body mass index (BMI) and also the basic performance including agility, VO_2max and training intensity. All parameters were measured and compared by using one-way analysis of variance. The basic physiological parameters and physical performance revealed no significantly difference. In other word, the comparison clearly showed the homogeneity of subjects in all groups (table 1).

Table 1 General characteristic of subjects in the four training groups.

	AI	AT	SI	ST
Age (yr.)	20.3±1.3	20.9±0.7	20.4±0.7	20.3±0.7
Body weight (kg)	68.0±7.5	67.5±9.5	62.3±6.3	69.2±9.8
Height (cm)	174.8±3.5	173.0±5.8	168.4±5.3	174.1±6.1
BMI ($kg \cdot m^{-2}$)	22.2±2.0	22.5±2.8	21.9±1.5	23.0±2.7
Agility (sec.)	14.1±0.2	14.6±0.1	14.1±0.3	14.7±0.1
V02max	43.42±5.22	42.37±0.9	43.62±8.5	41.70±2.7
Training Intensity(hr/wk)	9.22±2.55	9.51±2.33	9.43±2.17	9.17±2.74
Warm-up Stretching	1.12±0.13	1.17±0.14	1.17±0.12	1.18±0.14
Physical Fitness	2.08±0.15	2.18±0.11	2.18±0.12	2.19±0.13
Skill	2.03±0.16	2.08±0.15	2.17±0.12	2.17±0.10
Team Stagiest	3.15±0.14	3.19±0.15	3.18±0.12	3.17±0.10
Cool Down	1.07±0.11	1.11±0.11	1.12±0.10	1.10±0.09

Values represented mean ±SD. The altitude training with iron supplementation group (AI; N=10), altitude training group (AT; N=10), Sea-level training with iron supplementation group (SI; N=10), Sea-level training group (ST; N=10). No significant differences were found between groups for any variable.

Table 2 Comparative mean changes of performance and the change that the true difference represents a substantial improvement or impairment in HRV parameters in stand and supine position in AT/ST, AI/SI, AI/AT and AI/ST group. The percentage change of each HRV parameters were calculated by [POST-PRE]/PRE *100%.

	Change in performance %			Percentage chance of HRV parameters (%)	
			Difference90%CL	%	Qualitative
AT vs ST group	AT	ST			
RMSSD stand (ms)	-2.9±78.0	-13.3±51.8	11.0±67.1	22	unclear
RMSSD supine (ms)	6.7±47.7	2.9±27.3	-26.5±44.8	24	unclear
LF stand (ms^2)	4.5±86.1	-42.1±51.8	3.8±43.1	4	unclear
LF supine (ms^2)	11.7±104.9	-5.5±101.1	6.4±115.8	28	unclear
HF stand (ms^2)	8.8±102.5	0.2±135.0	-8.4±150.5	39	unclear
HF supine (ms^2)	19.9±108.6	19.9±77.7	0.0±107.1	30	unclear
LF/HF Ratio stand(ms^2)	3.0±108.1	-67.4±63.7	102.3±108.6	2	unclear
LF/HF Ratio supine(ms^2)	-0.1±81.9	-8.4±90.7	8.7±96.2	30	unclear
AI vs SI group	AI	SI			
RMSSD stand (ms)	25.7±63.6	0.6±58.4	28.6±61.6	11	unclear
RMSSD supine (ms)	-7.7±52.7	22.9±40.3	-26.5±44.8	81	likely
LF stand (ms^2)	20.8±111.9	-53.2±92.6	109.7±137.7	4	unclear
LF supine (ms^2)	-76.6±122.1	-31.0±89.5	-36.6±136.8	71	unclear
HF stand (ms^2)	33.6±128.5	-5.0±143.9	47.2±192.3	18	unclear
HF supine (ms^2)	-21.4±123.4	58.7±92.1	-55.2±135.9	87	likely
LF/HF Ratio stand (ms^2)	-28.3±103.5	-16.3±116.1	-14.8±140.9	52	unclear
LF/HF Ratio supine(ms^2)	-50.3±80.3	-88.5±67.7	46.6±82.6	7	unclear
AI vs AT group	AI	AT			
RMSSD stand (ms)	25.7±63.6	-2.9±78.0	19.1±74.8	19	unclear
RMSSD supine (ms)	-7.7±52.7	6.7±47.7	-11.1±48.6	50	unclear
LF stand (ms^2)	20.8±111.9	4.5±86.1	24.5±118.8	19	unclear
LF supine (ms^2)	-76.6±122.1	11.7±104.9	-58.2±145.7	89	likely
HF stand (ms^2)	33.6±128.5	8.8±102.5	-5.1±184.0	31	unclear
HF supine (ms^2)	-21.4±123.4	19.9±108.6	-31.7±149.2	61	unclear
LF/HF Ratio stand (ms^2)	-28.3±103.5	3.0±108.1	-50.0±161.5	83	unclear
LF/HF Ratio supine(ms^2)	-50.3±80.3	-0.1±81.9	-21.8±90.8	61	unclear
AI vs ST	AI	ST			
RMSSD stand (ms)	25.7±63.6	-13.3±51.8	26.1±56.0	10	unclear
RMSSD supine (ms)	-7.7±52.7	2.9±27.3	3.8±4.31	24	unclear
LF stand (ms^2)	20.8±111.9	-42.1±51.8	59.5±77.2	4	unclear
LF supine (ms^2)	-76.6±122.1	-5.5±101.1	6.4±115.8	28	unclear
HF stand (ms^2)	33.6±128.5	0.2±135.0	-8.4±150.5	39	unclear

HF supine (ms^2)	-21.4±123.4	19.9±77.7	0.0±107.1	30	unclear
LF/HF Ratio stand (ms^2)	-28.3±103.5	-67.4±63.7	102.3±108.6	2	unclear
LF/HF Ratio supine(ms^2)	-50.3±80.3	-8.4±90.7	8.7±96.2	30	unclear

±90% confidence limits; add and subtract this number to the mean effect to obtain the 90% confidence limits for the true difference. LF, low frequency reflecting sympathetic predominance; HF, high frequency reflecting parasympathetic predominance; LF/HF, sympathetic-parasympathetic neurovegetative balance; RMSSD, root mean square of the standard deviation of the R-R intervals.; ST, sea level training group; SI, sea level training with iron supplementation group; AT, altitude training group; AI, altitude training with iron supplementation group.

Table 3 Comparative mean changes of performance and the change that the true difference represents a substantial improvement or impairment in HRV parameters in stand and supine position in AT/ST, AI/SI, AI/AT and AI/ST group. The percentage change of each HRV parameters were calculated by [POST-PRE]/PRE *100%.

	Change in performance %			Percentage chance of HRV parameters (%)	
			Difference90%CL	%	Qualitative
AT vs ST group	AT	ST			
RMSSD stand (ms)	10.7±55.6	53.0±53.0	-34.5±50.5	89	likely
RMSSD supine (ms)	36.7±47.2	46.4±38.0	-9.2±38.7	47	unclear
LF stand (ms^2)	4.8±65.1	17.3±81.5	-11.8±77.0	49	unclear
LF supine (ms^2)	51.6±121.5	34.7±109.6	18.4±138.6	24	unclear
HF stand (ms^2)	12.4±96.5	91.8±96.9	-54.8±106.3	90	likely
HF supine (ms^2)	77.9±103.8	91.0±98.3	-12.2±104.4	41	unclear
LF/HF Ratio stand (ms^2)	5.9±64.8	-77.4±103.1	130.1±96.3	1	unclear
LF/HF Ratio supine(ms^2)	-21.3±51.4	-63.2±67.2	52.1±59.1	3	unclear
AI vs SI group	AI	SI			
RMSSD stand (ms)	24.3±69.0	33.8±47.1	-9.1±59.4	50	unclear
RMSSD supine (ms)	-31.1±91.5	29.0±64.5	-45.2±86.6	90	likely
LF stand (ms^2)	52.0±107.4	-1.3±84.7	70.6±125.3	8	unclear
LF supine (ms^2)	-84.6±118.6	-38.6±122.9	-36.8±164.4	71	unclear
HF stand (ms^2)	46.5±146.8	36.5±102.6	10.5±172.0	32	unclear
HF supine (ms^2)	-72.6±196.5	67.8±142.3	-75.5±286.3	93	likely
LF/HF Ratio stand (ms^2)	-8.7±80.7	3.4±86.0	-11.5±94.9	49	unclear
LF/HF Ratio supine(ms^2)	-78.4±96.5	-94.7±77.7	138.6±103.4	1	unclear
AI vs AT	AI	AT			
RMSSD stand (ms)	24.3±69.0	10.7±55.6	5.4±63.5	28	unclear
RMSSD supine (ms)	-31.1±91.5	36.7±47.2	-48.3±79.9	94	likely

LF stand (ms^2)	52.0±107.4	4.8±65.1	45.7±102.1	8	unclear
LF supine (ms^2)	-84.6±118.6	51.6±121.5	-72.0±158.1	97	likely
HF stand (ms^2)	46.5±146.8	12.4±96.5	6.0±171.2	39	unclear
HF supine (ms^2)	-72.6±196.5	77.9±103.8	-77.6±250.6	95	likely
LF/HF Ratio stand (ms^2)	-8.7±80.7	5.9±64.8	-35.5±98.0	76	unclear
LF/HF Ratio supine(ms^2)	-78.4±96.5	-21.3±51.4	51.0±86.8	7	unclear
AI vs ST	AI	ST			
RMSSD stand (ms)	24.3±69.0	53.0±53.0	-31.5±60.8	83	likely
RMSSD supine (ms)	-31.1±91.5	46.4±38.0	-9.2±38.7	47	unclear
LF stand (ms^2)	52.0±107.4	17.3±81.5	-11.8 ±76.0	49	unclear
LF supine (ms^2)	-84.6±118.6	34.7±109.6	18.4±138.6	24	unclear
HF stand (ms^2)	46.5±146.8	91.8±96.9	-54.8±106.3	90	likely
HF supine (ms^2)	-72.6±196.5	91.0±98.3	-12.2±114.4	41	unclear
LF/HF Ratio stand (ms^2)	-8.7±80.7	-77.4±103.1	130.1±96.3	1	unclear
LF/HF Ratio supine(ms^2)	-78.4±96.5	-63.2±67.2	52.1±59.1	3	unclear

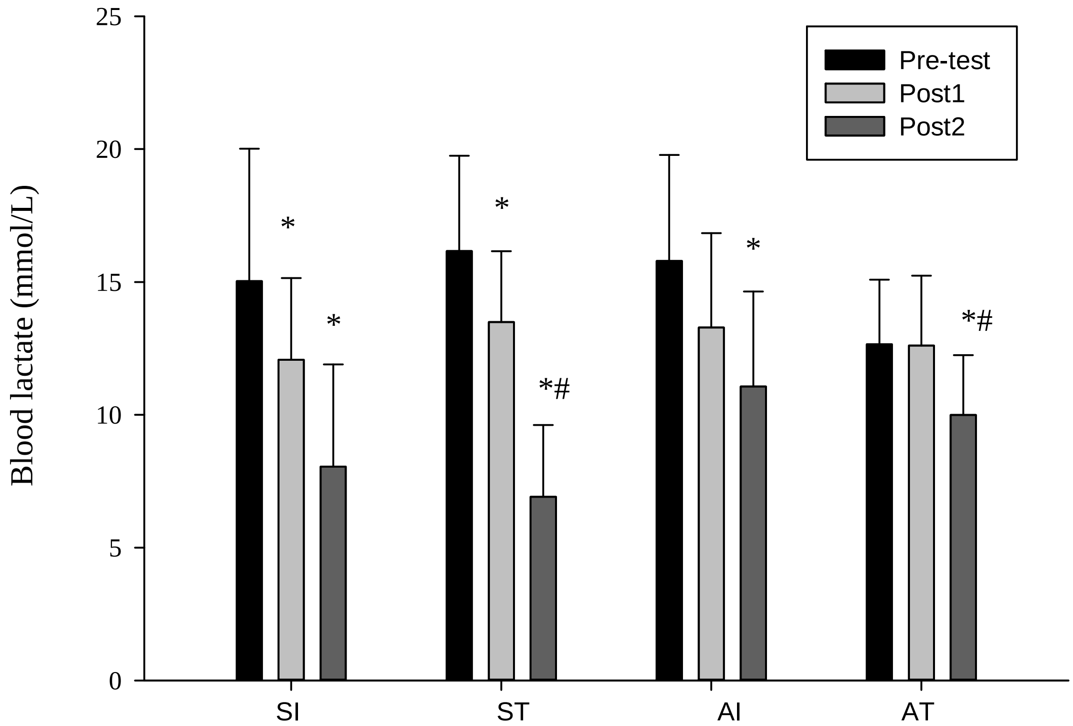
±90% confidence limits; add and subtract this number to the mean effect to obtain the 90% confidence limits for the true difference. LF, low frequency reflecting sympathetic predominance; HF, high frequency reflecting parasympathetic predominance; LF/HF, sympathetic-parasympathetic neurovegetative balance; RMSSD, root mean square of the standard deviation of the R-R intervals.; ST, sea level training group; SI, sea level training with iron supplementation group; AT, altitude training group; AI, altitude training with iron supplementation group

For the results, when compared the changes of performance and the change that the true difference represents a substantial improvement or impairment in HRV parameters in stand and supine position at PRE and POST1 time point, we found that the $LF_{suprine}$ was likely to increase in altitude training with iron supplement (AI) group when compared to the altitude training (AT). Likewise, $RMSSD_{suprine}$ and $HF_{suprine}$ revealed the decrease in AI group when compared to sea-level with iron supplement group (SI) (Table2).

Furthermore, when considered changes of performance and the change that the true difference represents a substantial improvement or impairment in HRV parameters in stand and supine position, we found that $RMSSD_{stand}$ and HF_{stand} trended to reduce in AT group when compared to ST group. Besides, $RMSSD_{stand}$ and HF_{stand} decreased in AI group when compared to ST group. Likewise, $RMSSD_{suprine}$ and $HF_{suprine}$ also decreased in AI group when compare to SI group. Similarly,

$RMSSD_{suprine}$, $LF_{suprine}$ and $HF_{suprine}$ declined in AI group when compared to AT group. (Table3)

Figure 1. Blood lactate content among the experiment groups (SI, ST, AI and AT) at the 3 time points; pre-test (PRE), 1st post test (POST1) and 2nd post test (POST2).



*= significantly difference when compared to PRE group ($p<0.05$)
#=significantly difference when compare POST1 and POST2 ($p<0.05$)

To elucidate the response of blood lactate among the experiment groups (SI, ST, AI and AT), we measured blood lactate at the 3 time points; pre-test (PRE), 1st post test (POST1) and 2nd post test (POST2) and compared mean by using one-way analysis of variance. Overall, blood lactate content was slightly decreased after training in all groups.

The blood lactate in both sea-level training group (ST and SI) significantly dropped after 8 weeks of training (POST1) and after 2 weeks of POST1 timing (POST2). In addition, in ST group, blood lactate content was dramatically decreased at POST2 time point.

In altitude training (AT and AI), blood lactate was showed differently response. The AT group maintained the blood lactate level at PRE and POST1 time point. However, blood lactate content significantly reduced at POST2. In the other hand, blood lactate content in AI group was showed significantly reduced only at POST1 time point.

Table 5 The average of Physical fitness content among the experiment groups (SI, ST, AI and AT) at the 3 time points; pre-test (PRE), 1st post test (POST1) and 2nd post test (POST2)

Physical Fitness Test	**AT**			**AI**			**ST**			**SI**		
	Pre	Post1	Post2	Pre	Post1	Post2	Pre	Post1	Post2	Pre	Post1	Post2
Runing(m)												
50m(sec)	7.24	6.46*	6.39*	6.99	6.30*	6.24*	7.17	6.60*	6.48*	6.69	6.25	6.20*
400m(min)	1.20	1.25	1.25	1.14	1.20	1.18	1.20	1.31	1.32	1.17	1.26	1.24
Jump												
Power(w)	313.3	900.8*	955.5*#	540.4	990.1*	1021.*#	314.2	937.8*	948.8*	280.7	838.0*	897.7*
Height(cm)	29.9	32.9*	35.7*#	30.4	32.0*	33.8*#	29.1	30.60	30.70	28.10	32.2*	33.10*
RHIET test (sec)	17.29	15.97*	15.81*	16.67	15.95*	15.7*#	16.95	16.47*	16.24*	16.86	16.20*	16.11*

*= significantly difference when compared to baseline (p<0.05)
#=significantly difference between POST1 and POST2 (p<0.05)

Table 5 revealed the average of physical fitness among the experimental groups (SI, ST, AI and AT) measured before (Pre) and after training (Post1 and Post2). For the AT group, the physical fitness test revealed that the altitude training significantly improved the running ability in all distance (50m, 100m, 400m and 2800m) when compared to the beginning of the test. However, this result clearly showed the difference after 10 weeks of training (Post2). Besides, the jump power and jump height also displayed the similar trend. The AT group increased the jump power and height after training. In addition, the agility in AT group also increased after training (Post1 and post2). Interestingly, running ability, jump ability and RHIET test values in Post2 significantly increased than those in Post1. These data indicated that they progressively increased after training.

For the AI group, the physical fitness of AI group dramatically improved the running ability when compared to the beginning of training. The running test in AI group clearly showed that running ability in Post2 higher than Post1 of all distance tested. Furthermore, when considered the jump ability in AI group, it showed that AI treatment significantly increased jump performance when compared to before training period. While, the agility and RHIET test also revealed the similar trend that the AI treatment significantly elevated these parameters.

For the ST group, the physical fitness test revealed that the sea-level training significantly improved the running ability in all distance (50m, 100m, 400m and 2800m) when compared to the beginning of the test. Moreover, the jump power, agility and RHIET test results displayed that the ST group significantly enhanced RHIET capacity of those two parameters after training in both Post1 and Post2 time point.

For the SI group, the running ability of SI group significantly improved after 8 weeks training. Besides, the SI group still improved their running ability at the Post2 time point. For the jump power parameters, jump height and RHIET test displayed the similar response to physical fitness test by which the SI athletics continuously elevated their jump ability and RHIET results. In addition, the agility test of SI subject also showed the significantly improved only Post2 time point.

Table 2 The average of Body composition and size lower body content among the experiment groups (SI, ST, AI and AT) at the 2 time points; pre-test (PRE) and 1st post test (POST1)

Body Composition	**AT**		**AI**		**ST**		**SI**	
	Pre	Post1	Pre	Post1	Pre	Post1	Pre	Post1
Body Composition								
BMI	22.5	21.9*	22.2	21.9	23.0	22.6	21.9	21.7
P.B.F.	18.4	14.9*	17.5	14.8*	18.9	16.1*	17.0	14.3*
L.B.M.	54.6	55.4	52.0	52.4	55.8	56.7*	51.6	53.0*
S.L.M.	50.7	51.6	55.9	56.2	51.8	52.7*	48.0	49.4*
Size Lower Body								
Thigh.R(cm)	54.4	53.7	54.8	54.9	53.5	53.7	50.5	49.8
Thigh.L(cm)	54.7	53.2*	54.5	54.2	52.8	53.0	50.0	50.1
Waistline(cm)	80.7	78.0	78.3	77.6	82.3	81.0	76.9	75.9
Hip(cm)	99.5	92.2	92.3	92.8	90.8	93.8	88.8	90.5
Calf.R(cm)	35.6	37.8*	36.3	37.7*	36.1	37.3	36.9	37.9
Calf.L(cm)	35.6	37.2*	36.4	37.6*	36.5	37.1	36.32	37.6

*= significantly difference when compared to baseline ($p<0.05$)

To elucidate the effect altitude and iron supplementation on body composition and size lower body content among the experiment groups, we measured the parameters included body mass index (BMI)body fat percentage (P.B.F), lean body mass (L.B.M.), soft lean mass (S.L.M.), thigh, waistline, hip and calf diameter at 2 time point which were pre-test (PRE) and 1st post test (POST1). Then we compared the average of each parameter between PRE and POST1 by using paired sample t-test analysis to determine the significant factors.

The results showed that altitude training (AI and AT) significantly improved BMI, P.B.F., both left and right calf after training. However, the average of left thigh was significantly decreased when compared to PRE. On the other hand, the sea-level training (SI and ST) revealed the similar response among the group. SI and ST group significantly reduced P.B.F. after training. While, L.B.M. and S.L.M. significantly increased in POST1 time post.

Figure 2 This figure represent the SpO_2 value when AI and AT group cycling under 15% O_2 condition stimulated by hypoxicator while SI and ST group cycling under the ambient condition.

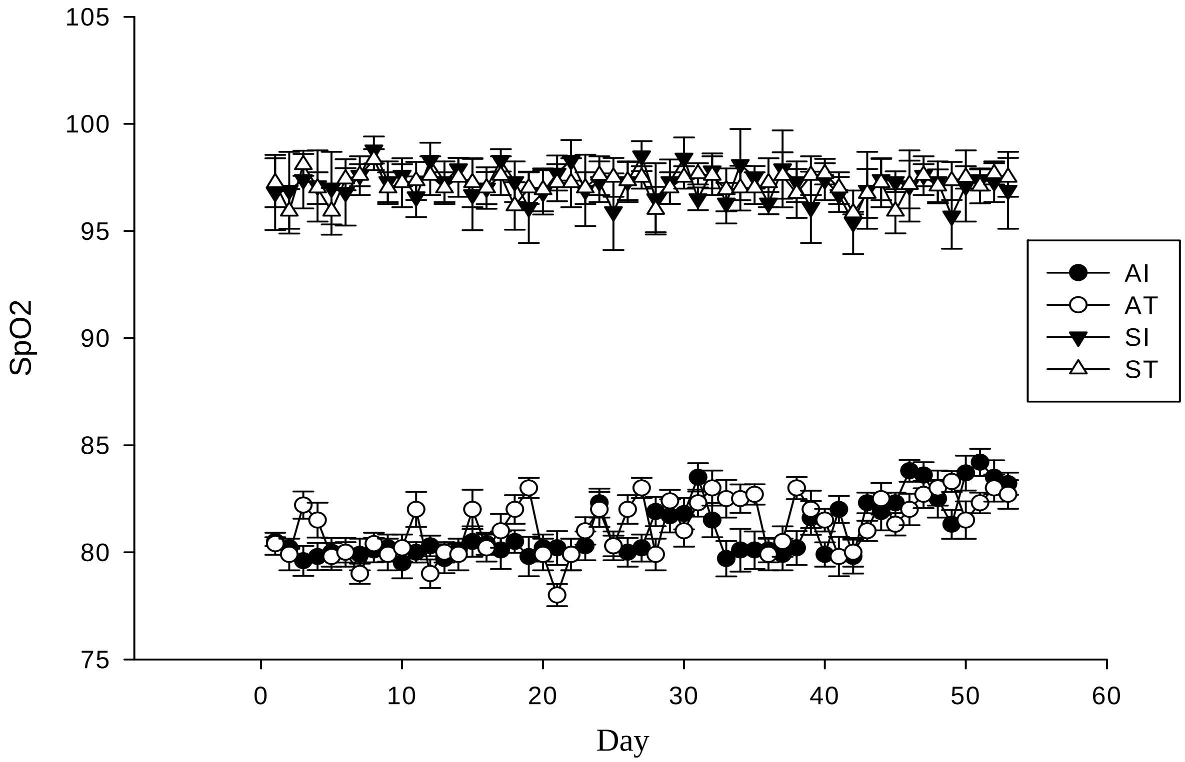


Figure 2 indicated that SpO_2 level in the sea-level groups (SI and ST) showed the dramatically higher when compared those in the altitude groups (AI and AT). However, the averages SpO2 in sea-level groups were 96-98% while the averages of SpO_2 in altitude subjects were 80-85%. Moreover, when trained at the sea-level, it showed no significantly difference among the subject group with and without iron application. On the other hand, in the altitude subject, they revealed the contrast response. After treated with ferrous sulfate for 30 days, the SpO_2 value showed the positively response to iron application.

Discussion

In the order to assess the impact of altitude training on physiological parameters and physical performance of soccer players, we separated the subjects into 4 groups according to math group method. Sea-level group (ST) was trained at 123meters above sea-level and the altitude training (AT) was trained at 825meters above sea-level. Moreover, we also assessed the response of iron supplementation on both sea-level training (SI) and altitude training (AI).

Then we observed physiological change in athletes by detected several hematological parameters. We found that both of altitude training, AT and AI, trended to increase oxygen concentration in blood (SPO_2) at rest during experimental period (figure2). With altitude training in several forms, athletes theoretically should acquire the beneficial effects of altitude acclimatization, particularly stimulation of the oxygen delivery system increasing in the total body haemoglobin for maximizing oxygen transport and utilization, and enhancing ability to train without reducing training intensity (Zhang et al, 2007). These studies found that the body will adapt by breathing more frequently to bring more oxygen in to the cells. Moreover, the body will be adapted by increasing erythrocyte to carry with oxygen. Normally, the body has hemoglobin as 13.4 grams per 100 cubic centimeters of blood at sea level. After the body exposed to higher altitude, hemoglobin increases to 17 g per 100 cubic centimeters of blood (Thoranin, 1979).

Heart rate variability (HRV) measured the interval time of heartbeat which reflected the balance of nervous system function. Moreover, in altitude training, HRV was used to monitor physical activity in athlete. In order to study the correlation altitude training and the HRV on athlete's performance, we conducted the series of physiological experiments and measured HRV parameters to elucidate the effects of altitude training. We performed the experiment in two differences level, the sea-level training (123 meters above sea-level) and altitude training (825 meters above sea-level). In addition, we also test the effect of iron supplementation by using ferric oxide supplementation.

Heart rate variability (HRV) is a monitoring tool used to detect changes in autonomic nervous control that may indicate slow or impaired adaptation to altitude. In our research, altitude training significantly reduced some of HRV parameters such as RMSSD stand and HF stand (table2). The effects of hypoxia when trained at altitude (altitude groups) and exercise training (sea level groups) on HRV showed difference results. An increase training load or stressor by hypoxia at altitude may negatively influence vagally-mediated HRV indices (Sandercock et al. 2005) as RMSSD stand and HF stand in both altitude groups (AT and AI) showed substantially decreased compared with sea-level group (ST group). The athletes in hypoxic environment that did not respond positively to the 8-weeks live high train high showed a withdrawal of vagally-mediated HRV parameters similar to Mourot and colleages (2004) observations, which suggests that HRV may be a useful tool at identifying athletes under too much stress, whether it is from training, or a combination of training and hypoxia. It seems likely that the effect of high training loads with the addition of hypoxic stress has a cumulative effect, such that the normal adaptation to such stressors is overwhelmed and sympathetic activity predominates. If recovery is not sufficient and training with hypoxia continue, the athlete has little chance of recovery and gradually shifts into a sympathetically innervated overstress-type syndrome causing a reduction in performance potential (Hamlin et al. 2011). However, the athletes' physical fitness (run 50,100,400m, jump height) did not correspond to these HRV results; our results may indicate the stressor from either hypoxic environment or training load not influence to muscle activity via somatic nervous system but HRV via autonomic nervous system. This is speculative and further research is required to elucidate these changes.

Cellular lactic acid was generated from anaerobic respiration, so blood lactate content reflected the hypoxia condition at the cellular level. When blood lactate content increased, it induced exhaustion in athletes. Moreover, the blood lactate level indicated exercise intensity. In our experiment, sea-level group (ST and SI) significantly reduced blood lactate content after training and still decreased after experiment period (figure1). However, compared to the sea-level groups, altitude training groups (AT and AI), blood lactate level presented slowly decreased immediately after 400m run at both day 1 and day 14 (figure1). These findings indicated the anaerobic metabolism involved in the athletes in altitude groups. It more likely that altitude groups worked harder, although get the same training program. Lactic acid is known as the chemical substance that activates growth hormone release and muscle hypertrophy later all (Manimmanakorn et al. 2013). Our results showed the thigh and calf muscles cross-sectional area increased significantly after training in AT and AI groups (Table 2). This may indicate the mechanism involve as described above. Theoretically, a professional soccer player should ideally be able to maintain a high level of intensity throughout the whole game. This study may suggest that the athletes had improve buffering capacity in their bodies as a results of RHIET trend to improve than sea level training although no significant were found (table 5). Some studies, however, have shown a reduction in distance covered, a lower fractional work intensity, reduced blood sugar levels and reduced lactate levels in the second half of games compared with the first half (Douglas, 1993). Other study reported that the increase in blood lactate concentration during submaximal and maximal exercise during altitude exposure has been related with the hypoxia – induced increase in epinephrine (Kayser 1996; Lundby et al., 2000). Furthermore, Spengler, Roos et al. (1999) observed the relation between blood lactate level and oxygen consumption in athletes during endurance exercise. They suggested that the two main reasons of decrease of blood lactate concentration were 1. The muscle itself reduced the production of lactic acid due to the decrease of energy demand and 2. Muscle uptakes lactate and catabolized to server as energy. In determining aerobic endurance is considered the most important element as well as for soccer game.

The body composition: BMI was only finding that changed after altitude training in AT groups (table 2). This may due to water loss during hypoxic environment training not fat mass, because of percent body fat were decreased significantly in all four groups.

In conclusion, our data reflected that altitude training more likely to induce autonomic nervous system via sympathetic outflow. The sympathetic and parasympathetic nervous systems are responsible for the regulations of several physiological responses including heart rate, respiratory rate and substrate utilization. The other important finding is blood lactate concentration which showed less reduction immediately after short distance running, in other word the athletes' muscle exposed to acidic environment but gave efficiency work through physical fitness. This phenomenon indicated our protocol (altitude training with soccer training program in this study) effective to improve anaerobic based performance (100m and 400m run). Finally, the body composition did not alter with this 8-weeks altitude training or live high train high program.

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