Artificial altitude training strategies: Is there a correlation between the haematological and physical performance parameters?

Diego Fernández-Lázaro1, Juan Mielgo Ayuso2, Alberto Caballero García3, Jorge Pascual Fernández4, Alfredo Cóordova Martínez3


Summary

Introduction: Exposure to intermittent hypoxia (IHE) which is used as a complement to conventional training to obtain improvements in key haematological indices to increase athletic performance.

Objective: We assessed haematological and physical performance changes by an IHE program in elite athletes (EA) living and training in moderate hypoxia.

Material and method: For a 4-week normobaric IHE treatment (90 minutes, 7 days a week, 10-13 % FIO2) was applied at 12 EC. Their physical-antropometric characteristics were established before the start of the study: Blood tests and physical tests were performed at 2 points in the study: a) on day 1, just before the start of the study (T1); b) on day 28, just at the end of the study (T2). The following were measured: reticulocytes (RET.), reticulocyte haemoglobin (Hb-RET), erythropoietin (EPO), complete haematological profile and iron metabolism. Physical performance was determined by evaluation of aerobic potency, anaerobic potency, and velocity and maximum oxygen consumption (CsO2 max).

Results: Between T1 and T2 there is a significant increase in EPO, RET and Hb-RET, as well as a non-significant increase in the haematological variables involved in erythropoiesis HEM, Hb and Hcto. Performance increased in all physical tests, speed (1.96±2.35 %), aerobic power (3.73±5.34 %), CsO2 max (3.36±4.35 %) and was significant in anaerobic power (p = 0.05 with 1.93±1.13 %).

Conclusions: The IHE program of 4 weeks’ duration in combination with training is able to stimulate hematological parameters such as EPO, RET, HEM, Hct, and Hb that demonstrate an activation of the erythropoiesis of the athlete and could be the cause of improvements in all performance tests, being only significant the increase in anaerobic potency.


Estrategias artificiales de entrenamiento en altitud: ¿Existe correlación entre parámetros hematológicos y de rendimiento físico?

Resumen

Introducción: La exposición a hipoxia intermitente (IHE) utilizada como complemento al entrenamiento convencional para obtener mejoras en los índices hematológicos claves para incrementar el rendimiento deportivo. Objetivo: Evaluar los cambios hematológicos y de rendimiento físico por un programa de IHE en atletas de élite (AE) que viven y entrenan en hipoxia moderada.

Material y método: Se aplicó un tratamiento de IHE normobárico de 4 semanas de duración (90 minutos, 7 días a la semana, 10-13 % FIO2) a 12 AE. Se establecieron sus características físico-antropométricas antes del comienzo del estudio: Las analíticas de sangre y las pruebas físicas se realizaron en 2 momentos del estudio: a) en el día 1, justo antes de comenzar el estudio (T1); b) en el día 28, justamente al final estudio (T2). Se midieron: reticulocitos (RET.), hemoglobina reticulocitaria (Hb-RET), eritropoyetina (EPO), el perfil hematológico completo y el metabolismo del hierro. El rendimiento físico se determinó mediante la evaluación de la potencia aeróbica, la potencia anaeróbica y el consumo máximo de oxígeno (CsO2 max).

Resultados: Entre los T1 y T2 existe un incremento significativo de EPO, RET y Hb-RET, además de un aumento no significativo en las variables hematológicas involucradas en la eritropoyesis HEM, Hb y Hcto. Se incrementó el rendimiento en todas las pruebas físicas, de velocidad (1.96±2.35 %), de potencia aeróbica (3.73±5.34 %), de CsO2 max (3.36±4.35 %) y fue significativo en la potencia anaeróbica (p = 0.05 con un 1.93±1.13 %).

Conclusiones: El programa de IHE de 4 semanas de duración en combinación con el entrenamiento es capaz de estimular parámetros hematológicos, como la EPO, RET, HEM, Hct, y Hb que demuestran una activación de la eritropoyesis del deportista y que podrían ser la causa de las mejoras en todos los test de rendimiento, siendo únicamente significativa el aumento potencia anaeróbica.

Correspondence: Diego Fernández Lázaro
E-mail: diego.fernandez.lazaro@uva.es
Introduction

For long-distance athletes, resistance and/or aerobic capacity is a major element in sporting performance, which is why factors that improve the transportation and use of oxygen on a muscular level take on particular relevance. For this reason, trainers and athletes introduce diverse strategies into their conventional training methods that are able to induce adaptations to improve the functionality of muscular, sanguineous, cardiovascular, respiratory and endocrine-metabolic performance. A particularly outstanding strategy is the use of continued exposure to hypoxia - typical of altitude training - triggering a series of physiological responses and adaptations that are beneficial to athletes' performance. For this to happen, athletes must live and train at altitude for at least 20-day periods, which is the time needed for acclimatisation, the primary training phase, the recovery phase and the preparatory phase for returning to sea level, all required to induce improvements in sporting performance at sea level. These periods have a negative influence on the intensity of training, and entail a decline in performance levels. In order to overcome these disadvantages of altitude training, in recent years new devices have been used that aim to simulate the physiological effects of altitude.

Simulated altitude-condition training strategies used among elite athletes are: intermittent hypoxic exposure (IHE), which is applied through passive stays in rooms with a hypoxic atmosphere, or by breathing air with a lower oxygen concentration; and intermittent hypoxic exposure during training (intermittent hypoxic training, IHT), which consists in training under hypoxic conditions.

The aim of both methods (IHE and IHT) is to simulate the erythropoiesis of the athlete and generate adaptations that improve the haematological profile, with the final outcome of an increase in the blood's capacity to transport oxygen. This series of physiological responses and adaptations starts with erythropoiesis production (EPO), which entails an increase in the amount of haemiaties (HEM) and in the total mass of haemoglobin (Hb), and consequently the level of haematocrit (Hct) increases. This change in the athlete's haematological values, allows for an improvement of the physiological parameters linked to performance, such as the anaerobic performance-threshold, and aerobic metabolism (reduced trial time, increase of $\text{C}_2 \text{max}$ and increase of thresholds).

IHT programmes appear to be considerably more beneficial than those of IHE in stimulating erythropoiesis and sporting performance, because exercise in hypoxia plays an extremely important role in the set of haematological and physiological adaptations. However, IHT entails greater wear, fatigue, immunosuppression, and muscle catabolism during the training periods than training performed under normoxic conditions. This makes recovery time between training sessions longer, which could alter a classic training system. All this increase in organic stress caused by IHT, could lead to the modification of our training methods established over 20 years ago, and that we consider suitable given results obtained, with athletes that were Olympic, world and European champions in mid to long distance athletic competitions. Furthermore, in our study the Elite Athletes (EA) lived and trained in Soria, at 1,100-1,200 metres of altitude above sea level, considered “Medium Altitude” with a resting oxygen percentage saturation (% $\text{SaO}_2$) of 95%.

This situation led us to propose the analysis of the influence of IHE on haematological changes and on specific physical performance trials, on classic EA training, mid and long distance, with athletes that compete on out-door tracks, that are constantly exposed to a moderate hypoxic situation, entailing a double hypoxic stimulus. This study is new, as far as we know, as in other research studies the athletes are only subject to a single hypoxia stimulus (IHT or IHE).

Material and method

We have studied the effect of IHE on elite athletes (EA) during the pre-competitive training period of one season. The study protocol was approved by the University of Valladolid (Spain) ethics committee (reference 03/2010-11), and we adhered to the recommendations put forward in the Helsinki Declaration. We performed the analytical control on 2 study times: a) on day 1, just before starting the study (T1); b) on day 28, just on the final study (T2).

Subjects

A total of twelve (n=12) elite males from the Soria Centre of High Training and Sports Promotion (CAEP) and the Spanish National Team participated in the study. All the EA were volunteers and were informed about the research protocol. The physical characteristics of the EA are displayed in Table 1. From the total of 12 EA, 8 competed in 800 and 1,500 metre flat specialities, 3 in the 5,000-metre flat speciality, and 1 in the 3,000-metre obstacle speciality. All the subjects signed an informed consent form and completed a medical questionnaire, as well as a cardiovascular pulmonary examination and an electrocardiograph before entering the study. None of the subjects smoked or drank alcohol, or took medication able to alter their haematological response. Concomitant pathologies were ruled out with the clinical history and the medical examination. The EA followed a similar diet throughout the season, and in particular, followed the same diet during the study, which was constantly supervised by the CAEP doctor. All the athletes followed the same training programme (Table 2), which consisted in 2 daily sessions, from Monday to Saturday. The morning session comprised specific training (2 hours) and after an hour the athletes performed the hypoxia session. The af-

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>EA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>26.12±2.90</td>
</tr>
<tr>
<td>Weight (kilograms)</td>
<td>63.37±9.72</td>
</tr>
<tr>
<td>Height (centimetres)</td>
<td>175.87±9.12</td>
</tr>
<tr>
<td>Body Mass Index (BMI) (Weight/Height$^2$)</td>
<td>20.49±8.83</td>
</tr>
<tr>
<td>Fat percentage (%) (Yuhasz)</td>
<td>8.93±1.21</td>
</tr>
</tbody>
</table>

The data is expressed Average ± Standard Deviation.

Table 1. Physical and anthropometric characteristics of the elite athletes (EA)
Table 2. Training programme.

<table>
<thead>
<tr>
<th>Day</th>
<th>Morning</th>
<th>Afternoon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monday</td>
<td>Lactic capacity</td>
<td>Continuous aerobic capacity work</td>
</tr>
<tr>
<td>Tuesday</td>
<td>Aerobic power</td>
<td>Continuous aerobic capacity work</td>
</tr>
<tr>
<td>Wednesday</td>
<td>Resistance strength</td>
<td>Continuous mixed work</td>
</tr>
<tr>
<td>Thursday</td>
<td>Lactic power</td>
<td>Continuous aerobic capacity work</td>
</tr>
<tr>
<td>Friday</td>
<td>Resistance speed</td>
<td>Continuous aerobic capacity work</td>
</tr>
<tr>
<td>Saturday</td>
<td>Mixed aerobic-anaerobic</td>
<td>Continuous aerobic capacity work</td>
</tr>
<tr>
<td>Sunday</td>
<td>Aerobic capacity</td>
<td>Rest</td>
</tr>
</tbody>
</table>

ternoon session comprised 1 hour of continuous and mixed training. On Sundays, they only performed the morning training session and the hypoxia session. The study lasted for 4 weeks, 3 weeks of high load (high intensity training) and a week of lower load work. This is the week in which we carried out the performance tests, the same tests that were performed before starting the hypoxia study.

Analytical control

We followed the World Anti-Doping Agency (WADA) regulations to collect and transport samples (www.ama-wada.org). All of our samples were collected under baseline conditions and on an empty stomach with a period of at least 12 hours fasting since the last meal. All the blood samples were taken at 08:30 and all the participants rested comfortably in a sitting or lying position. The Vacutainer system was used (10 ml for serum, 5 ml and 3 ml with EDTA). Immediately after extraction, the tubes were inverted 10 times and were stored in a sealed box to be stored at 4ºC. The temperature during transportation to the laboratory was controlled using a specific label (Libero Ti1, Elpro, Buchs, Switzerland), which was used to measure and register the temperature.

The samples were transported under suitable conditions and the time taken to deposit the samples at the laboratory was 30 minutes after extraction. Delays did not affect the analytical quality of the parameters studied. The EDTA (anti-coagulant) samples were homogenised for 15 minutes before being analysed, as recommended by the WADA. The tubes containing blood plus EDTA were centrifuged at 2,000 rpm for 15 minutes. The plasma was extracted using a Pasteur pipette then transferred to a sterile storage tube, kept at -20ºC until the analysis.

The leucocytes (LEU), monocytes (MON), lymphocyte (LIM), haemoglobin (Hb) and haematocrit (Hct) were established in a System Coulter Counter MAX-M model haematological counter. To analyse the serum iron (sFe), the Synchron CX model automatic chemical analyser from the Beckman laboratory was used; to establish the ferritin (FER), duplicated aliquots of serum were needed, using the standardised IRMA commercial kit from the Bio-Rad laboratory.

To determine the erythropoietin (EPO), an immunometric and chemoluminescent trial was used in solid phase using the Immulite 2000 Epo analyser (Diagnostic Products Corporation). The reticulocytes (RET) were measured through fluorescence using flow cytometry (Beckman Dickinson, Beckman Coulter). To quantify the contents of the reticulocyte haemoglobin (RET-Hb), the XE-2100 analyser (Sysmex) was used.

The percentage changes in the plasmatic volume (% ΔPV) were calculated using the Van Beaumont equation\(^\text{15}\). Furthermore, the haematologic indicator values were adjusted to the changes in the plasmatic volume using the following formula: Corrected value = Uncorrected value x ((100 +% ΔPV) / 100)\(^\text{15}\).

Intermittent hypoxia exposure (IHE) protocol

The EA were in resting conditions and sitting comfortably whilst they received the daily IHE session over a 4-week period, breathing through a hand-held mask for 90 minutes each day. Intermittent breathing was administered in 5-minute bursts of hypoxic conditions followed by 5 minutes of normoxic environmental air. They received a normobaric hypoxic gas through a GO\(_{2}\) Altitude hypoxic device (Bio-medtech, Victoria, Australia). To allow for sufficient adaptation time, and in accordance with the manufacturing instructions, the oxygen concentration in the hypoxic gas was reduced progressively (Table 3). The hypoxic conditions of this protocol entailed subjecting the EA to altitudes classified as “High Altitude” (+4,000-5,000 metres) and “Very High Altitude” (+5,000 metres)\(^\text{15}\). The peripheral oxygen saturation for each individual was measured automatically using the GO\(_{2}\) Altitude hypoxia device, or manually by a research assistant with a finger pulse oximeter (INVIPOX LTD800, Diemer, Biscay, Spain). None of the subjects were acclimatised or exposed previously to altitude or hypoxia, other than that they lived in Soria (1,100 m). Given that this study was performed during the important pre-competition period of the season, the IHE was administered during the training recovery times.

Performance trials

The physical performance of the EA was assessed using the individual test time that was taken on the first day of the study (T1), when the hypoxia treatment had not yet begun, and on the final day of the study (T2) after 4 weeks of IHE. The assessment of the aerobic, power, anaerobic...
power and speed, were performed on the athletics track at distances of 1,000 metres (m), 400 m and 60 m, respectively. To establish the maximum oxygen consumption (\(\text{CsO}_2\text{max}\)), a modified Bruce treadmill protocol was used\(^{18}\). The trial included a 5-minute warm-up on a monitored treadmill, at a constant speed of 9 km h\(^{-1}\). We performed constant speed increases of 1 km h\(^{-1}\) every 2 minutes, until the EA reached exhaustion. An automated gas analyser was used (Vmax 29, Sensormedics, USA) to register the respiratory parameters every 20 seconds, whilst the athletes breathed environmental air. The trial ended when the EA could no longer keep up the pace set on the treadmill. The \(\text{CsO}_2\text{max}\) (ml kg\(^{-1}\) min\(^{-1}\)) for any 20-second interval was registered as the individual’s \(\text{CsO}_2\text{max}\).

### Statistical analysis

The statistical analyses were performed using the IBM Statistical Package (SPSS Version 22) and Graphpad Prism (Graphpad Software Version 6.01 San Diego, CA). The data was expressed as average ± standard deviation (SD). The differences in the hematologic parameters were assessed using the paired Student t test parametric to identify significant differences between T1 and T2 independently. After this, the normality of the data was confirmed using the Shapiro-Wilk test to decide to use the parametric analysis. Significant differences were considered for \(p <0.05\).

### Results

#### Haematology

The % \(\Delta\text{PV}\) in the EA between T1 and T2 was a reduction of 4.5%, with all the values analysed in T2 of the study adjusting to this result. The haematological variables and the performance parameters analysed followed a normal distribution.

Analysing the haematological variables (Table 4) that present the pre (T1) and post (T1) data of the training mesocycle, reveals that in the white series: LEU, MON, LIN, there are no significant differences in any of the variables. Regarding the red series: HEM, HB and HCT there is a slight increase between T1 and T2, but there are no significant differences between the two time periods. Likewise, the same occurs in the control parameters of the iron metabolism: SFe and FER.

Figure 1 reveals that the RET increase from the first time in T1 = 0.94 ± 0.38 % to the later time in T2 = 1.03 ± 0.40 % with significant differences \((p = 0.041)\) between both assessment times in the study. This behaviour is reproduced for the EPO hormone \((T1 = 6.18±1.59 \text{ mU/mL} \text{ and } T2 = 7.05 ± 1.43 \text{ mU/mL} \text{ with } p =0.010)\) and for the RET-Hb represented in Table 4.

#### Performance tests

Improvements in performance can be seen in all the tests carried out (Table 5) \((p = 0.059)\) by 1.96 ± 2.35 % in the speed test. The analysis of anaerobic power with the 400-metre trial, revealed significant improvement \((p = 0.05)\) with 1.93 ± 1.13 %. Although the 1,000-metre test analysing aerobic power did not reveal significant improvements \((p = 0.112)\), it did reveal the largest percentage of improvement with 3.73 ± 5.34 %. Finally, the \(\text{CsO}_2\text{max}\) improvement \((p =0.054)\) was 3.36 ± 4.35 %.

###Table 4. Haematological study variables

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time (T)</th>
<th>Average ± SD</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucocytes (x10³/µL)</td>
<td>T1</td>
<td>5.29±0.88</td>
<td>0.055</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>6.18±1.28</td>
<td></td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>T1</td>
<td>8.81±1.48</td>
<td>0.270</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>8.31±0.88</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>T1</td>
<td>37.59±9.65</td>
<td>0.911</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>37.90±7.89</td>
<td></td>
</tr>
<tr>
<td>Red Blood Cells (106 µL⁻¹)</td>
<td>T1</td>
<td>4.96±0.54</td>
<td>0.427</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>5.03±0.45</td>
<td></td>
</tr>
<tr>
<td>Haemoglobin (g.dL⁻¹)</td>
<td>T1</td>
<td>15.25±1.58</td>
<td>0.227</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>15.50±1.24</td>
<td></td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>T1</td>
<td>44.51±3.66</td>
<td>0.598</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>45.67±2.28</td>
<td></td>
</tr>
<tr>
<td>Reticulocyte haemoglobin (pg)</td>
<td>T1</td>
<td>342.13±10.08</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>356.75±14.57</td>
<td></td>
</tr>
<tr>
<td>Serum iron (µg. dL⁻¹)</td>
<td>T1</td>
<td>106.91±31.53</td>
<td>0.222</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>161.20±122.74</td>
<td></td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>T1</td>
<td>82.66±38.28</td>
<td>0.246</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>89.84±40.47</td>
<td></td>
</tr>
</tbody>
</table>

The data is expressed Average ± Standard Deviation.
The differences were assessed using the paired Student t test parametric.

###Figure 1. Precursory erythropoiesis variables.

The data is expressed Average ± Standard Deviation.
The differences were assessed using the paired Student t test parametric.
Artificial altitude training strategies: Is there a correlation between the haematological and physical performance parameters?

Table 5. Physical performance trials

<table>
<thead>
<tr>
<th>Test</th>
<th>Time (T)</th>
<th>Average ± SD</th>
<th>p</th>
<th>% of improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed (Sec) 60 m.L</td>
<td>T1</td>
<td>6.20±1.21</td>
<td>0.059</td>
<td>1.96±2.35</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>5.29±0.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaerobic power (seconds) 400 m.L</td>
<td>T1</td>
<td>46.65±18.54</td>
<td>0.050</td>
<td>1.93±1.13</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>45.70±18.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerobic power (minutes) 1000 m.L</td>
<td>T1</td>
<td>2.43±0.25</td>
<td>0.112</td>
<td>3.73±5.34</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>2.32±0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum O₂ consumption (ml/min/kg)</td>
<td>T1</td>
<td>83.16±3.14</td>
<td>0.054</td>
<td>3.63±4.35</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>86.18±4.80</td>
<td></td>
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</tbody>
</table>

The data is expressed Average ± Standard Deviation. The differences were assessed using the paired Student’s t-test parametric.

Discussion

The most relevant findings are: the significant increase of EPO hormone secretion, and of specific precursors of the hematopoietic process such as the RET. These increases of 14.7% and of 9.5% for EPO and RET respectively, cause a favourable increase of the haematological parameters: HEM, Hb, and Htc. Bonetti et al.19 have reported benefits in cyclist performance as a result of erythropoiesis stimulation19. These advantages translate as improvements in all performance tests (Figure 2) performed on the EA of this study after exposure to normobaric IHE.

The EA use IHE in combination with the training session, with IHE allowing the athletes to “Live high and train low.” There is a normobaric IHE study that presents discrepancies in the effectiveness of these programmes, due, most probably, to the duration of the session in which the athlete is exposed to IHE and the link to EPO stimulus, in which individuals can respond or not and undergo different responses to acclimatisation.20

Our study reveals similar results to those found by Knaupp et al. who observed changes in EPO secretion after just 5 minutes with a FIO2 of 10.5% and these changes are significant when the exposure lasts for 120 minutes21. As well as this study, Klaursen et al. found a 28% increase in the erythropoiesis hormone with a hypoxia programme at 10% of FIO2 and a 2-hour duration per normobaric IHE session.22 Likewise, Villa et al. reported an increase in EPO hormone secretion after applying a normobaric IHE programme on cyclists during the cycle tour of Spain 2001 (La Vuelta), but no modifications to the Hct, Hb or the HEM were observed1. Available literature includes a previous study of normobaric IHE that presents discrepancies in the effectiveness of these programmes, due, most probably, to the duration of the session in which the athlete is exposed to IHE and the link to EPO stimulus, in which individuals can respond or not and undergo different responses to acclimatisation.

Our study reveals similar results to those found by Knaupp et al. who observed changes in EPO secretion after just 5 minutes with a FIO2 of 10.5% and these changes are significant when the exposure lasts for 120 minutes. As well as this study, Klaursen et al. found a 28% increase in the erythropoiesis hormone with a hypoxia programme at 10% of FIO2 and a 2-hour duration per normobaric IHE session. Likewise, Villa et al. reported an increase in EPO hormone secretion after applying a normobaric IHE programme on cyclists during the cycle tour of Spain 2001 (La Vuelta), but no modifications to the Hct, Hb or the HEM were observed. Available literature includes a previous study of normobaric IHE, which shares the most similarities to our study, in terms of the hypoxia exposure protocols, results obtained, and limitations, as it also lacked a demographic control group. This study by Hellemans et al. of resistance athletes, observes that along with the increase of EPO, there was also an increase in the erythropoietic response of RET (29%), Hb (4%) and Hct (5%). The benefits of normobaric IHE on the haematology of athletes specialising in rowing and cycling are completed with two research studies by the Bonetti et al. group, who obtained increases in the RET and the Hb after a similar normobaric exposure time to the protocol used in this research study.

Analysing the normobaric IHE studies that are not in line with the results obtained in our study or with those described above, we find that two of them do not reveal any modifications in any of the haematological parameters among resistance athletes and swimmers. Other studies, such as that performed by Julian et al. on runners, do not reveal any alterations on EPO levels or on any haematological parameter. More recently, Ramos et al. – after applying this normobaric IHE programme – also failed to see any increases in any of the determining haematological variables for stimulating erythropoiesis in trained cyclists.

A larger number of favourable haematological responses are obtained in hypobaric IHE studies than in normobaric IHE studies, as described in the review by Ramos-Campos et al. These results obtained with exposure to hypobaric IHE have a greater capacity to stimulate EPO secretion and to increase the main haematological variables that induce improvements to athletes’ performance, and are similar to those observed in our normobaric IHE study. Perhaps the advantage given by the stimulus of the drop in partial pressure of O₂ in hypobaric exposure can be found in this study in the stimulus resulting from the athletes living and training in Soria, at medium altitude and moderate hypoxia. It is possible that this double hypoxic stimulus to which our EA are subjected, could have an additional effect that influences the advantageous outcomes for the EA featuring in our study.

It should be highlighted that some studies that reveal significant improvements in EPO hormone production, or in the haematopoietic precursors, such as RET, also simultaneously present significant increments to the haematological parameters of HEM, Hb and Hct, after exposure to normobaric or hypobaric IHE. However, our EA reveal insignificant increases in HEM (1.41%), Hb (1.64%) and Hct (2.60%). The reasons behind this could be the impact of the sporting practice on the haematological parameters that have been used as health and performance indicators (HEM, Hb and Hct), which vary depending on their sport-specific training. The differences were assessed using the paired Student’s t-test parametric.
the physical exercise being carried out, the intensity, duration and also the degree of training of the elite cyclists\textsuperscript{14} and athletes with a high degree of training\textsuperscript{16}. Therefore, high workloads from training or competing and strong psychophysical strains of the athletes and cyclist are the factors leading to drops in haematological variables, which can also remain under the lower limit of the established physiological ranges\textsuperscript{23,26}. Furthermore, they are only reverted when physical activity is stopped\textsuperscript{15}. This is the line followed by Villa et al., who despite reporting significant increases in the EPO, do not observe modifications in HEM, Hb or Hct in the group exposed to IHE, but do demonstrate a drop in these variables in the control group without exposure to IHE, which can be interpreted as a result of the physical efforts performed during the study, taken during the professional cycle trial, the “Vuelta España”\textsuperscript{15}. On this basis, if could be affirmed that IHE may have a protective role against training or competition loads (they increase in this pre-competition period among our EA), and that they avoid the drop of blood indicators (they even increase in our study), caused by the high physical demands of elite sport, which could end up leading to over-training among the athletes, resulting in a sharp drop in sporting performance.

FER is the main iron-storing protein and therefore influences the effectiveness of the erythropoiesis process. Just as with the previous blood parameters that we have studied, results are diverse depending on the IHE protocol used\textsuperscript{23}. In this study, among rugby players, an insignificant increase of 8.4\% has been observed in the FER, along the same lines as results reported by Hinckson et al., who observed an increase of 10.5\% in the concentration of this protein\textsuperscript{15}.

Achieving haematological adaptations in the organism linked to an increase in sporting performance is the main aim of the IHE application. We believe that the response measured by an increase in the EPO secretion that stimulates erythropoiesis and improves the blood oxygen transportation capacity has been fulfilled in this study. This is because in all the tests performed, despite appearing to be modest percentages between 2-3\%, the athletes improve upon their previous scores, after training combined with IHE exposure for 4 weeks. For EA that use the “Live high and train low” strategy, this could facilitate an improvement of scores of between 0.8 – 1\% in competitions lasting between 45 seconds and 4 minutes. Although this improvement appears minimal, it is not irrelevant. For example, an improved score of between 0.4 – 0.7\% means a greater possibility of winning an international 1,500 athletics trial by 10 to 20\%\textsuperscript{15}.

The research results regarding the effectiveness of the IHE programmes on trial time are diverse. Our results present similarities with the factors leading to drops in haematological variables, which can also remain under the lower limit of the established physiological ranges\textsuperscript{23,26}. Furthermore, they are only reverted when physical activity is stopped\textsuperscript{15}. This is the line followed by Villa et al., who despite reporting significant increases in the EPO, do not observe modifications in HEM, Hb or Hct in the group exposed to IHE, but do demonstrate a drop in these variables in the control group without exposure to IHE, which can be interpreted as a result of the physical efforts performed during the study, taken during the professional cycle trial, the “Vuelta España”\textsuperscript{15}. On this basis, if could be affirmed that IHE may have a protective role against training or competition loads (they increase in this pre-competition period among our EA), and that they avoid the drop of blood indicators (they even increase in our study), caused by the high physical demands of elite sport, which could end up leading to over-training among the athletes, resulting in a sharp drop in sporting performance.

When we analyse aerobic performance – via $C_{O_2,\text{max}}$ and the 1,000-metre trial – we observe higher percentages in improvement than with anaerobic performance. Perhaps the increase observed in both Hb and Hct could allow for a greater O\textsubscript{2} delivery and its muscle absorption, generating an improvement in $C_{O_2,\text{max}}$\textsuperscript{44}. In this respect, $C_{O_2,\text{max}}$ depends on three systems: respiratory, cardiac and muscular. The first two are central factors, such as the capacity to transport O\textsubscript{2} and heart production, whilst the last factor is peripheral, such as the use of O\textsubscript{2} by the muscle\textsuperscript{16}. The organism defends itself from a lack of O\textsubscript{2} by taking in more air, i.e. by increasing the breathing rate and the volume of air inhaled. Increasing the volume of air entering the lungs by time unit makes it easier to eliminate CO\textsubscript{2}. With this comes an improvement of the partial alveolar oxygen pressure, by which oxygen is diffused more easily into the blood, and as a consequence, the partial pressure of oxygen dissolved in the arterial blood (PaO\textsubscript{2}) is greater. In situations of hypoxia, sympathetic-adrenal activity is stimulated, resulting in an increased heart rate (HR). As a consequence of this, the cardiac expenditure is increased, making the heart pump a larger volume of blood per unit of time\textsuperscript{46}.

Another possible explanation of the aerobic improvements, could be due to the adaptations achieved through IHE such as the increase of the density and length of the capillaries; the increase of density or the mitochondrial oxidative action; and the over-expression of the hypoxia-inducible factor (HIF-1\alpha)\textsuperscript{47}. The capacity to generate more power for a particular $C_{O_2,\text{max}}$, or the capacity to use less O\textsubscript{2} to perform a specific power could be due to mechanical efficiency. This is defined in terms of O\textsubscript{2} expenditure to perform an exercise. Improvements of 3-10\% are produced in the economy of the exercise with altitude training. This causes lower ventilation expenditure, given the priority use of carbohydrates for phosphorylation and also the improvement of mitochondrial efficiency, which is interpreted as an improvement of $C_{O_2,\text{max}}$\textsuperscript{48}.

Our $C_{O_2,\text{max}}$ results are closer to the IHT exposure programmes in which the $C_{O_2,\text{max}}$ can increase around 4\%, to the IHE programmes that do not have a positive influence on the $C_{O_2,\text{max}}$\textsuperscript{10}. These results can be justified because our EA train in moderate hypoxia. In contrary to the effects on $C_{O_2,\text{max}}$, IHE programmes do produce improvements in the trial time, as we have highlighted earlier, which could suggest that there may be different mechanisms involved in sporting performance. In our study, the fact we have obtained improvements in both aerobic performance tests ($C_{O_2,\text{max}}$ and 1,000 metres via IHE) could be because the EA are subject to a second hypoxic stimulus: they live and train in Soria, which could allow them to activate the different mechanisms involved in improving the performance of the EA.

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Conclusion

In this study the EA subjected to the two hypoxic stimuli, such as continuous exposure to medium altitude and resting normobaric IHE, enable for the stimulation of haematological parameters such as the EPO, RET, Hct, HEM and Hb, which demonstrate an activation of the erythropoiesis of the athlete and which leads to an improvement in the athletes’ aerobic and anaerobic performance as a consequence of an improved oxygen transportation capacity in the blood. Furthermore, this double hypoxic stimulus can improve the results obtained in previous normobaric IHE research studies, reaping all the benefits obtained from other hypobaric IHE or IHT programmes.

Limitations

In our study the main limitation is the lack of a control demographic group, and also the sample size is small. Both limitations are a consequence of the difficulty in obtaining groups of athletes with the anthropometric, physical and sporting characteristics of our professional athletes, and that also live at medium altitude. The inclusion of this control group would provide a foundation to examine if there is a cause-effect relationship between the use of IHE and the possible hematological fluctuations and the variations of the specific performance tests.

Acknowledgements

The authors would like to thank the Castile and Leon Health Sciences Studies Institute (ICScYCL) for its support and collaboration throughout the research process of this study.

Conflict of interest

The authors do not declare a conflict of interest.

Bibliography


