

## HAEMATOLOGICAL CHANGES PRODUCED BY EIGHT WEEKS OF INTERMITTENT HYPOXIA EXPOSURE PROGRAM IN CYCLIST

### MODIFICACIONES HEMATOLÓGICAS PRODUCIDAS POR UN PROGRAMA DE EXPOSICIÓN A HIPOXIA INTERMITENTE DE OCHO SEMANAS DE DURACIÓN EN CICLISTAS

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#### SUMMARY

**Introduction:** Exposure to intermittent hypoxia (IHE) is a frequent complement for the high-level athlete to improve performance, due to improvements in hematologic key indices for performance in endurance sports. The aim of the study was to check the changes produced by a normobaric IHE in trained cyclists.

**Methods:** Included in this study 16 elite-sub23 cyclists. They were divided into an experimental group (GH) (n = 8) (Age: 23,38 ± 3,3 years; Size: 178,75 ± 7,6 cm, Weight: 75,41 ± 9,7 Kg) and a control group (CG) (n = 8) (Age: 27,13 ± 4,6 years, Size: 174,06 ± 7,1 cm, Weight: 60,48 ± 3,7 kg). Was measured through blood tests hematocrit (%), hemoglobin (g / dl), erythrocytes (x 10<sup>6</sup>/μl), the ferritin (ng / ml), reticulocytes (%) mean corpuscular hemoglobin (MCH) (pg), the mean corpuscular hemoglobin concentration (CHCM) (g / dl), mean corpuscular volume (MCV) (fl), the serum iron (mcg / dl) and hormone erythropoietin (EPO) (mU / ml). Following the completion of the blood analytical was applied a 8 weeks normobaric IHE (40-60 min duration, 4 days a week, 11-14% FiO<sub>2</sub>).

**Results:** Between the time pre-and post-treatment, there is a maintenance variables erythrocytes, hemoglobin, reticulocytes, EPO and serum iron. Hematocrit decrease in GH between the time pre (46,02 ± 1,4%) and post-treatment (43,02 ± 1,3%) with significance of p<0,05. It also found differences in CHCM between GH (34,28 ± 0,8 g/dl) and GC (33,22 ± 0,9 g / dl) in the time after treatment at the p<0,05.

**Conclusions:** The implementation of this intermittent hypoxia program not increases hematological variables for stimulation of erythropoiesis.

**Key words:** Cycling. Intermittent Hypoxia Exposure. Erythropoietin. Hematocrit.

#### RESUMEN

**Introducción:** La exposición a hipoxia intermitente (IHE) es un frecuente complemento al entrenamiento del deportista de alto nivel para incrementar su rendimiento debido a las mejoras en índices hematológicos claves para el rendimiento en deportes de resistencia. El objetivo del estudio fue comprobar los cambios producidos por un programa de IHE normobárica en ciclistas entrenados.

**Metodología:** Formaron parte de este estudio 16 ciclistas de categoría élite y sub-23, divididos aleatoriamente en un grupo experimental (GH) (n=8) (Edad: 23,38 ± 3,3 años; Talla: 178,75 ± 7,6 cm; Peso: 75,41 ± 9,7 Kg) y un grupo control (GC) (n=8) (Edad: 27,13 ± 4,6 años; Talla: 174,06 ± 7,1 cm; Peso: 60,48 ± 3,7 Kg). Se midió a través de analítica sanguínea el hematocrito (%), la hemoglobina (g/dl), los hematíes (x 10<sup>6</sup>/μl), la ferritina (ng/ml), los reticulocitos (%) la hemoglobina corpuscular media (HCM) (pg), la concentración de hemoglobina corpuscular media (CHCM) (g/dl), el volumen corpuscular medio (VCM) (fl), la sideremia (mcg/dl) y la hormona eritropoyetina (EPO) (mU/ml). Tras la realización de la analítica sanguínea se aplicó un tratamiento de IHE normobárica de 8 semanas de duración (40-60 min de duración, 4 días en semana, 11-14 % FiO<sub>2</sub>).

**Resultados:** Entre el momento pre y post-tratamiento, existe un mantenimiento en las variables hematíes, hemoglobina, reticulocitos, EPO y sideremia. Se produce un descenso en la variable hematocrito en el GH entre los momentos pre (46,02 ± 1,4 %) y post-tratamiento (43,02 ± 1,3 %) con una significación de p<0,05. También se encuentran diferencias en la CHCM entre el GH (34,28 ± 0,8 g/dl) y GC (33,22 ± 0,9 g/dl) en el momento post-tratamiento a nivel de p< 0,05.

**Conclusiones:** La aplicación de este programa de IHE no incrementa las variables hematológicas determinantes para estimular la eritropoyesis en ciclistas entrenados.

**Palabras clave:** Ciclismo. Exposición a hipoxia intermitente. EPO. Hematocrito.

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## INTRODUCTION

Altitude training is a frequent complement used by the high-level athlete in order to increase their performance at sea-level<sup>1</sup>. In an attempt to produce adaptations that derive in a competing performance increase, many endurance athletes carry out preparations at high altitude during part of the year<sup>2</sup>. However, scientific evidence confirms that this practice's efficiency is very low, since the responsible mechanisms for producing the adaptations have not been clarified.

Historically, altitude training implied athletes having to live and train at altitude during 2-3 weeks<sup>3</sup>, however, this kind of programmes reduces the training intensity<sup>4</sup>, thus reducing their performance. In order to avoid these disadvantages, new methods and devices aroused, during the 90s in Scandinavia and the USA that could enhance performance through altitude training. Nowadays, different altitude training or exposure strategies vary from the traditional "live high-train high" (LHTH) to the contemporary "live high-train low" (LHTL). Besides, intermittent hypoxia exposure at rest (IHE) and intermittent hypoxia exposure during training (IHT) are made. Despite the existing substantial differences among the aforementioned methods, all strategies have the same goal or aim, improving the athlete performance at sea-level.

The subjacent mechanism of the training under hypoxia conditions effects is still discussed nowadays. The hematologic capacity increase is the most popular improvement, but it might not be the main or only factor that helps in the performance improvement. Other main factors (ventilatory, hemodynamic parameters or neutral adaptations) or peripheral factors (storing or economy muscular capacity) play an important role.

Intermittent or periodic hypoxia exposure (IHE) is defined as the exposure to hypoxia during intervals ranging from seconds to hours and a frequency ranging from days to weeks. These intermittent hypoxia periods are separated by intervals of normoxia or lower hypoxia intensity<sup>5</sup>. IHE, combined with training sessions under hypoxia, is known as intermittent hypoxia training

(IHT)<sup>2,6</sup>. Interval hypoxia intermittent training (IHIT) is a method by which during a training session hypoxia and normoxia periods are alternated<sup>6</sup>. In the published experimental designs with endurance athlete, a high variety in the use of protocols and their length, frequency and intensity are observed.

The use of IHT or IHE makes it necessary to determine the minimum exposure time to stimulate eritropoyesis. Only short exposures or stimulus are necessary to stimulate the EPO-production<sup>7-11</sup>. Therefore, we can assert that IHE and IHT are valid methods to increase the EPO and erythrocytes segregation significantly, hence enhancing the performance and VO<sub>2</sub>max in endurance athlete and, at the same time, eliminating all negative effects that prolonged natural altitude has on athletes, such as fatigue, muscular mass decrease or immunodepression.

In the literature that studies the IHE programme application with a progressive increase in the hypobaric hypoxia from 90 minutes to 3 hours, improvements related with the eritropoyesis stimulation were observed, through a significant increase in the number of reticulocytes, erythrocytes, haemoglobin and hematocrits<sup>8</sup>, EPO<sup>12</sup> or improvements in the ventilatory response during a hypoxic submaximal exercise<sup>13</sup>.

The IHE with normobaric hypoxia studies show an increase in EPO, reticulocytes (29%), haemoglobin (4%) and hematocrit (5%)<sup>11</sup>. Other studies<sup>14,15</sup> don't observe any changes in hematologic variables after the programme on athletes and swimmers.

If we focus on the findings that are related to the IHE effects on endurance sports performance, we observe contradictory results. There are studies in which the athlete's aerobic endurance is increased, hence improving the task time<sup>11</sup> and in other cases enhancing the generated power in the anaerobic threshold<sup>8</sup> or the VO<sub>2</sub>max and the peak ventilation during exercise<sup>16</sup>. However, other measured parameters in different studies are not modified in a significant way, such as the VO<sub>2</sub>max or the test length<sup>8</sup>. In other researches no changes are produced in the organism response to maximum and

submaximum exercises neither in athletes<sup>14,16,17</sup> nor in anaerobic effort<sup>18</sup>.

This diversity of results made us consider analyzing the hematologic changes produced in elite and U-23 category cyclists after a classic training complemented with a normobaric intermittent hypoxia exposure programme, compared to a classic training with no exposure to hypoxia. Therefore, we determined that the study hypothesis is that a classic training programme complemented with IHE modifies in a significant way the hematologic values related with erythropoietic stimulation.

## MATERIAL AND METHOD

The study was made among elite category cyclist divided in an experimental (GH) and a control group (GC). The GH was subjected to an 8-week normobaric

intermittent hypoxia exposure programme which complemented their regular training, while GC subjects were not submitted to the IHE treatment and only made their road training. A first evaluation was carried out before the programme start and another after its end.

*Participants:* 16 elite and U-23 category cyclists took part in this study, randomly divided (through www.randomizer.org) in an experimental group (GH) (n=8) and a control group (GC) (n=8). All subjects were part of a sports group located in the region and followed the same training programme. The inclusion criteria to be allowed in the study were that cyclist had to have at least 5 years of experience in sports. Also, the sports practice should be of a competitive level and they should not show any disease or injury at the beginning of the study. Descriptive characteristics of the sample are shown on Table 1.

**TABLE 1. Descriptive characteristics of the sample**

	n	Age (years)	Height (cm)	Weight (Kg)	Fat (%)	VO2max (ml/Kg/min)
GH	8	23,38 ± 3,3	178,75 ± 7,6	75,41 ± 9,7	11,57 ± 2,9	63,68 ± 4,4
GC	8	27,13 ± 4,6	174,06 ± 7,1	71,95 ± 8,5	10,87 ± 1,9	60,48 ± 3,7

Age, height, weight, fat, and VO2max. Mean ± standard deviation is shown.

*Variables:* The parameters obtained from the blood test were hematocrit (%) or blood volume percentage occupied by red blood cells, haemoglobin (g/dl), which is a heteroprotein that transports oxygen from the respiratory organs to the tissues. Red blood cells ( $\times 10^6/\mu\text{l}$ ), ferritine (ng/ml), which is the main protein that stores iron in vertebrates and reticulocytes (%) or young red blood cells, whose presence in peripheral blood indicates a higher bone marrow activity, were also quantified. Mean corpuscular haemoglobin (HCM) (pg) or haemoglobin mass contained in a red blood cell, and mean corpuscular haemoglobin concentration (CHCM) (g/dl) calculated by the haemoglobin and the hematocrit quotient, were also measured.

Finally, the mean corpuscular volume (VCM) (fl) or red blood cells volume, the blood iron (mcg/dl) or seric iron concentration and the erythropoietin hormone (EPO) (mU/ml), or hormone responsible for the eritropoyesis stimulation, were calculated.

*Instruments:* The blood test was carried out in a clinical laboratory, in which the samples were analyzed in a System 9000 Coulter Counter hematologic (Menarini Diagnósticos, Spain) following the calibration and control methodology proposed by the manufacturer. To complete the programme, the GO<sub>2</sub> Altitude hypoxicator for four people (Biomedtech Australia, Melbourne, Australia) was used, which simulates heights between 2700 and 6500 m (9-15% of O<sub>2</sub>).

*Procedure:* Once the subjects were chosen and the sample was randomized, the calendar was elaborated informing each subject of the place, date, time and nature of each test. Afterwards, they were asked their informed consent on a document signed by the project director and the subject himself, under the ethic directives on investigation on human beings dictated in the World Medical Association declaration of Helsinki and updated by the American Physiology Society in 2008.

The blood analytic was then performed in a clinical laboratory to obtain the hematologic variables. On the following day the 8 week intermittent hypoxia program was started, in which the percentage of inhaled oxygen was progressively reduced and the session duration was increased from 40 to 60 min. During the first two months, the hypoxia program frequency was 4 days a week, Monday, Tuesday, Thursday and Friday. Exposure to hypoxia was made in a sports performance and readaptation laboratory in the sports sciences Faculty of Toledo. Cyclists arrived at the laboratory to conduct their hypoxia session in a structured way according to a preestablished schedule. The session was at all times supervised by the laboratory staff.

**TABLE 2. Intermittent hypoxia protocol**

Week	Duration	O2 % in air	O2 saturation %
1	40	14	90
2	50	14	90
3	50	13,5	88
4	50	13	88
5	60	12,5	87
6	60	12	86
7	60	11,5	85
8	60	11	85

Oxygen saturation or control factor of the hypoxia program intern load was set at a ratio from 90 to 85%, progressively diminishing during the treatment. Oxygen saturation was monitored by a pulse oximeter placed on the patient's right hand index finger.

During the treatment the standard intermittent hypoxia program was used, on which 5 minutes long hypoxic air inspiration intervals were alternated with normoxic air inhalation intervals of a similar length<sup>11,17,19-20</sup>. This height training program lasted for 8 weeks during which the session's length, percentage of oxygen in air, and oxygen saturation percentage were modified as detailed on figure 2.

Once the program was finished the blood test was made again following the aforementioned procedures.

*Training program performed by cyclists:*  
The season program of the studied group was divided into 2 macrocycles. The first macrocycle was 34 weeks long while the second one's length was 21 weeks. The

study was carried out during the first macrocycle of the season.

The planning model of the study was the parallel-complex, which uses regular training loads, also known as linear or diluted. This planning model is considered traditional or classic and has its greatest exponent in Matveiev<sup>21</sup>. Planning is divided in a preparatory period and a competitive period. The training conducted by the study subjects belongs to the precompetitive period of the I macrocycle of the season, correspondent to the winter phase. Planning is shown on Figure 1, where the study developed in the III perfecting and pre-competition mesocycles is put into context. Each mesocycle was composed of 4 microcycles. Perfecting III mesocycle, whose main aim was the Anaerobic Threshold development and has as secondary goals the Aerobic Threshold, Aerobic Capacity and Resistance to Aerobic Strength development, consisted of three load and one recovery microcycles (C-C-C-R), whereas the pre-competition mesocycle, whose main aim was the Competition Rhythm training, and had as secondary goals the athlete's Anaerobic and Aerobic Threshold maintenance and Resistance to Aerobic Strength work, consisted of an adjustment, two load and a recovery microcycle (A-C-C-R). Each subject trained individually in the areas established in the first tests.

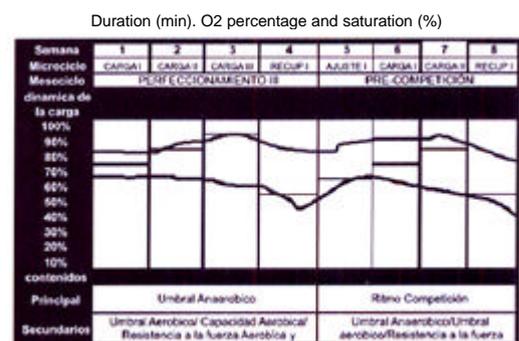


FIGURE 1. Planning of the training period where the study is conducted

The training load quantification was carried out by TRIMPS (Training Impulse), on which the considered parameters were the duration and intensity of the exercise being the load level quantitatively indicated<sup>22</sup>. This unit has been used to describe the exercise load in the different cycling categories, such as time trial and road cycling in the big

professional cycling tours<sup>23,24</sup>. Each microcycle quantification is shown on Table 3.

Athletes were given a training diary where they wrote down all information related to their training, time spent, average and maximum data gathered during the training seasons, felt sensations, as well as ergogenic aids ingested each day.

*Data analyze techniques:* For the analysis of the data obtained in the study the SPSS statistic package version 17.0 for Windows has been used. At first, descriptive values

were obtained for all studied variables, including mean, standard deviation, maximum, minimum and range. Afterwards, the variables normality was determined by the Shapiro Wilk test for samples under  $n=30$ . Later, a multifactor analysis (MANOVA) was conducted on parametric variables, applying the Bonferroni post-hoc, to determine the intragroup and intergroup differences. An ANCOVA was used to determine the intragroup differences due to the applied training load factor, by this way we eliminate the heterogeneity caused on dependent variables by the training's influence.

TABLE 3. Quantification of training microcycles in which the program is followed

Mesocycle	Perfectioning III				Pre-competition I			
Microcycle	Load I	Load II	Load III	Rec I	Adjustment I	Load I	Load II	Rec I
Trimps (GH)	1678 ±18	1857±12	2056±24	1171±21	1598±34	1677±22	2211±16	1271±17
Trimps (GC)	1629±20	1851±16	2060±12	1198±8	1593±28	1703±30	2188±21	1202±23

The applied statistic tests run on non-parametric variables consisted firstly of a Mann-Whitney U test for independent samples to establish the intergroup differences before and at the end of the programme. A Wilcoxon test for 2 related samples was later applied, before and after the treatment, to analyze the intragroup differences. Signification level for all studied variables was set at  $p<0,05$ .

Analyzing hematologic variables, we observe that regarding, red blood cells ( $\times 10^6/\mu\text{l}$ ) there are no significant differences between the GC and the GH neither before the treatment ( $p=1$ ) nor after it ( $p=1$ ), as shown on Figure 2. There are also no intergroup differences found between the different moments of study on this variable.

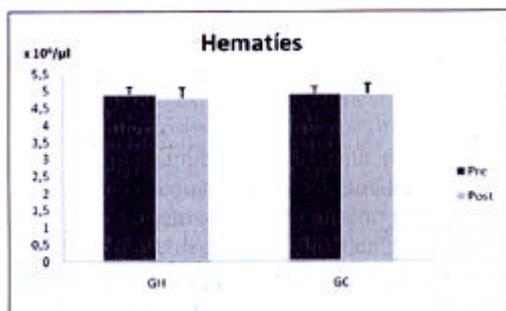


FIGURE 2. Red blood cells ( $\times 106/\mu\text{l}$ ) in GH and GC in the moments before and after the intervention

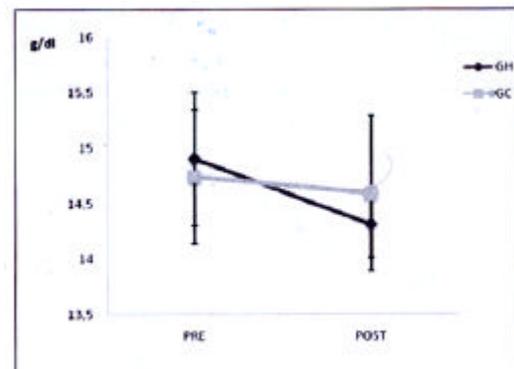


FIGURE 3. Dynamics of the variable hemoglobin (g / dl) at different measuring moments in the GH and GC

## RESULTS

Variables haemoglobin, red blood cells, HCM, VCM, CHCM, ferritine, reticulocytes, erythropoietin, and descriptive variables (age, height, weight and  $\text{VO}_2\text{max}$ ) followed a normal distribution. However, hematocrit and GH blood iron were non parametric variables.

Likewise, there is no difference observed on variable haemoglobin (g/dl) in the GH (Pre:  $14,9 \pm 0,6$  g/dl Post:  $14,74 \pm 0,3$  g/dl) or the GC (Pre:  $14,31 \pm 0,6$  g/dl; Post:  $14,59 \pm 0,7$  g/dl) at the different moments, as shown on figure 3. Being the GH compared with the GC at the pre ( $p=0,228$ ) and post-treatment ( $p=1$ ) moment there isn't any difference observed on this variable either.

Reticulocytes (%) remain unchanged between the moments prior to and after the treatment, both in the GH (Pre:  $0,48 \pm 0,2$  %; Post:  $0,51 \pm 0,1$ %) and in the GC (Pre:  $0,51 \pm 0,3$ %; Post:  $0,8 \pm 0,6$ %), being no significant differences found neither between the evaluation moments (GH:  $p=1$ ; GC:  $p=0,622$ ) nor between groups (pre-treatment:  $p=1$ ; post-treatment:  $p=0,549$ ) (Figure 4).

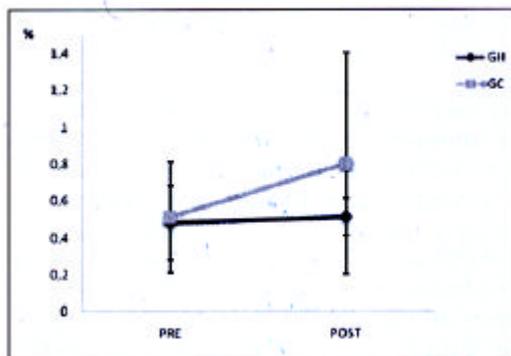


FIGURE 4. Dynamics of the variable reticulocytes (%) at different measuring moments in the GH and GC

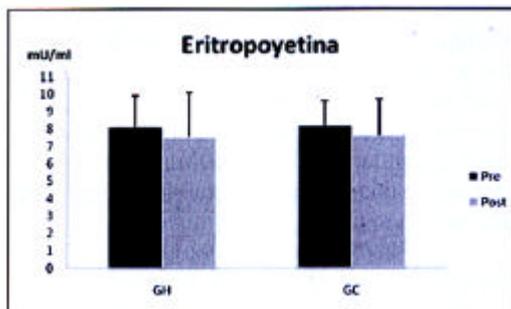


FIGURE 5. Erythropoietin (mU / ml) in GH and GC in the moments before and after the intervention

When focusing on the erythropoietin hormone (mU/ml), as we can observe on Figure 5, values for both measuring moments (GH:  $p=1$ ; GC:  $p=1$ ) as well as for both investigation groups (pre-treatment:  $p=1$ ; post-treatment:  $p=1$ ) remain constant.

Variable blood iron (mcg/dl) is not modified in any group or measuring moment. Therefore, the value in GH before the program is  $71,71 \pm 37$  mcg/dl and  $72,59 \pm 34,4$  mcg/dl once it was finished. In the GC the value was  $96,67 \pm 42,1$  mcg/dl at the moment prior to the treatment and  $99,66 \pm 49$  mcg/dl after it.

Furthermore, we observe on Figure 6 that, in the GH, there is a drop on variable hematocrit (%) in the pre ( $46,02 \pm 1,4$ %)

and post-treatment moments ( $43,02 \pm 1,3$  %), with a signification of  $p<0,05$ . In the control group no significant differences are found between the different evaluation moments ( $p=1$ ). No differences are found either between the GH and the DC in the pre-treatment ( $p=0,324$ ) or post-treatment ( $p=1$ ).

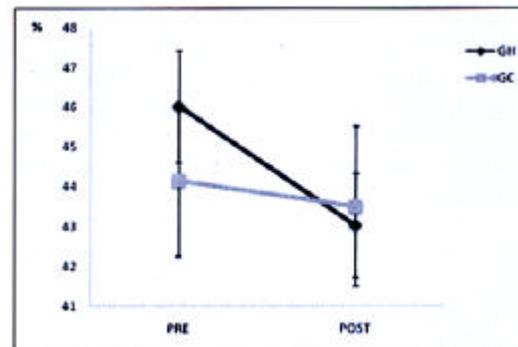


FIGURE 6. Dynamics of variable hematocrit (%) at different measuring moments in the GH and GC

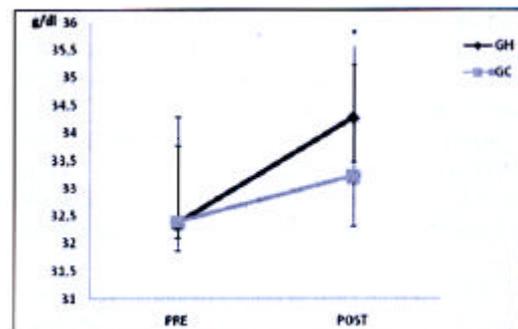


FIGURE 7. Dynamics of the variable MCHC (g / dl) at different measuring moments in the GH and GC

In the variable mean corpuscular haemoglobin concentration (CHCM) (g/dl) we observe an increase in the GH between the moments prior to ( $32,37 \pm 0,5$  g/dl) and after the programme ( $34,28 \pm 0,8$  g/dl) at a level of  $p<0,001$ . Differences are also found on this variable between the GH ( $34,28 \pm 0,8$  g/dl) and the GC ( $33,22 \pm 0,9$  g/dl) in the moment after the treatment with a signification of  $p<0,05$  (Figure 7).

Finally, there are differences in variable ferritine (ng/ml) in the moment prior to the treatment between both groups (GH:  $66,04 \pm 40,5$  ng/ml; GC:  $137,69 \pm 41$  ng/ml) with a significance of  $p<0,01$ . These differences remained the same once the program was finished (GH:  $68,41 \pm 32,2$  ng/ml; GC:  $145,16 \pm 32,1$  ng/ml), making no differences being found between the

different measuring moments neither in the GH nor in the GC.

## DISCUSSION

No favorable hematologic modification has been observed in this study after an 8 week long exposure to intermittent hypoxia program with an oxygen percentage from 14% to 11% and a saturation from 90 to 85%, on elite and U 23 category cyclists. Therefore, the formulated study hypothesis, by which a classic training program complemented with IHE does not modify in a significant way the hematologic values related with erythropoietic stimulation, is rejected.

In the last years, the interest aroused by height training on cyclists has increased<sup>25</sup>. Many researches show how intermittent hypoxia stimulates the eritropoyesis and produces benefits on different athlete<sup>26-28</sup>. This assertion doesn't coincide with the results obtained in our study, or other studies of the literature<sup>17,29-30</sup>, in which no positive effects appeared on the measured hematologic variables. These discrepancies on these programs effectiveness are due fundamentally to the length of the session during which the subject is exposed to hypoxia and its relationship with the erythropoietin hormone stimulation. Knaupp<sup>9</sup> observes an increase of 50% on this hormone 4 hours after a 2 hour long exposure to hypoxia session.

Among the previous researches that analyzed this kind of methods effectiveness was the Rodriguez study *et al*<sup>7</sup>, in which a significant increase is obtained in hematocrit (42,15 % to 45,1%), red blood cells ( $5,16 \times 10^6/\mu\text{l}$  to  $5,79 \times 10^6/\mu\text{l}$ ), reticulocytes (0,5 % to 1,1 %) and haemoglobin concentration (14,2 g/dl a 16,7 g/dl) with a hypobaric intermittent hypoxia programme of 9 days at a height of 4000-5000 metres during 3 hours a day.

Along the lines of this study we find the Eckard research<sup>31</sup>, who exposed active subjects to a hypobaric intermittent hypoxia program simulated at 3000 to 4000 meters high during 5 hours and a half a day, observing a significant rise in erythropoietin. In another study, Klaursen, *et al*<sup>32</sup> found an increase of 28% in the erythropoietin hormone with a hypoxia program of 10% of

O<sub>2</sub> and a length of 2 hours per normobaric intermittent hypoxia session. These results don't coincide with those obtained in our study, were no changes in the erythropoietin hormone are produced.

These methods are also used as a means of acclimatization on mountaineers, such as the Casas study, *et al*<sup>33</sup>, in which 6 elite climbers were exposed to 17 session of 3 hour long simulated hypobaric hypoxia at 4000 metres of height, increasing the haemoglobin from 14,8 g/dl a 16,4 g/dl. Consequently, it seems well-grounded to put into practice these sorts of methods to achieve a response to erythropoietic acclimatization, which could be compared to that produced by the use of exogenous erythropoietin<sup>34,35</sup>. Furthermore, this study, along with that of Rodriguez, *et al*<sup>7</sup> manages to increase the haemoglobin concentration. These data is not found in our study, were haemoglobin remains constant throughout the research study. This may be due to the fact that the individual response on experimental subjects is very variable in this kind of studies, in which many participants may or may not respond to height and experience acclimatization responses to this sort of programs<sup>8</sup>. Finally, Rodas, *et al*<sup>36</sup>, find an increase in reticulocytes, haemoglobin and hematocrit, after a 2 week hypobaric hypoxia program, with 6 sessions a week and 3 hours per session.

These improvements obtained in the literature with hypobaric hypoxia are not obtained with normobaric hypoxia, were all measured blood parameters remain unchanged<sup>14,17</sup>, except in the Hellemans studies<sup>11</sup>. In our study, we don't obtain similar results to those with hypobaric hypoxia either, and instead the parameters remain unchanged in a similar way to the found in the normobaric hypoxia studies.

On the other hand, studies can be found in the bibliography, like that of Katayama, *et al*<sup>30</sup>, or Lundby, *et al*<sup>37</sup>, were no modifications in hematologic parameters are found after a hypobaric hypoxia program. In the first one, the authors used a 90 minutes long protocol at a height of 4500 m, with a frequency of 3 times a week during 21 days, being no changes in haemoglobin, hematocrit, red blood cells, reticulocytes, erythropoietin or ferritin produced. In the second of the mentioned,

a 2 hour long protocol at a height of 4100 meters was used, being no changes in the mentioned hematologic parameters observed.

Along these lines we find another study with intermittent short-length hypobaric hypoxia. The exposure was of an hour at a height of 4000 m combined with a physical training in a group of high level triathletes. The authors found no adaptation signs attributed to the intermittent hypoxia<sup>38</sup>.

Sports practice effect on hematologic parameters hasn't been taken into account among the different hypoxia studies. Thus, changes on hematocrit, haemoglobin and plasma volume modifications haven't borne in mind the training load performed during the hypoxia program. Overload and persistent performance decrease, with or without other psychological and physical symptoms<sup>39</sup>, directly affect the blood markers, producing a drop in the plasma volume<sup>40</sup>. This also originates a fall in haemoglobin, hematocrit and a series of hormonal changes induced by training, and that over the long term can lead to a fall in the organism's iron fixation capacity, a drop in its quantity and therefore an anemia<sup>41</sup>. This assertion makes us think in the possibility that cyclists who participated in our study may suffer an overload or accumulation process that would justify the obtained values in blood parameters. Recent studies show how in trained athletes drops in various hematologic variables are produced throughout seasons when intensity and volume of workout is high<sup>42</sup>, being differences in those parameters between sedentary subjects and athletes<sup>43</sup>. More specifically, it has been proved that since the beginning of the season there is a fall in the hematocrit in professional Danish cyclists that only reverts with the interruption of training or transitory periods<sup>44</sup>, which justifies the fall in hematocrit in cyclists of our study that is derived from the training process.

Differences among the obtained results can be ascribed to the different heights used as stimulus, the height simulation technology and the difference in the exposure time per session. Authors like Katayama, *et al*<sup>30</sup> speculate about the optimal hypoxia exposure time that would mean a sufficient stimulus to increase the eritropoiesis. Rodriguez, *et al*<sup>8</sup> also assert that the

minimum dose for the hypoxia session to be a stimulus that goes beyond the adaptation threshold and produces an intense reaction or increase in erythropoietin is 90 minutes.

Katayama, *et al*, studies<sup>28</sup> conclude that intermittent exposure to hypoxia in one hour long sessions produce the same adaptations as 3 hours long sessions. Studies like that of Hellemans<sup>11</sup> or Julian, *et al*<sup>17</sup> use 60 minute long sessions, applying similar protocols to those in our study, and obtaining improvements in hematologic variables.

With all these studies we can assert that the session length is a key factor. The increase in time of hypoxia exposure, as a stimulus for the increase of the oxygen transportation to the muscles, could generate positive effects in the cyclists of our study, since the short length of the sessions doesn't produce any apparent changes in the cardiorespiratory or metabolic system<sup>7</sup>. On the other hand, the training level of the sample must be taken into account, since in low trained subjects the adaptation time to lower oxygen availability conditions increases<sup>7</sup>, which may directly affect subjects of our study, who were highly trained. Therefore, hypoxic stimulus is not enough to produce adaptations on the studied cyclists' organisms, which makes us find similar results between the GH and the GC.

The modulation and adaptation channels of the transportation system of oxygen -which is used to enhance sea level performance, by the increase in the capacity of transporting oxygen in blood<sup>4</sup>-, seem to be clearly described. However, these changes are usually small, since this is a system that hardly suffers alterations and these require a certain amount of time to be reached and to become stabilized. It should be emphasized that the improvement in this system not only affects hematologic parameters and sports performance, but it is equally profitable in the processes of recuperation after interval or successive loads, as well as in the high training loads assimilation while at the same time improves health and life quality of the people following these programs.

The applicability of this kind of intermittent hypoxia programs is oriented towards pre-

acclimatization at extreme heights in climbers and improvement in performance and physical capacity as it has been shown in previous studies<sup>7,33,45-46</sup>. It has also an application related to the health and physical condition of sedentary, active subjects and affected by diverse pathologies<sup>47</sup>.

## CONCLUSIONS

Bearing into mind the hematologic data obtained in our investigated sample, the application of this intermittent hypoxia program does not increase the main hematologic variables for erythropoiesis which enhance the trained cyclist's performance. There are no significant differences between the GH and the GC that make it possible to determine that the used IHE program causes beneficial effects

on cyclists that conduct it. The used protocol, sample size and individual differences towards height acclimatization may have interfered in these results. Furthermore, the program described in the study doesn't cause the necessary stimulus in the sample to produce an adaptation that will derive in an increase in the measured hematologic variables.

It is also observed that the exhaustive and systematic training combined with the exposure to intermittent hypoxia in this group of cyclists negatively affects the athlete blood parameters, showing a significant drop in the variable hematocrit. Finally, in further studies it would be interesting to direct this kind of programs to other populations, such as sedentary or ill subjects, to check if this type of stimulus means an effective treatment in the pathologic processes control.

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