Effect of hypobaric hypoxia on hematological parameters related to oxygen transport, blood volume and oxygen consumption in adolescent endurance Training athletes

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A B S T R A C T

Background/Objective: To analyze the effect of altitude on hematological and cardiorespiratory variables in adolescent athletes participating in aerobic disciplines.

Methods: 21 females and 89 males participated in the study. All were adolescent elite athletes engaged in endurance sports (skating, running and cycling) belonging to two groups: permanent residents in either low altitude (LA, 966 m) or moderate altitude (MA, 2640 m). Hematocrit (Hct), hemoglobin concentration ([Hb]), total hemoglobin mass (Hbt), blood, plasma and erythrocyte volumes (BV, PV and EV), VO2peak and other cardiorespiratory parameters were evaluated.

Results: Sex differences were evident both in LA and HA skating practitioners, the males having higher significant values than the females in oxygen transport-related hematological parameters and VO2peak. The effect of altitude residence was also observed in Hct, [Hb], Hbt and EV with increased (14%–18%) values in the hematological parameters and higher EV (5%–24%). These results matched the significantly higher values of VO2peak measured in MA residents. However, BV and PV did not show differences between LA and MA residents in any case. Sports discipline influenced neither the hematological variables nor most of the cardiorespiratory parameters.

Conclusions: LA and MA adolescent skaters showed sex differences in hematological variables. Endurance-trained male adolescent residents at MA had an increased erythropoietic response and a higher VO2peak compared to their counterparts residing and training at LA. These responses are similar in the three aerobic sports studied, indicating that the variables described are highly sensitive to hypoxia irrespective of the sports discipline.

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1. Introduction

In endurance athletes, the oxygen supply to the active muscle is the main limiting factor of VO2peak and, as oxygen is transported by hemoglobin, Hbt and BV determine oxygen transport capacity in blood and, therefore, VO2peak. The oxygen transport capacity can be modified by different variables such as altitude and physical training. Thus, exposure to altitude has specific biological effects in humans since acclimatization to environmental hypoxia initiates a series of metabolic and muscle-cardiorespiratory changes that influence oxygen transport and utilization. Continuous residence at
moderate altitude (2000–2500 m) improves oxygen transport capacity due to an increase in hematocrit (Hct) induced by erythropoietin (EPO). When exercising at altitude, the human body must respond to two stressful stimuli: hypoxia and exercise. As oxygen supply to active muscles is reduced at altitude, particularly during exercise involving large muscle groups, fatigue occurs at lower work rates and involving lower oxygen consumption, indicating that, under hypoxic conditions, muscle metabolism is more limited by oxygen availability than at sea level. Whatever the origin of the hypoxic stimulus (environmental or pathological), the final consequence to the organism is an insufficient availability of oxygen at the tissue level, which implies that the demand of the tissue exceeds its oxygen supply. The main regulator of oxygen homeostasis is hypoxia-inducible factor-1 (HIF-1), which acts as a transcriptional activator. HIF-1 target genes code proteins that enhance O2 delivery and mediate acclimatization or adaptive responses to O2 deprivation. Like in other tissues, skeletal muscle homeostasis at rest is challenged during hypoxic exposure, either acutely or chronically. However, unlike in other tissues, muscle function can be compromised if exercise is performed during hypoxic exposure. Physical activity by itself can contribute to the loss of the balance between oxygen supply and consumption and, together with the hypoxic stimulus, it can trigger, mediated by HIF-1, changes in gene expression leading to the enhancement of angiogenesis, modification of vascular tone, glucose and iron metabolism, promotion of cell proliferation, oxidative metabolism and oxygen transport, ultimately mitigating the harmful effects of chronic hypoxia.

Most studies on the relationship between Hbt and VO2peak have been conducted on trained adults and only a few on adolescents. Since there is no conclusive evidence on how simultaneous factors such as exposition to hypoxia and aerobic endurance training affect children and adolescents, we engaged in research whose main goals were to study the behavior of blood oxygen transport-related parameters and VO2peak throughout childhood and puberty in boys and girls and the hormonal regulation of erythropoiesis. As part of this research, the aim of our work here was to investigate the effects of moderate altitude residence on oxygen transport-related hematological parameters and on VO2peak in adolescent endurance athletes participating in different sport disciplines.

### 2. Methods

#### 2.1. Participants

A total of 110 adolescents (21 females and 89 males) participated in the study with the descriptive characteristics shown in Table 1. The study was developed in accordance with the Helsinki Declaration concerning the ethical principles of human experimentation and approved by the local Ethical Committee. Because the athletes were adolescents, their parents or legal tutors received clear and simple information from the researchers about the study procedures, the benefits of their participation, as well as the risks of the methodologies used. The voluntary participation of the athletes was authorized by the signing of consent forms by both parents and athletes. Athletes were free to withdraw from the experimental protocol at any time.

The participants reported having lived and been born at low altitude (LA) in the city of Tuluá, (Colombia) at 966 m or moderate altitude (MA) in the city of Bogotá (Colombia) at 2640 m, and not having moved to a different altitude for more than a week in the last year. The individuals belonged to endurance modalities of inline skating, athletics running and cycling. To be included in the study, they must have had a minimum sporting history of one year with a minimum frequency of 3 times per week. The mean training load (in hours per week) was similar in LA and MA participants (Table 1) to exclude that differences in training load could be responsible for possible differences in the analyzed parameters. The stage of sexual maturation of all participants was determined according to Tanner and only individuals classified as stage III or stage IV were included in the study. To assess sex differences, female and male subjects were considered in the skating modality, whilst to evaluate the effect of sports discipline only males participating in skating, athletics and cycling were considered. No participant reported consuming iron, folic acid or other supplements that could affect the study variables. Exclusion criteria included: presenting signs of disease or diagnoses that impeded the performance of the procedures or that interfered with the behavior of the study variables and having suspended physical training for more than a week in the last two months prior to the study evaluations.

#### 2.2. Procedures

Participants were recommended not to perform any physical exercise in the 24 h prior to the evaluations. To avoid methodological errors in the comparison of results, the same equipment was used in the LA and MA laboratories and the tests were carried out by the same evaluators. The procedures were performed on four different consecutive days as follows:

**Day 1. Anthropometrical measurements, medical and fitness examination, and assessment of biological maturation.** A sport medicine doctor performed a medical evaluation on the participants to verify

### Table 1

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Age, sex, anthropometric parameters and training load of the participants in the study. Sample sizes (n) are indicated grouped by sex and condition.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>LA</strong></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>13.0 ± 1.8</td>
</tr>
<tr>
<td>Minimum</td>
<td>11</td>
</tr>
<tr>
<td>Maximum</td>
<td>17</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>154.6 ± 7.4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>46.0 ± 6.8</td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>19.2 ± 2.2</td>
</tr>
<tr>
<td>Training load (hours per week)</td>
<td>14.7 ± 3.7</td>
</tr>
</tbody>
</table>

**BMI** — body mass index; **LA** — low altitude; **MA** — moderate altitude. Asterisks indicate statistically significant differences after running a Student’s t-test (**, P < 0.001; *, P < 0.05) within the same altitude when comparing males vs. females. Letter a superscript indicates a statistically significant difference (P < 0.05) between males “Cycling MA” and “Skating MA” after running a one-way ANOVA.
their health condition, inquired about the existence of risk factors for sports practice, and performed a 12-lead electrocardiogram at rest. Primary and secondary external sexual characteristics were evaluated to classify participants as stage III or IV according to the size of the breasts, genitals, testicular volume, and the development of pubic and axillary hair.

Day 2. Blood sampling for the determination of hemoglobin concentration, hematocrit and reticulocyte count. This procedure was carried out following the protocols established by the World Anti-Doping Agency (WADA) for the collection, transport, storage, and analysis of blood samples. After remaining in a sitting position and resting for 10 min, participants' blood samples (4 mL) were obtained from the antecubital vein and stored in EDTA tubes. The concentration of hemoglobin ([Hb]), hematocrit ([Hct]) and reticulocyte counts were measured using an automated hematology analyzer (Sysmex XT2000i, USA).

Day 3. Determination of total hemoglobin mass and blood, plasma, and erythrocyte volumes. Total hemoglobin mass (Hbt) was determined according to the optimized CO-rebreathing method as described by Prommer and Schmidt. After familiarization with the equipment (SpiCO, Blood tec GmbH, Bayreuth, Germany), the participants remained in a sitting position for 5 min, after which two capillary blood samples (75 μL) were taken from the ear lobe and processed in a gas analyzer (OSM 3 Radiometer Hemoximeter, Denmark), to obtain the percentage of carboxyhemoglobin (% HbCO). Subsequently, the subjects inhaled a bolus of CO (95%) with a dose adjusted to 0.6 mL kg⁻¹ instead of the 0.8–1.2 mL kg⁻¹ used for adults and filling the rebreathing bag with pure oxygen also reduced to approximately 2 L. The subjects breathed this mixture for 2 min and % HbCO values were obtained at minutes 6 and 8. The determination of Hbt and blood volume (BV), with co-evaluation of CO volume and % HbCO, and subsequent plasma (PV) and erythrocyte volume (EV) was done according to the equations described by Prommer and Schmidt and adjusted for barometric pressure at LA and MA.

Day 4. Incremental tests. Peak oxygen consumption (VO₂peak), maximum heart rate (HRmax), respiratory quotient (RQ) and the percentage of VO₂peak attained at the ventilatory threshold (%VT1) were determined after an incremental test until exhaustion. The athletics runners were evaluated on a treadmill (HP Cosmos Quasar, Germany) whilst skaters and cyclists on a cycle ergometer (Monark Ergomedic 839, Sweden). In both cases, oxygen consumption was measured using a Cosmed ergospirometer (Quark CPET Model, Italy). The subjects were accustomed to the laboratory conditions and to the evaluation protocols prior to the definitive tests. Since there are no protocols for the determination of VO₂peak in children and adolescents that take into account their biological differences and the particularities of each sports discipline, pilot tests were carried out to establish the tolerance and relevance of the proposed protocols. Fig. 1 shows the characteristics of the incremental tests performed. In all tests, a 3-min warm-up was performed with a load intensity lower than the initial one. The test was terminated when the subject made the signal of maximum exhaustion, could not maintain the gesture of running or balance (in treadmill tests), or could not maintain an adequate pedaling cadence (in cycle ergometer tests). The following criteria were established to consider having reached VO₂max: 1) reaching a plateau in oxygen consumption, despite the increase in the intensity of the load, 2) heart rate greater than 180 beats per minute or a stabilization in the final stages, and 3) an RQ > 1.1. During the progressive and incremental tests performed on the pediatric population, only a small minority reached a stabilization (plateau) of oxygen consumption until voluntary exhaustion. For this reason, VO₂peak is considered the highest oxygen consumption rate at exhaustion and is recognized as the best individual indicator of aerobic fitness in children and adolescents. Thus, in our study, when the subject reported maximum exhaustion even though they did not meet the aforementioned criteria, it was considered that the subject had reached their VO₂peak.

2.3. Statistical analyses

The normality of the data (Shapiro-Wilk test) and the equality of the variances (Levene test) were checked before applying the statistical tests of comparison between groups. Parametric comparison tests were applied for all cases. Student t-tests were used to assess the effect of sex (female vs male in skaters) at each altitude, and the effects of altitude (LA vs MA) in each group. To evaluate the effects of the sports disciplines in the male groups, one-way ANOVA tests were run and Holm-Sidak post hoc test used for multiple comparisons when statistical differences were found. Data are expressed in all parameters with the arithmetic mean and standard deviation (SD) and P-values are given throughout the text, tables and figures, considering significant statistical differences at P < 0.05. In the box-and-whisker plots, the box represents the interquartile range and shows the first and third quartiles separated by the median. The mean is represented by a black dot, and the whiskers represent the minimum and maximum values. All data were statistically analyzed using SigmaPlot 11 (Systat Software, Inc., San Jose, CA, 2008-2009).

3. Results

3.1. Effect of sex

Comparisons between male and female skaters showed significantly higher values in males in all the measured hematological parameters related to oxygen transport capacity ([Hct], [Hb] and Hbt) as well as in blood, plasma, and erythrocyte volumes (Figs. 2 and 3). These differences were evident both under LA and MA conditions.

Regarding the parameters measured after the maximum incremental tests, significant sex differences were obtained in VO₂peak both at LA and MA (Fig. 4A) and in HRmax at MA (Fig. 4B) but no statistical differences were observed neither in HRmax at LA (Fig. 4B), in RQ (Fig. 4C) nor in %VT1 (Fig. 4D).

3.2. Effect of altitude

Results from Fig. 2 show that MA induced significant higher values in all the measured hematological parameters related to oxygen transport capacity both in males and females and in the three sports disciplines considered, with the only exception of Hbt in male skaters where no significant differences were found between LA and MA (Fig. 2C). Absolute Reticulocyte Count (ARC) was significantly increased in MA in the three sports disciplines and both in male and female skaters (Fig. 2D). A similar behavior was observed for EV with significant differences elicited by altitude in all groups except for male skaters (Fig. 3C). However, no differences were found either in BV or in PV between altitudes in any of the athlete groups analyzed (Fig. 3A and B). Regarding the parameters related to VO₂peak, MA resident athletes had better results than their LA counterparts with significant increases in VO₂peak and % VT1 in all groups (Figs. 4A and D) and significant decreases of HRmax in female skaters and male runners (Fig. 4B). Altitude had no significant effect on RQ in any group of athletes (Fig. 3C).

3.3. Effect of sports discipline on male athletes

No statistically significant differences were found between male skaters, athletics runners and cyclists which demonstrates that the
Fig. 1. Maximal incremental test protocols for the evaluation of VO2peak in every sports discipline. Skaters and cyclists were evaluated on a cycle ergometer whilst athletics practitioners on a treadmill. Initial load (w) and speed (km/h) were adjusted according to sex and age. Treadmill inclination and load and speed increments are indicated in each case.

Fig. 2. Box and whisker plots showing the blood oxygen transport-related parameters and absolute reticulocyte count in residents and practitioners of the different sport disciplines at low and moderate altitude. A, hematocrit (%); B, hemoglobin concentration (g.dL⁻¹); C, hemoglobin mass (g.kg⁻¹); D, absolute reticulocyte count (ARC, 10⁵ cells.L⁻¹). The P-values obtained after running a Student’s t-test between Low Altitude and Moderate Altitude conditions are shown on the upper whiskers. Asterisks (*) and crosses (†) indicate statistically significant differences after running a Student’s t-test within the same altitude when comparing males vs. females in skating practitioners (one symbol: P < 0.05; two symbols: P < 0.01; and three symbols: P < 0.001). Statistically significant differences after running a one-way ANOVA were only found for males’ comparisons between skating and cycling in Low Altitude (P = 0.017).
Sports disciplines performed at the same altitude can influence in the same manner the hematological parameters related to oxygen transport capacity (Fig. 2), BV and PV (Fig. 3A and B). However, lower EV were observed in runners at LA in comparison to skaters and cyclists, this difference being statistically significant between skaters and runners (Fig. 3C). Runners also showed significantly lower VO2peak values at MA (Fig. 4A), higher HRmax at LA (Fig. 4B) and lower RQ at LA (Fig. 4C) than skaters. Cyclists had significantly
higher RQ than runners at both altitudes and higher than skaters at LA (Fig. 4C). Finally, cyclists had higher %VT1 than skaters at MA (Fig. 4D).

4. Discussion

Exposure to hypobaric hypoxia triggers a series of systemic responses mediated by the hypoxia-inducible factor 1 (HIF-1), which acts as the main regulator of gene expression in response to the decrease in oxygen partial pressure at tissue level.11–21 The maintenance of a chronic hypoxic stimulus develops several acclimatization responses making it possible to continue life at altitude with minimum functional repercussions. In humans, these responses are well known and have been thoroughly described.22–27 Based on these responses, several training programs have been developed where the limitation in the availability of environmental oxygen constitutes a stimulus that enhances physiological effects related to greater capacity to transport oxygen, aimed at improving performance at sea level.18–20 The results of the present study show that the modifications occurring in the hematological parameters involved in oxygen transport in adult athletes who reside and train at moderate altitude, compared to those who do so at low altitude, are also evident in the population of adolescent athletes of both sexes. The increase in ARC also indicates that erythropoiesis took place in adolescent athletes, as deduced from the increases in ARC in MA subjects. These differences are significant in the three sports analyzed and have a consequence in VO2peak, showing that those subjects who reside and train in MA have a greater capacity to develop aerobic exercise. Since training loads were not statistically different between LA and MA participants in all sports modalities, we assume that altitude training is responsible for the results reported here. Finally, no remarkable differences were observed in most data between the three sports modalities either at LA or at MA. This finding is in agreement with the results of Heinicke et al.31, who found similar Hbt and blood volume values in elite athletes of endurance disciplines.

4.1. Effect of sex

A total of 29 female skaters were included in this study and compared with altitude-matched male skaters (Table 1). Sex had a strong significant effect on hematological parameters (Figs. 2 and 3) and VO2peak (Fig. 4A), the females showing lower values than males. A pioneering work by Kjellberg et al.32 studying the relationship between Hbt, BV and the degree of physical training in adults showed that females had from 21% to 26% lower Hbt and BV values than males, both in untrained and trained subjects. Since then, it has been well known that women have 10% lower [Hb]33 values than males, both in untrained and trained subjects. Since 1982, these results have not found studies on skaters. Heinicke et al.31 did not find any significant difference between Hbt and BV measured in a total of 29 female skaters and 20 male skaters living and training in LA. We found similar values in Hbt and BV in 14 male and female Icelanders,43 who found similar Hbt and blood volume values in elite athletes of different sports and age groups (Figs. 2C and 3C). This finding is consistent with the results of Heinicke et al.31, who found similar Hbt and blood volume values in elite athletes of endurance disciplines.

According to a recent study carried out in cross-country skiers,38 these sex differences begin to appear in the maturation process approximately at 13 years old and are clearly evident when the boys and girls reach the age of 15 years old. Similarly, our latest research involving a large sample of children and adolescents from both sexes concluded that the associated effects of endurance training on Hbt and BV is only observed after the onset of puberty.12 Our results, shown in Figs. 2–4, are consistent with these previous findings since the mean range of age of the skater girls and boys participating in our study was from 13 to 15 years old (Table 1). The higher Hbt and BV obtained in Tanner stage III and IV adolescent males will be the result of an increase in erythropoiesis stimulated by testosterone39,40 and, since Hbt influences VO2max via its relationship with BV,1 this would explain the higher VO2peak values obtained in male skaters. Regarding the erythropoietic role of testosterone, it is interesting to highlight the study by Plunjevic Gligoroska et al.37 who did not find, in females with an age span from 8 to 18 years, significant differences between age groups in RBC variables, showing significant age- and sex-specific differences only within males and adolescents. This is also consistent with the positive correlation between total hemoglobin mass and testosterone found in a recent study by our group.41

4.2. Effect of altitude

Our results show that adolescent athletes resident at MA have significantly higher values in the analyzed parameters related to blood oxygen transport (i.e., Hct, [Hb], Hbt, ARC, and EV) than their counterparts at LA, with the only exception being male skaters in Hbt and EV where no statistically significant differences were found (Figs. 2C and 3C). These results are consistent with others performed in young adult elite athletes involving different Live High-Train High (LH-TH) schedules at MA and several sports disciplines. Thus, Garvican-Lewis et al.42 found that distance runners training LH-TH (altitude <2000 m) for 3 weeks had significant increases in Hbt, Hct and ARC compared to runners who lived and trained at sea level. Similarly, authors from the same group47 reported that elite cyclists living and training for 3 weeks at 2760 m increased significantly Hbt compared to their sea level counterparts. Heinicke et al.43 also found significant increases in Hct, [Hb], Hbt and EV in a small sample of male (n = 6) and female (n = 4) elite biathlon athletes after 3 weeks of training at MA (2050 m). Although we have not found studies on skaters, Gough et al.45 also reported significant increases in blood oxygen-related transport parameters in young males and females in swimmers after a temporary training camp. Regarding studies focused on the adolescent population, comparable results have been reported for elite swimmers46 and skiers,47,48 although a wide inter-individual variability was clear in the erythropoietic response in elite adolescent swimmers.49 To our knowledge, only a few studies have dealt with MA elite athlete permanent residents and, apart from our recent report50 none involved the adolescent population. Schmidt et al.49 found, in a sample of male professional cyclists resident at MA (2600 m) with a mean age of 29 years, significant increases in Hbt and BV when compared to sea level counterparts, leading them to the conclusion that Hbt and BV were synergistically influenced by training and altitude exposure. Accordingly, Böning et al.51 reported increased Hbt, Hct, [Hb], ARC and EV in male runners with a mean age of 25 years. The same authors found in 23-year-old female runners and cyclists resident at MA (2600 m) that there was an increase in Hct, [Hb] and Hbt resulting from the effects of both altitude and subject’s training level.52
PV did not show significant differences in any paired group between MA and LA, whilst BV did show marginal increased values (P < 0.088) in cyclists (Fig. 3A). These findings contrast with those reported by Böning et al.25 in MA female residents that had lowered PV, leading to a decrease in BV, as compared to sea level residents. Henicke et al.28 also reported PV significant reductions (14%) in a sample population (n = 9) permanently resident at 3550 m when compared with sea level residents. Conversely, and according with our results, Schmidt et al.50 found unchanged PV in male cyclists living under chronic hypoxia (2600 m). However, comparisons between our results and those commented above should be made with caution for two main reasons. Firstly, the altitude condition of our LA group (residents at 960 m) is different from a sea level condition. Secondly, there is an important age difference between the previous studies and the participants evaluated from our work. This is an important consideration because, despite the fact that in adult female runners no statistical differences were detected in any of the blood volume fractions between sea level and 960 m,51 the response of the adolescent population, which go through a transition phase of the development between childhood and adulthood, is yet unclear.

Although the oncotically mediated mechanism responsible for PV contraction during short-term hypoxia acclimatization has recently been elucidated,54 no hypothesis explaining PV behavior after long-term acclimatization has been considered in recent excellent reviews.55,56 In any case, our results corroborate previous clues which indicate that the best correlations between VO2peak and blood constituents are found for Hbt and EV rather than for BV or PV.7

Considering the results shown in Figs. 2 and 3, we can conclude that the increase in the hematological variables in the MA group is not due to hemoconcentration but rather to a chronic acclimatization process due to elevated RBC production (i.e., erythropoiesis). The relevance of these results is derived from the fact that the sample is from a poorly studied population (elite adolescent athletes) showing the effects of altitude on paired results for sports discipline and sex group. It is well known that the hypobaric hypoxia stimulus induces the proliferation of RBC57,58 allowing, in our case, an improved oxygen transport capacity to overcome the low ambient oxygen tension that occurs in MA. Hypoxia initiates a HIF-mediated signaling pathway by stimulating the production of erythropoietin (EPO) in the kidney, which binds to its receptor (EPOR) on erythroid progenitors in the bone marrow to stimulate their survival, proliferation, and differentiation. Erythropoiesis consists of the absorption of large amounts of iron by the marrow, which are used in the synthesis of Hb. In the liver, HIF-1 stimulates iron absorption by suppressing the gene encoding hepcidin, which is an inhibitor of ferroportin, the main protein responsible for intestinal iron absorption. HIF-1 also activates the hepatic synthesis of transferrin, the main plasma protein responsible for the transport of iron from the intestine to the bone marrow through the transferrin receptor. Thus, HIF-1 directly regulates the expression of 5 gene products (EPO, EPOR, hepcidin, transferrin, and transferrin receptor) involving 5 different organs (kidney, liver, intestine, blood, and bone marrow) to control erythropoiesis. Additionally, considering the origin and residence at MA of the adolescents participating in the study, these hematological differences can also be attributed in part to the fact that the phenotype of physiological responses to hypoxia of residents of the Andean region exhibits an altitude-dependent pattern of Hb increase.69 Thus, the homeostatic mechanisms triggered by HIF-1 signaling compensate for the reduced availability of oxygen with an increase in Hbt, which is considered one of the main reasons why the natives of high-altitude areas have better sports performance at altitude.7 Considering that the maximum performance, expressed as VO2peak, depends on the oxygen consumption in muscle and oxygen transport in the blood, the results shown in Figs. 2 and 3C would explain the higher VO2peak found in adolescent MA athletes (Fig. 4A). These findings are consistent with those obtained by Saunders et al.61 in adult men and women. These authors found, in an extensive study involving 145 elite endurance athletes from different disciplines and various forms of altitude training (including LH-TH), a strong cross-sectional relationship between Hbt and VO2max, indicating that exposure to moderate altitude is effective in increasing VO2max and Hbt by approximately 3%. Our data show greater increases (ranging from 9% to 23%), presumably demonstrating the higher impact of long-term MA residence. These findings could be especially relevant since they indicate that, like at sea level, the erythrocyte-related parameters are the determining factors for endurance performance in adolescent elite athletes at MA. This conclusion derives some support from the fact that the limiting factor for exercise performance in trained subjects is the oxygen transport system.31

Fig. 4 also shows consistently lower %VT1 and lower HRmax in female skaters and male runners which could seem not to correspond with the reported VO2peak increases. Maybe this reduction in %VT1 could be attributed to a higher sympathetic activity triggered by the hypoxic exposure which subsequently increases the ventilator drive.62 The RQ did not have significant differences between the MA and BA groups (Fig. 4C) indicating that both groups behaved similarly to the strenuous exercise imposed by the incremental test. The intensity of exercise at treadmill or cycle ergometer loads when VO2peak is reached, generates the release of lactic acid into the blood from glycolytic activity of muscle cells, which leads to an increase in exhaled CO2 through hyperventilation as a buffering response. This well-known behavior at sea level (see for example, Seidenberg et al.63) has also been described in both acute and chronic hypoxia conditions.64

4.3. Effect of sports discipline in male athletes

In male adolescents residing at the same altitude, sports discipline did not influence the behavior of hematological variables (Fig. 2), although a slight decrease in BV and PV (Fig. 3A and B) and a significant decrease in EV (Fig. 3C) was evident in runners when compared with skaters. The results of the present study are coincident with the findings of Malczewska-Lenczowska et al.65 in young adults, who reported very similar Hbt levels both in males and females trained for endurance sports such as running, cycling and cross-country skiing. Similarly, Heinicke et al.31 found no significant differences either in Hbt or EV in elite cyclists, runners and triathletes. Thus, our results indicate that, despite the minor influence of endurance training on Hbt, EV and BV levels before puberty,66 in pubertal subjects the hematological variables behave physiologically the same as in adult individuals when faced with the hypoxic stimulus and in relation to sports performance.

When analyzing the values obtained from the incremental tests (Fig. 4), differences between sports disciplines are scarce and only significant in some comparisons. Runners showed lower VO2peak values at MA (Fig. 4A) and higher HRmax at LA (Fig. 4B) than skaters and cyclists. These differences (significant only when compared with skaters) could be a result of the different characteristics of the incremental tests performed, since runners did them on a treadmill and skaters and cyclists on a cycle ergometer (Fig. 1). A more consistent difference between sport disciplines was evident in RQ, the cyclists having higher values than skaters and runners at both altitudes (Fig. 4C). This could be a consequence of the physiological differences between running and cycling.67 For example, Scott et al.68 described, during a bout of the same intensity in running and cycling, similar total energy expenditure but with differences...
in the extent of aerobic and anaerobic energy transfer, with a greater relative contribution of the glycolytic component in cycling. A higher glycolytic metabolic contribution would increase the RQ, as is shown in our results.

4.4. Limitations of the study

The sample size of the subject's categories in which the study was divided ranged from n = 8 to n = 21. Thus, in some comparisons, the low sample size is a main limitation of the study. However, the low SD in age, sex, and anthropometric parameters and the similar age ranges analyzed in the different categories (Table 1) could minimize this limitation, which is the consequence of the difficulty in finding elite sport adolescent practitioners training and living at different altitudes.

5. Conclusion

Male elite adolescent skaters in the Tanner III and IV stage of maturation showed higher values in blood oxygen-related transport parameters than females, both at low and moderate altitude, indicating that sex differences in erythropoiesis have already appeared at this maturation stage. Male adolescent moderate altitude residents (2640 m) engaged in elite skating, running and cycling had an increased erythropoietic response, resulting in a higher VO2peak, compared to subjects from the same disciplines who reside and train at low altitude (966 m). These findings agree with those reported in the scientific literature for adult elite athletes leading to the conclusion that hypoxia and aerobic training elicits almost the same responses in subjects in Tanner III and IV stages of maturation as it does in adults. Moreover, these responses are similar in low and moderate altitude residents involved in the three aerobic sports studied confirming that the variables described are highly sensitive to the effect of altitude hypoxia irrespective of the sports discipline.

Authorship

Conception and design of study: E.M. Mancera-Soto, E. Cristancho; acquisition of data: E.M. Mancera-Soto, M.L. Chamorro-Acosta; D.M. Ramos-Caballero; analysis and/or interpretation of data: M.L. Chamorro-Acosta, J.R. Torrella. Drafting the manuscript: M.L. Chamorro-Acosta, J.R. Torrella.; revising the manuscript critically for important intellectual content: E.M. Mancera-Soto, E. Cristancho, D.M. Ramos-Caballero. Approval of the version of the manuscript to be published (the names of all authors must be listed): E.M. Mancera-Soto, M.L. Chamorro-Acosta, D.M. Ramos-Caballero, J.R. Torrella, E. Cristancho.

Declaration of competing interest

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