Hematological and Ventilatory Responses to a 3900 M Altitude Sojourn in an Elite Wheelchair-marathoner

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ABSTRACT

This case study aimed to report blood markers and resting respiratory rate (RR) oscillations at sea level, during a 5-wk 3900 m altitude sojourn, and after returning to sea level in a 36-year-old professional wheelchair marathoner. Outcome measures plasma erythropoietin (EPO) concentration, hemoglobin, reticulocytes count, erythrocytes and hematocrit as well as RR were measured upon wakening 7-weeks pre-altitude, 7-days pre-altitude, 35 hours after arrival to altitude, on days 8, 15, 21, 28 and 35 at altitude, 6 and 16 days after returning to sea level. EPO increased up to 259 % (31.6 U l⁻¹) 35 hours upon arrival at altitude and decreased below pre-altitude level (12.2 U l⁻¹) on the 21st day of the camp (8.7 U l⁻¹), reaching the lowest values 16 days after returning from altitude (1.9 U l⁻¹). All blood parameters, except for reticulocytes, increased (range: +17.9 to +23.8%) after 35 days of altitude exposure. Compared to pre-altitude, RR increased during the first week of exposure to hypoxic conditions and remained elevated throughout the camp until the fifth week (5.1±0.4 vs. 9.1±1.6 and 6.6±0.8 breaths min⁻¹; Cohen’s d = +3.4 and +2.4, respectively). A 5-wk high-altitude sojourn triggered polycythemia and elevations in RR (as indicators of effective hypoxic acclimatization) in a professional wheelchair-marathoner.

Keywords: altitude training, EPO, erythropoiesis, marathon, ventilatory response.

I. INTRODUCTION

At a terrestrial altitude of 4000 m, larger increases in red cell volume (RCV) occur after a 4-week stay compared to shorter sojourns (Rasmussen et al., 2013). Moreover, at moderate altitude (1800-2500 m) hemoglobin (Hb) concentration gains are of 1% week⁻¹ (Berglund, 1992), while RCV increases in a range of 5 to 9% (Levine & Stray-Gundersen, 1997). In addition, plasma erythropoietin (EPO) concentration generally expands after only 2 h upon hypoxic exposure and generally continues for up to 3-4 days (Eckardt et al., 1989), with values resuming to baseline 1-4 weeks after removal of the hypoxic stress (Chapman et al., 2014).

To date, only one study has analyzed the acute effect of 4-week ~4000 m altitude exposure on performance in a cohort of middle-distance high school runners (Buskirk et al., 1987). These authors observed a 20-24% impairment of performance, in distances ranging from 1 to 2 miles by the camp completion. Additionally, Chapman et al. (2014) showed a worsened 3000-m time trial performance after returning to sea level in high school runners who lived and trained 4-week at 2800 m, likely explained by a ventilatory de-acclimatization despite a ~7% increase in RCV. Moreover, after 17 days of training at 3100 m altitude, middle-distance collegiate runners increased their maximal oxygen uptake (VO₂max) by a 5 % and extended (24.1%) their time to exhaustion in constant speed treadmill test (Dill & Adams, 1971).

To consider, the observations with based-endurance athletes training at > 3000 m elevation are scarce in the literature and were done with able-bodied participants (Buskirk et al., 1967; Dill & Adams, 1971),...
however, data from a professional wheelchair-marathoner, after carrying out a 5-week Live High-Train High (LHTH) ~4000 m altitude sojourn highlighted: i) a minimal perturbation (~3%) in 2000 m interval repetitions done at 4090 m elevation compared to sea level, ii) a 3.4% improvement in 3000 m performance after returning to sea level, and iii) an enhancement of the power output by 13.6% after the training camp (Sanz-Quinto et al., 2019). Unfortunately, physiological mechanisms triggering these performance gains after returning to sea level remain unclear.

Additionally, and considering the remote scenarios at altitude, and the practicability of monitoring and tracking several physiological variables for coaches and physicians, the use of smart T-shirts has been reported as a reliable tool to assess respiratory rate (RR) among elite triathletes (Saugy et al., 2014). In fact, the assessment of resting RR may be useful to determine if athletes cope well with the chronic stress of high-altitude (~4000 m) exposure (i.e., ventilatory sensitivity).

Therefore, and considering the more and more professionalism among Paralympic wheelchair endurance athletes (e.g., Tokyo Paralympics huge media impact), the aim of this study was to analyze changes in red blood cell parameters and resting RR as markers of altitude acclimatization, in response to a 5-week LHTH altitude training camp (3900–4100 m) in a professional wheelchair marathoner, which can elucidate valuable data for coaches and physicians engaged to this athletics population.

II. MATERIAL AND METHODS

A. Participant

One male professional wheelchair marathoner with upper limb affection (i.e., tetraparesia), diagnosed with the most common neuromuscular disease “Charcot Marie Tooth” (Banchs et al., 2009), IPC-Athletics class T52 (Individuals with quadriplegia-tetraparesia), holding thirteen world records, took part in the study. The Participant main features were: age, 36 yrs; body-height, 176 cm; body-mass, 52.6 kg; VO$_{2\text{max}}$, 52 ml kg$^{-1}$ min$^{-1}$. The participant provided written informed consent to be a research subject in this case study. Research conformed the Declaration of Helsinki, being approved by the Ethics Research Committee of the University (project identification code #DPS.MMR.02.15).

B. Design

Repeated observations were made on a single athlete during:
1) 6-weeks leading phase at sea level (16 m altitude), including 74 training sessions and the completion of 1280 km;
2) pre-altitude week (W$_{1}$);
3) 5-week altitude camp in Puno, Peru (3900–4100 m above sea level) (W$_{1}$–5);
4) post-altitude week (W$_{1}$) (Fig. 1). The details of training have been previously published (Sanz-Quinto et al., 2019).

| Phase     | Lead-in       | W$_{1}$ | Specific Training | W$_{1}$ |
|-----------|---------------|---------|-------------------|---------|
| Timing    | 6 w           | 1d      |                   | 7d      |
| Sessions  | 74            | 6       |                   | 6       |

Fig. 1. Structure of experimental design.

C. Measurements and Data Collection Procedures

Blood tests were conducted at 8 am, under fasting conditions, after a day of rest. Blood was withdrawn: i) the first day of season or 7-weeks before the altitude camp (T$_{–7\text{ weeks}}$); ii) 7 days before the altitude camp (T$_{7\text{ days}}$); iii) during the altitude camp on days eight, fifteen, twenty-one, twenty-eight and thirty-five (T$_{8,15,21,28,35\text{ days}}$); iv) 6 days after returning from altitude (T$_{6\text{ days}}$). During first week at altitude (days 1 to
In order to avoid hemoconcentration values, no blood markers assessment was planned, except for Plasma erythropoietin (EPO), due to plasma volume contraction occurred during early acclimatization (Siebenmann et al., 2017). EPO was assessed: i) 7 days before the altitude camp (T-7 days); ii) 35 hours upon altitude arrival (T35h); iii) on the 21st day at the altitude camp (T21 days); iv) 16 days after returning from altitude (T16d). Hb and erythrocytes count were measured with a Coulter T840 Counter (Coulter Electronics, Krefeld, Germany). Hematocrit (Hct) was determined with microcentrifugation. Reticulocytes (Ret) were measured with flow cytometry (Epics XL, Beckman). For serum EPO 7 mL blood sample was centrifuged (3000 rpm, 10 min, 4 degrees Celsius) and measured (Erythropoietin ELISA, IBL, Hamburg, Germany). Ferritin was measured photometrically (EIAgen Ferritin Kit, Adaltis, Freiburg, Germany).

Moreover, urine specific gravity was measured at 7:30 am (Mission® U500, ACON Laboratories, San Diego, California), as a way to verify if blood test values were influenced by hemoconcentration/dilution. In fact, urine specific gravity was maintained inside normal range (≤1.20) (Stover et al., 2006) throughout the altitude sojourn (1.014±0.006 to 1.019±0.002).

D. Breathing Frequency Assessment

A Hexoskin wearable body metrics shirt (Hexoskin, Carré Technologies Inc. San Francisco, CA, USA), which was found to be valid and reliable (Tanner et al., 2015), was used for assessing resting RR. Measurements were obtained in supine position during 5 min after awaking, with the average value for the last 60 s retained for final analysis.

E. Iron Supplementation

The athlete ensured iron deposits through the daily intake of 105 mg of ferrous sulphate (FeSO4) (Ferogradumet®, Ross, Abbott Científica) 30 min before breakfast (Constantini et al., 2017). Oral supplementation commenced from the starting point of the season (7-wk pre-altitude) as previously recommended by Stellingwerff et al. (2019). In addition, an individualized nutritional program supported optimal energy balance to the athlete throughout the altitude sojourn (Sanz-Quinto et al., 2019).

F. Statistics

Blood parameters and ferritin data are presented as raw values (Table I), while RR data are presented as mean ± SD (Table II). ΔEPO, Aerythrocytes, ΔRet, ΔHb, ΔHct and Δferritin were calculated with reference to T-7 days, which was assigned the value of 100%. Repeated-measures analysis of variance (ANOVA) (time) were carried out. Effect size (d) associated with change in RR was calculated using Cohen’s d and was interpreted as trivial (≤0.19), small (0.20-0.49), medium (0.50-0.79), and large (≥0.80) (Hopkins et al., 2009). An alpha level of 0.05 was stated for statistical significance. Statistical analyses were performed using the SPSS version 22.0 (SPSS, Inc., Chicago, IL, USA) software and Statgraphics (STSC, Inc., Rockville, MD, USA) version 16.1.17.

III. RESULTS

From T-7 days to T35 days, erythrocytes, Hb and hematocrit (Hct) increased by 17.9%, 21.2% and 23.8%, respectively. Moreover, reticulocytes (Ret) decreased by 38.3% from T-7 days to T35 days, and by 64.7% from T-7 days to T6 days. At T35 days, Erythrocytes, Hb and Hct were enhanced by 8.0%, 8.8% and 12.1%, respectively compared to T-7 days. Moreover, EPO increased by 259.0% at T35 days, while lower values were observed at T21 days (-28.7%) and T16 days (-84.4%) compared to T-7 days. In addition, compared to T-7 days, ferritin values were 55.5% lower at T8 days (247 vs. 110 ng mL⁻¹) but 39.3% lower (150 ng mL⁻¹) at T35 days and they remained 12.2% lower at T16 days (Table I).

### Table I: Blood Markers Pre-Altitude during the Sojourn and After Returning to Sea Level

|       | T-7 days | T35 days | T21 days | T28 days | T35d | T16d |
|-------|----------|----------|----------|----------|------|------|
| Ery   | 5.04     | 4.62     | 5.06     | 5.17     | 5.04 | 5.28 |
| %     | 100      | 100      | 100      | 100      | 100  | 100  |
| Hb    | 14.3     | 13.7     | 15.3     | 15.6     | 15.3 | 16.1 |
| %     | 100      | 100      | 100      | 100      | 100  | 100  |
| Δ%    | +9.5     | +11.9    | +9.1     | +14.3    | +17.9| +8.0 |
| Ret   | 12.4     | 16.7     | 15.0     | 12.6     | 12.4 | 12.0 |
| %     | 100      | 100      | 100      | 100      | 100  | 100  |
| Δ%    | -10.2    | -22.6    | -25.8    | -28.2    | -38.3| -64.7 |
| Hct   | 42.3     | 40.4     | 46.0     | 47.0     | 46.0 | 48.0 |
| %     | 100      | 100      | 100      | 100      | 100  | 100  |
| Δ%    | +13.9    | +16.3    | +13.9    | +18.8    | +23.8| +12.1 |
| EPO   | 284      | 247      | 110      | 126      | 132  | 122  |
| %     | 100      | 100      | 100      | 100      | 100  | 100  |
| Δ%    | -55.5    | -49.9    | -46.6    | -50.6    | -39.3| -12.2 |

T-7 days, blood test first day of season or seven weeks before the camp; T-35 days, blood test six weeks after starting season or one week before the camp; T35 days, EPO test thirty-five hours after arriving to altitude; T21, T28, T35 days 8, 15, 21, 28, and 35; T16d, blood test six days after returning from altitude; T35d, EPO test sixteen days after returning from altitude; Ery, erythrocytes ×10⁶

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Compared to sea level, resting RR was higher during the 5-week altitude training camp (pooled values; 7.7±1.8 vs. 5.3±0.6 breaths min⁻¹). The RR increased at W₁ (9.1±1.6 breaths min⁻¹, d = -3.4) and W₂ (9.3±2.1 breaths min⁻¹, d =-2.8) compared to W₄ (5.1±0.4 breaths min⁻¹). Resting RR decreased throughout the camp from W₃ onwards, yet values remained significantly higher at any altitude time point when compared to sea level (Table II, Fig. 2.).

Table II: Resting Respiratory Rate (RR) Pre-, During and Post-altitude

| RR | W₁ | W₂ | W₃ | W₄ | W₅ | W₆ |
|----|----|----|----|----|----|----|
| Cohen’s d | 5.1±0.4 | 9.1±1.6 | 9.3±2.1 | 7.0±0.8 | 6.4±0.8 | 6.6±0.8 | 5.5±0.8 |

W₁, pre-altitude week; W₂, W₃, W₄, W₅, Weeks at altitude; W₆, post-altitude week; RR, respiratory rate upon waking in resting conditions expressed as breaths min⁻¹.

Differences from W₁ (p<0.001); Differences from W₂ (p<0.001); Differences from W₃ (p<0.001); Differences from W₄ (p<0.001); Differences from W₅ (p<0.001); Differences from W₆ (p<0.001); Cohen’s d, difference in mean scores over time divided by pooled SD in reference to the previous time point.

IV. DISCUSSION

This case study of a professional wheelchair marathoner reports the effects of a 5-week LHTH sojourn at 3900-4100 m altitude on red blood cell parameters and resting RR. Our novel findings are: 1) this intervention induced polycythemia; 2) erythrocytes, Hb and Hct values peaked towards the end of the camp; 3) resting RR increased immediately when the athlete was exposed to altitude compared to pre-altitude, and it remained elevated throughout the camp.

It has been previously observed in untrained individuals that 6-weeks training at sea level prior to an altitude sojourn increased Ret (+25.8%) (Schmidt et al, 1991). This goes in line with our observations from the 6-week leading phase (+34.7%). However, the Ret oscillations after a high-altitude sojourn remain unclear with recently published research, pointing out to a possible Ret count decrease after descending from a 19-day sojourn at 3450 m altitude elevation (Klein et al., 2021), which goes in line with our findings (-64.7% at Tₑ₀), in contrast with opposite findings (Rice et al., 2001). Therefore, more research is needed on Ret response after descending to sea level, and its role on enhancing endurance-athletes VO₂max.

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In contrast with observations among elite biathletes living and training at 2050 m altitude for 16 days, in which no changes in EPO from pre- to post-intervention were observed (Heinicke et al., 2005). In this study, the EPO suppression observed at \( T_{21 \text{ days}} (-29\%) \) and \( T_{4 \text{ days}} (-84\%) \) might explain the 8.5\% decrease in erythrocyte values from \( T_{5 \text{ days}} \) to \( T_{6 \text{ days}} \). Likewise, EPO suppression might explain the 38.3\% Ret decrease at altitude. Furthermore, the decrease in erythrocytes, Hb and Hct observed at \( W_1 \) compared to \( W_2 \) could be the consequence of an excessive number of high physiological demanding sessions completed during \( W_2 \) (Sanz-Quinto et al., 2019). In fact, and in line with our observations, a 4.6\% descent in Hb after an intense mesocycle compared to a greater mesocycle volume was observed in a group of elite cyclists (Zapico et al., 2007).

Contrastingly, in a study with elite athletes, training and residing at moderate altitude (2100 m), who combined low intensity sessions with a low number of intense sessions (5) during a 3-week LHTH sojourn, failed to elicit sufficiently severe hypoxemia levels (-96\%) to trigger any change in Hb and Hct. Furthermore, a decreasing trend (\( \Delta -1.8\% \)) in erythrocytes was reported compared to the first day of altitude exposure (Sperlich et al., 2016). This lead us to think that both, enough elevation and a minimum of high physiological demanding sessions are required to stimulate polycythemia. Regarding the magnitude of the elevation, a recently published study conducted with 52 athletes and 50 sedentary inhabitants from two towns of Ethiopia (Guna, 3100 m elevation and Addis Ababa, 2400 m elevation), reported significant differences in the mean hemoglobin, being 6.2\% greater among male runners residing and training at the highest town (17.47 vs. 16.39 g dl\(^{-1}\)). However, no differences were observed in the erythrocytes (5.58±0.3 vs. 5.56 ± 0.3×10\(^6\) mm\(^3\)) (Zelalem Tilahun et al., 2021). Therefore, this study could help us to understand the greater oscillations from pre-altitude to post-altitude in Hb compared to erythrocytes, observed in our study participant.

The greater Hb and erythrocyte levels at \( W_4 \) and \( W_5 \) compared to \( W_{1,2,3} \), are in line with previous meta-analysis results showing that peak blood parameters normally occur by the fourth week of altitude exposure (Rasmussen et al., 2013). Furthermore, greater Hb, erythrocytes and Hct values observed one week after returning to sea level confirm findings from previous LHTH studies conducted at 2050 m altitude (Heinicke et al., 2005).

In this study, the magnitude of decrement in ferritin observed at altitude exceeded that of a previous study (-56 vs. -15\%) with elite biathletes (Heinicke et al., 2005). However, the differences in altitude elevation (3900 vs. 2050 m) could partly explain this discrepant finding. Therefore, amounts greater than 105 mg per day of ferrous sulphate should be prescribed to athletes residing and training at ~ 4000 m, practice which is encouraged even for athletes training at moderate altitude (Constantini et al., 2017; Stellingwerff et al., 2019).

Noteworthy, decrease in resting RR at \( W_3 \) occurred concomitantly with 12-14\% increases in erythrocytes and Hb in reference to \( W_1 \), highlighting the negative relation between RR and hematological adaptations. In fact, hyperventilation helps to cope with exercise under extreme hypoxic environments (Schoene et al., 1984), facilitating the oxygen diffusion from the alveoli to the capillaries, and it is also related with a positive early acclimatization to high altitude exposure (Hackett et al., 1982). Therefore, the increase in RR found in our athlete is an homeostatic regulation in an attempt to preserve arterial oxygen saturation levels.

Finally, potential explanations for performance increments (3000 m time trial and power output) observed in our wheelchair athlete after returning to sea level (Sanz-Quinto et al., 2019) are: 1) a slower decay in hematological adaptations resulting from chronic hypoxic exposure after returning to sea level (Constantini et al., 2017), 2) a faster ventilatory acclimatization (Chapman et al., 2014) during exercise after returning to sea level compared to able-bodied runners and, 3) a lower impairment of the neuromuscular function once resuming training at sea level (Constantini et al., 2014)), 4) Our participant completed more than 10 training camps in the Breckenridge-Keystone area (~2900 m, Colorado, USA) before the Peruvian Altiplano training camp, while athletes from Buskirk et al., (1967) had no previous experience at this elevation. In support of the third assumption, the improvement in the 3000 m trial has been associated with a greater mechanical efficiency at a submaximal speed in this athlete (Brizuela Costa et al., 2009), indicating that neuromuscular function was probably not impaired after the altitude sojourn.

The main limitation of this study was the non-use of the CO-rebreathing technique, previously described by Schmidt & Prommer (2005), thus not allowing us to determine the total haemoglobin mass and hemodynamic parameters as blood volume and plasma volume. In order to minimize an hemococoncentration / dilution status, we assessed each morning urine specific gravity, which was maintained inside normal range (≤ 1.20) (Stover et al., 2006) throughout the altitude sojourn (1.014±0.006 to 1.019±0.002) (Sanz-Quinto et al., in press).
V. CONCLUSION

Residing and training at ~4000 m terrestrial altitude was a practical tool for a wheelchair marathoner who has huge experience of training and residing at the greater threshold of moderate altitude, in order to increase his VO₂max in preparation for competitions, as a result of the induced greater polycythemia triggered at higher elevations. Furthermore, resting RR assessment could be added to the athlete’s toolbox to confirm that positive adaptations to altitude occur, especially during the acute acclimatization phase. Interestingly, this elevation might be beneficial for enhancing performance after returning to sea level among wheelchair marathoners, in contrast with the ~2500 m threshold proposed for able bodied runners. Nevertheless, more research is needed, and this hypothesis will have to be tested in future studies with a greater sample size.

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CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

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