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DOCTORAL THESIS

Santiago J. Sanz Quinto

Universidad Miguel Hernández de Elche
Programa de Doctorado en Deporte y Salud
Directores: D. Manuel Moya Ramón
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La presente tesis doctoral es un compendio de cuatro artículos previamente publicados:

- Sanz-Quinto S, López-Grueso R, Brizuela G, Flatt AA, Moya-Ramón M. Influence of Training Models at 3,900-m Altitude on the Physiological Response and Performance of a Professional Wheelchair Athlete: A Case Study. J Strength Cond Res. 2019;33(6):1714-1722. DOI: 10.1519/JSC.0000000000002667.
- Santiago Sanz-Quinto, Gabriel Brizuela, Raúl López-Grueso, Ian Rice, Manuel Moya-Ramón. Influence of training load on mood disturbance at sea level and 3900 m altitude: A case study of a wheelchair athlete. Sports (Basel). 2018;6(4). DOI: 10.3390/sports6040122.
- Sanz-Quinto S, Brizuela G, López-Grueso R, Flatt AA, Aracil-Marco A, Reina R, Moya-Ramón M. Monitoring heart rate variability before and after a marathon in an elite wheelchair athlete: A case study. J Sports Sci Med. 2018;17(4):557-562.
- Santiago Sanz-Quinto, Manuel Moya-Ramón, Gabriel Brizuela, Ian Rice, Tomás Urbán, Raúl López-Grueso. Nutritional strategies in an elite wheelchair marathoner at 3900m altitude: a case report. J Int Soc Sports Nutr. 2019;16(51):1714-1722. DOI:10.1186/s12970-019-0321-8.

Además, se incluyen otros dos trabajos en proceso de revisión:

- Santiago Sanz-Quinto, Girard O, López-Grueso R, Brizuela G, Moya-Ramón M. Hematological and ventilatory responses to 3860 m altitude training in a wheelchair marathoner. Under review in Int J Sports Physiol Perform.

- Santiago Sanz-Quinto, Manuel Moya-Ramón, Gabriel Brizuela, Ian Rice, Raúl López-Grueso. Hydratus status assessment in an elite wheelchair marathoner training at 3900 m altitude: a case study. Under review in J Sport Med Phys Fit.

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Doctoral Thesis

A dissertation presented by

Santiago J. Sanz Quinto

Elche, 2020

El Dr. Manuel Moya Ramón, profesor Contratado Doctor en la Universidad Miguel Hernández de Elche, hace constar que el trabajo de investigación titulado "THE EFFECT OF A *LIVE HIGH – TRAIN HIGH* SOJOURN AT 3,060-4,090M TERRESTRIAL ALTITUDE ON PHYSIOLOGICAL, PSYCHOLOGICAL, BODY COMPOSITION, AND PERFORMANCE VARIABLES IN A PROFESSIONAL WHEELCHAIR MARATHONER" realizado por el doctorando Santiago José Sanz Quinto, ha sido supervisado bajo su dirección y autorizado para su depósito y posterior defensa como Tesis Doctoral en esta Universidad ante el tribunal correspondiente.

Lo que firmo para los efectos oportunos en:

Elche, 10 de febrero de 2020

Fdo.: Manuel Moya Ramón

Universidad Miguel Hernández de Elche

El Dr. D. Francisco Javier Moreno Hernández, coordinador del programa de doctorado en Deporte y Salud de la Universidad Miguel Hernández de Elche.

AUTORIZA

Que el trabajo de investigación titulado: "The effect of a *live high – train high* sojourn at 3,860-4,090M terrestrial altitude on physiological, psychological, body composition, and performance variables in a professional wheelchair marathoner" realizado por D. Santiago José Sanz Quinto, bajo la dirección del Dr. D. Manuel Moya Ramón, sea depositado y posteriormente defendido como Tesis Doctoral en esta Universidad ante el tribunal correspondiente.

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Tesis Doctoral presentada por:

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Dirigida por el Dr. D. Manuel Moya Ramón

El director **El doctorando**

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List of abbreviations

A session (8km <VT1 + technique drills + 5 x 80 m strides + 20 x 400 m VT2 Recovery 75 s + 2 km <V1)

AMS (acute mountain sickness)

AM SG (urine specific gravity upon awaking)

ANOVA (analysis of variance)

B session (2 hours at aerobic threshold)

BF (breathing frequency)

B_H (baseline in hypoxia or first week at altitude)

B_N (baseline in normoxia or pre-altitude week)

C session (8 km <VT1 + technique drills + 5 x 80 m strides + 6 x 2000 m VT2 Recovery 120 s + 2 km <VT1)

CHO (carbohydrate)

CL (confidence limits)

CV (coefficient of variation)

DP (diastolic blood pressure)

EPO (plasma erythropoietin)

FiO_2 (inspired oxygen fraction)

Fluid (daily total fluid intake)

Fluid Vol.1 (fluid intake for the morning session)

Fluid Vol.2 (fluid intake for the afternoon session)

FO (functional overreaching)

FP (flexible planning)

GI (gastrointestinal)

HB (hydric balance)

Hb (hemoglobin concentration)

Hb_{mass} (hemoglobin mass)

Hb_{mass} (hemoglobin mass)

Hct (hematocrit)

HF (High frequency 0.15-0.40 Hz)

HR (heart rate)

HRR (heart rate recovery)

HR_{rest} (resting heart rate)

HRV (heart rate variability)

HVR (hypoxic ventilatory response)

Hypoxia inducible factor-1 (H1F-1)

IP (inflexible planning)

LF (low frequency 0.04-0.15 Hz)

LF/HF (ratio LF to HF)

LHTH (live high – train high)

LHTL (live high – train low)

LL (leftover liquid)

MAP (mean arterial pressure)

Na^+ (sodium)

NFO (non-functional overreaching)

NN (successive normal sinus)

OTS (overtraining syndrome)

PM SG (urine specific gravity assessed two hours after dinner)

pNN50 (The mean number of times an hour in which the change in NN intervals exceeds 50ms)

POMS (profile of mood states questionnaire)

Post (week after returning from altitude)

$Post_{11}$ (11 days after returning from altitude)

$Post_{12}$ (12 days after returning from altitude)

Pre_{-3} (3 days before altitude exposure)

Pre_{-4} (4 days before altitude exposure)

RBC (red blood cells)

RCV (red cell volume)

$RD_{-6,-5,-4,-3,-2,-1}$ (previous days to race day)

$RD_{+1,+2}$ (following days to race day)

Ret (reticulocytes)

RMR (resting metabolic rate)

rMSSD (the square root of the mean squared differences of successive R to R intervals)

RPE (ratings of perceived exertion)

R-R (successive heart beats)

RV (reference value)

SDNN (standard deviation of R-R)

SG (urine specific gravity)

SL (sea level)

SP (systolic blood pressure)

SWC (the smallest worthwhile change

TMD (total mood disturbance)

TL (training load)

T_{-7days} (7 days before altitude sojourn)

$T_{-7weeks}$ (7 weeks before altitude sojourn)

$T_{8,15,21,28,35\ days}$ (altitude camp on days eight, fifteen, twenty-one, twenty-eight and thirty-five)

$T_{+6\ days}$ (6 days after returning from altitude)

$T_{+16\ days}$ (16 days after returning from altitude)

V_E (ventilation)

VLF (very low frequency (0.00-0.04 Hz)

VO_2 (oxygen uptake)

VO_{2max} (maximal oxygen uptake)

VS (volume suggested)

VT1 (first ventilatory threshold)

$W_{1,2,3,4}$ (weeks of specific training at altitude)

W1 to W13 (weeks regarding psychological assessment study)

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Abstract

Background: Since the late 60´s, altitude training has been a common method used among marathoners and middle/long distance athletes to enhance their maximal oxygen uptake (VO_{2max}) by the concomitant increase in red cell volume (RCV) and hemoglobin mass (Hb_{mass}) observed after returning from altitude. However, due to the performance impairment in the specific training sessions (i.e., speed maintained at the second ventilatory threshold intensity) observed at altitude relative to sea level (SL), which becomes worse as altitude increases, there are no studies in the literature, unless except one published 52 years ago, in which athletes were exposed to 4000 m terrestrial altitude. Moreover, no studies about altitude training in Paralympic Sport have been published. Considering this, the first aim of this research was to analyze the physiological and psychological response and, performance effects of a 5-week 3860-4090 m terrestrial altitude training camp in an elite-wheelchair athlete marathoner with Charcot Marie Tooth disease (CMT), previous to his participation in the Boston and London Marathons (studies 1 and 2).

On the other hand, and considering both altitude sojourns were programmed as preparation scenarios for the London and Boston Marathons, and also considering the practical application for coaches and physicians in charge of wheelchair athletes performance optimization, the second aim of this research was: 1) to analyze the autonomic nervous system response assessed non-invasively as cardiac autonomic vagal response oscillation with heart rate variability (HRV) assessment before, during and, after an international wheelchair marathon, comparing data to existing literature from able-bodied triathletes, athletes and XC skiers 2) to verify if HRV response after

relocation in a new time-zone is similar to able-bodied athletes 3) to compare the cardiovascular response of a world-class wheelchair marathoner with world-class paraplegics and able-bodied marathoners (study 3) in the same athlete.

Methods: A professional wheelchair athlete diagnosed with CMT, featured by an affection in both, upper and lower limbs, completed two 5-week training camps at 3860-4090 m terrestrial altitude in two consecutive years. In the first camp, named inflexible planning (IP), the athlete completed every predefined training session and the average weekly distance covered was 200 km. In the second camp, named flexible planning (FP), specific sessions were performed depending on a reference value (RV) of his HRV, but the weekly distance covered was less (140 km). Vagally-mediated-HRV markers as the root mean square of the successive differences (rMSSD), oxygen saturation (SO_2) and resting heart rate (HR_{rest}) fluctuations during normoxia and hypoxia conditions were studied, comparing differences between FP and IP. A pre- and post-altitude performance assessment was carried out in IP and FP with an incremental test in which power output and also a 3000 m time trial were estimated. In addition, the assessment of the following outcomes were carried out in FP (pre-altitude, during altitude sojourn, and post-altitude): systolic blood pressure (SP), diastolic blood pressure (DP), plasma erythropoietin (EPO), erythrocytes, reticulocytes count (Ret), hemoglobin (Hb), hematocrit (Hct), resting breathing rate (BF), profile of mood states, body mass, specific urine gravity upon wakening (SG AM) and pre-bed (SG PM), diuresis, daily Fluid intake, hydric balance (HB) after each training session, overall energy intake, overall carbohydrates (CHO), proteins and fat intake and daily sodium (Na^+) intake.

Secondly, and regarding the marathon study, ten days before the marathon day (RD_{-10}) an incremental ergometer test was performed to estimate the

second ventilatory threshold (VT2) and the heart rate (HR) plus oxygen uptake at VT2 intensity. Six days before the marathon (RD_{-6}), and two days after racing (RD_{+2}), the day-to- day HRV upon awakening was measured and rMSSD was chosen as cardiac vagal autonomic control indicator. The logarithmic expression of the rMSSD (Ln rMSSD) was averaged across all days pre-travel (RD_{-6} – RD_{-2}) to serve as baseline (BL). During the marathon, HR was monitored with a HR monitor, pooled every 5000 m (0-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30- 35, 35-40) and the last 2195 m (40-42.2).

Results: A greater suppression, related to greater fatigue in vagally-mediated markers throughout altitude sojourn was observed by a greater coefficient of variation of the rMSSD (rMSSDCV) in IP compared to FP; in addition, FP showed faster restoration of rMSSD upon returning to SL. Moreover, after a significant decrease observed in SO_2 in both models upon altitude arrival, there was a faster enhancement in SO_2 in FP compared to IP in different time points of the sojourn, showing faster acclimatization in FP. HR_{rest} increased at altitude in both models, showing a slight reduction after returning from altitude compared to pre-altitude. However, no within-models analysis differences were observed. Moreover, SP and DP were enhanced at altitude and went back to pre-altitude values after returning to SL. Both models facilitate an increase in the power output generated by the athlete, and also improve the 3000 m time trial, which it was slightly better after FP. Regarding blood marker results in FP, an increase in erythrocytes, Hb and Hct. was osbserved towards the end of the camp, which was even greater than at pre-altitude after arriving to SL. An increase over 200 % was observed in EPO 35-hours after arriving to altitude. However, a suppression in EPO was observed by mid-sojourn and it reached the lowest values after returning to SL. BF was enhanced at altitude compared to SL and a slight decrease was observed as hypoxic exposure became chronic. A decrease in

vigor, and an increase in fatigue were observed at altitude compared to SL when SO_2 and SP were considered as covariates. Moreover, total mood disturbance (TMD) increased significantly when the greatest training load (TL) was reached at SL, while it increased throughout altitude exposure, remaining high at post-altitude compared to pre-altitude. A decrease in body mass was observed after arriving to altitude. However, a significant increase was observed from the second to the fifth week of exposure. Moreover, body mass returned to within pre-altitude values after returning from altitude. Athlete´s CHO and protein intake was significantly greater at altitude compared to SL, while no differences among lipids intake was observed among conditions. Both fluid intake and diuresis were enhanced at altitude, especially during early acclimatization. In fact, lower SG AM was observed during the first week at altitude relative to pre-altitude. SG PM, while not significantly, showed a lower trend than SG AM, and both were inside the range suggested for optimal hydration status ($\leq$ 1.020). Sodium intake throughout the camp was inside the normal range suggested as optimal (1300 to 2500 mg $\cdot$ d^{-1}); however, Na^{+} didn´t reach the amounts recommended (0.5 to 0.7 g $\cdot$ L^{-1}) for any type of training session. HB was positive after all sessions, except one resistance session in which ambient temperature was abnormally high. However, nine sessions in which HB was over the + 2 % body mass gain were observed, most of them occurred under cold ambient temperatures in which sweat rate might be diminished. Ultimately, we observed almost the same performance in 2000 m interval repetitions at altitude compared to SL.

In the study of the physiological assessment during a marathon, a slight decrease in the Ln RMSSD the day after arriving to new time-zone relative to BL was found. Moreover, rMSSD was suppressed the day after marathon and back within BL values 48-hours after the marathon. During the race the

athlete showed similar heart rate to the one reported by elite-paraplegic and elite-able-bodied marathoners; however, the oxygen uptake (VO_2) assessed in the incremental laboratory test before the marathon revealed greater values (relating to the marathon intensity sustained) compared to the values reported from paraplegic marathoners and lower to the ones reported by able-bodied athletes. Interestingly, from 30 km to the finish line the athlete was able to maintain an intensity over the second ventilatory threshold, fact that has not been reported in elite-able-bodied marathoners.

Conclusions: Both training models, IP and FP set at 3860-4090 m terrestrial altitude, bring on an improvement in performance after returning from altitude; however, and despite a ~40 % lower TL imposed in FP, this model showed a slightly greater performance than IP, which might be attributed to a lower accumulated fatigue as reflected by the lower suppression of vagally-mediated HRV marker and its faster restoration after returning from altitude.

In the study of the marathon we observed: 1) similar HRV response after relocation in a new time-zone compared to able-bodied athletes; 2) similar pre-race HRV response to that observed in elite able-bodied triathletes and athletes; 3) similar HRV vagally-mediated marker suppression 24-hours after marathon compared to athletes participating in long-endurance events; 4) similar HRV vagally-mediated markers rebound as observed in cross-country skiers 48-hours after finishing the race; 5) similar HR response throughout the marathon than elite-paraplegics and elite-able-bodied marathoners. However, our athlete was able to maintain a HR corresponding to >VT2 from the 30 km to the end of the race (42.2 km).

Keywords: Altitude training, hypoxia, hypoxemia, HRV, sympathoexcitation, marathon, environmental physiology

Resumen

Contexto: El entrenamiento en altitud ha sido desde finales de la década de los sesenta un método común frecuente de entrenamiento entre maratonianos y atletas de media/larga distancia, con la intención de mejorar su consumo máximo de oxígeno (VO_{2max}), dado el incremento del volumen de células rojas (RCV) y la masa total de hemoglobina (Hb_{mass}) observado tras regresar de altitud. Sin embargo, dada la disminución de rendimiento en sesiones específicas (velocidad mantenida a intensidad de segundo umbral ventilatorio) observada en altitud, comparado con el nivel del mar (SL) e incrementándose esa perturbación a medida que la altitud se incrementa, no se encuentran estudios en la literatura, excepto uno publicado hace 52 años, donde los atletas se expusieron a 4000 m de altitud terrestre. Además, no se han publicado estudios sobre entrenamiento en altitud en el Deporte Paralímpico.

Considerando todo lo anteriormente expuesto, el primer objetivo de este trabajo de investigación fue analizar la respuesta fisiológica y psicológica, además de los efectos sobre el rendimiento de una estancia de cinco semanas a 3890-4090 m de altitud terrestre en un atleta de élite en silla de ruedas con Charcot Marie Tooth (CMT), realizada previamente a su participación en las maratones de Boston y Londres (estudios 1 y 2).

Por otra parte y, teniendo en cuenta que ambas concentraciones en altitud fueron programadas como escenario de preparación de las maratones de Londres y Boston y, dada su aplicabilidad en entrenadores y doctores a cargo de la optimización del rendimiento de atletas en silla de ruedas, el segundo objetivo de este trabajo de investigación fue analizar en el mismo atleta: 1) La respuesta del sistema nervioso autónomo de forma no invasiva, mediante

la oscilación de la respuesta autonómica cardíaca vagal con la variabilidad de la frecuencia cardíaca (HRV) antes, durante y después de una maratón en silla de ruedas internacional, comparando los resultados con la literatura existente en atletas, triatletas y esquiadores de fondo de la población general, 2) comprobar si la respuesta de la HRV tras la reubicación en una nueva franja horaria, es similar a la de atletas de la población general, 3) comparar la respuesta cardiovascular de un atleta de élite mundial en silla de ruedas a atletas del mismo nivel competitivo parapléjicos y de la población general (estudio 3).

Método: Un atleta profesional en silla de ruedas diagnosticado con CMT, caracterizado por una afección de sus extremidades inferiores y superiores, completó dos concentraciones de cinco semanas de duración a 3860-4090 m de altitud terrestre en dos años de forma consecutiva. En la primera, denominada programa inflexible (IP), el atleta completó todas las sesiones de entrenamiento, previamente establecidas, siendo la distancia media semanal recorrida de 200 km. En la segunda concentración, llamada programa flexible (FP), las sesiones específicas se realizaron en función de si se alcanzaba un valor referencial (RV) de su HRV, disminuyendo la distancia recorrida semanalmente a 140 km. Se estudiaron las fluctuaciones en normoxia e hipoxia de marcadores vagales de la HRV como la raíz cuadrada de la media de la suma de las diferencias al cuadrado de los intervalos R-R (rMSSD), la saturación arterial de oxígeno (SO_2) y la frecuencia cardíaca de reposo (HR_{rest}), comparando las diferencias entre FP e IP. Un estudio del rendimiento previo y posteriormente a la exposición a altitud fue llevado a cabo en IP y FP mediante un test incremental, en el cual se estimó la potencia generada, y un test contrarreloj de 3000 m. Además en FP (previamente, durante y tras la estancia en altitud), se llevaron a cabo mediciones en las siguientes variables: presión sistólica sanguínea (SP), presión diastólica

sanguínea (DP), eritropoyetina plasmática (EPO), eritrocitos, recuento de reticulocitos (Ret), hemoglobina (Hb), hematocrito (Hct), frecuencia respiratoria en reposo (BF), perfil de los estados de ánimo, masa corporal, gravedad específica de orina al levantarse (SG AM) y antes de acostarse (SG PM), diuresis, ingesta diaria de líquido ingerido, equilibrio hídrico (HB) tras cada entrenamiento, ingesta energética total, ingesta total de carbohidratos (CHO), proteínas y lípidos e ingesta diaria de sodio (Na^+).

En segundo lugar y, en referencia al estudio de maratón, diez días antes del evento (RD-10), fue realizado un test incremental en ergómetro para determinar el segundo umbral ventilatorio (VT2), así como la frecuencia cardíaca (FC) y consumo de oxígeno a intensidad de VT2. Seis días antes de la maratón (RD-6) y dos días tras la competición (RD+2), se midió la HRV diariamente al levantarse y la rMSSD se consideró el indicador de control autonómico cardíaco. La media de la expresión logarítmica de la rMSSD (Ln rMSSD) de los días previas al viaje (RD-6 – RD-2) sirvió como línea basal (BL). Durante la maratón, la FC fue monitorizada y los datos fueron agrupados cada 5000 m (0-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30- 35, 35-40), así como en los últimos 2195 m (40-42,2).

Resultados: Una mayor reducción de los marcadores vagales relacionados con una mayor fatiga se observaron en altitud. Además, este hecho medido sobre el coeficiente de variación en la rMSSD (rMSSDCV) mostró valores más heterogéneos en IP comparado con FP, acompañado en este último modelo por una recuperación más rápida de la rMSSD al regresar a SL. Tras una disminución significativa en la SO_2 en ambos modelos al llegar a altitud, un incremento más rápido de la SO_2 se observó en FP comparado a IP en diferentes puntos temporales de la estancia, mostrando una aclimatación más rápida en FP. La $HR_{rest,}$ SP y DP se incrementaron en altitud en ambos modelos, mostrando una ligera reducción tras regresar de altitud comparada a

registros previos a altitud, aunque sin diferencias significativas entre modelos. Ambos modelos facilitaron un incremento en la potencia generada por el atleta y también mejoraron su tiempo en los 3000 m, siendo ligeramente mejor tras FP. En referencia a los parámetros sanguíneos (recordar que sólo fueron registrados durante FP), se observó un incremento en los eritrocitos, Hb y Hct en el estadio final de la estancia, siendo significativamente mayores que en los resultados obtenidos previos a la estancia. Un incremento superior al 200 % se observó en la EPO treinta y cinco horas después de llegar a altitud, reduciéndose drásticamente hacia la mitad de la estancia y alcanzando sus valores mínimos al regresar a SL. La BF se incrementó en altitud respecto a SL y una ligera disminución se observó en la fase de exposición crónica. Una disminución en la dimensión vigor y un incremento en la dimensión fatiga se observó en altitud comparado a SL cuando SO_2 y SP se consideraron como covariables. Además, la alteración del estado de ánimo total (TMD) aumentó significativamente cuando se alcanzó la mayor carga de entrenamiento (TL) en SL, mientras que el TMD se incrementó a lo largo de la exposición a altitud, permaneciendo elevado tras regresar de ésta. Una disminución de la masa corporal se observó al llegar a altitud, sin embargo, un incremento significativo ocurrió de la segunda a la quinta semana en altitud, además la masa corporal regresó a valores registrados previos a altitud, al regresar a SL. La ingesta de CHO y proteínas fue significativamente superior en altitud comparada a SL, mientras que no se observaron diferencia en la ingesta de lípidos entre ambas condiciones. Tanto la ingesta diaria de fluido y la diuresis aumentaron en altitud, especialmente en la aclimatación, de hecho, observamos una menor SG AM durante la primera semana en altitud, en referencia a valores previos a la estancia. Aunque no hubo diferencias significativas, la SG PM mostró un patrón más bajo que SG AM y ambas estuvieron dentro del rango sugerido

para un estado óptimo de hidratación (≤ 1.020). La ingesta diaria de sodio estuvo dentro del rango normal sugerido como óptima (1300 a 2500 mg · d^{-1}), sin embargo, el Na^+ no alcanzó las cantidades recomendadas (0.5 a 0.7 g · L^{-1}) en ningún tipo de entrenamiento. El HB fue positivo tras todas las sesiones, excepto una sesión donde la temperatura fue anormalmente elevada, sin embargo, observamos nueve sesiones donde el HB fue superior al + 2 % de la masa corporal. La mayoría de estas sesiones ocurrieron con temperaturas muy bajas, donde la tasa de sudoración puede estar disminuida. Por último, observamos prácticamente el mismo rendimiento en repeticiones de 2000 m en altitud y SL.

En el estudio de maratón, encontramos un descenso en el Ln RMSSD el día después de llegar a la nueva zona de cambio horario, en relación a BL, además, la rMSSD descendió el día posterior a la maratón, regresando a valores similares al BL 48 horas después de la maratón. Durante la prueba el atleta mostró una frecuencia cardíaca (HR) similar a atletas de élite parapléjicos y sin ningún tipo de afección, sin embargo, su consumo de oxígeno (VO_2) registrado en el test de laboratorio efectuado días antes de la competición, desveló valores mayores (considerando la intensidad a la que corrió la prueba) que los mostrados en el grupo de atletas parapléjicos, aunque menores a los maratonianos sin afección. Cabe destacar que, del kilómetro 30 a la línea de meta el atleta fue capaz de mantener una intensidad superior a VT2, algo que no se ha observado en maratonianos de la población general.

Conclusiones: Ambos modelos IP y FP desarrollados a 3860-4090 m de altitud terrestre, facilitaron una mejora en el rendimiento tras regresar de altitud. Sin embargo y a pesar de una TL ~40 % inferior, FP mostró mejoras en el rendimiento ligeramente superiores a IP, que pueden atribuirse a menor

fatiga acumulada, reflejada por una menor reducción de marcadores vagales de la HRV y una mayor recuperación de estos tras regresar de altitud.

En el estudio de maratón observamos: 1) Similar respuesta en la HRV tras la reubicación en una nueva franja horaria comparada a atletas de la población general; 2) Similar HRV previa a la prueba comparada a la observada en triatletas y atletas de élite de la población general; 3) Similar supresión de marcadores vagales de la HRV 24 horas después de la maratón, comparado a atletas de la población general que han participado en eventos de resistencia de larga duración; 4) Similar recuperación "rebote" de los marcadores vagales de la HRV 48 horas después de la maratón, comparada a esquiadores de estilo nórdico de la población general; 5) Similar HR durante la maratón comparada a atletas de élite parapléjicos y maratonianos sin afección, sin embargo nuestro atleta fue capaz de mantener una HR correspondiente a una intensidad superior a VT2 desde el kilómetro 30 a meta (42.2 km).

Palabras clave: Entrenamiento en altitud, hipoxia, HRV, excitación simpática, maratón, fisiología medioambiental.

Chapter I:

Thesis Document

1. GENERAL INTRODUCTION

1. GENERAL INTRODUCTION

1.1. Altitude training

1.1.1. Origin and evolution

Since the 1968 Olympic Games (Mexico DF, 2250 m altitude), training under hypoxic conditions has become more and more popular among athletes from different disciplines. [1] At the very beginning athletes were residing and training at the same altitude scenario, no matter the intensity or duration of training session. This altitude training model is known among coaches and athletes as live high - train high (LHTH). In addition, it was almost thirty years after the beginning of altitude training, when Stray-Gundersen and Levine highlighted positive results in performance in middle-distance athletes after returning from altitude, when athletes were following the live high - train low (LHTL) model. [2] These authors compared SL performance in three groups of well-trained middle-distance athletes: 1) athletes living and training at SL (low-low group), 2) athletes living and training at 2500 m terrestrial altitude (LHTH), and 3) athletes living and performing lower intensity sessions below or close to the first ventilatory threshold (VT1) at 2500 m altitude, while more intense sessions were done at lower altitude (1250 m) (LHTL). Results from this study only showed improvement in a 5000 m test in the LHTL group, comparing pre-altitude times with times after returning from altitude. To consider, altitude training scenarios are not always close to the athlete`s residences, and sometimes many athletes cannot afford to spend three to four weeks out of home, so the use of normobaric hypoxic tents or houses for sleeping, simulating altitude conditions by decreasing the inspired oxygen fraction (FiO_2), and increasing the nitrogen concentration is well established among the endurance athlete community. [3] Furthermore, not all athlete´s hypoxic training targets are based on the enhancement of the

erythropoietic response, and for this reason, different live low – train high (LLTH) approaches have emerged in the last decade for intermittent endurance disciplines, and disciplines in which the glycolytic metabolism is predominant and several physiological mechanisms can be highlighted: 1) the increase of muscle perfusion and oxygenation, 2) the improvement of fast-twitch fibers behavior, 3) the increase of intramuscular vasodilation by the increase of nitric oxide, and 4) the enhancement of the phosphocreatine resynthesis [4] which seems to be improved with the repeated-sprint training in systemic hypoxia, [5] and also with the hypoxia induced by voluntary hypoventilation at low-lung volume. [6] Furthermore, new strategies for inducing local ischemia might be considered as novel hypoxic training strategies: 1) blood flow restricted exercise, [7] and 2) ischemic preconditioning. [8]

1.1.2. Altitude training physiological target in endurance disciplines

The main aim of altitude training for endurance disciplines is to increase the total volume of red blood cells (RBC) and hemoglobin mass (Hb_{mass}) to improve the arterial blood oxygen-carrying capacity, increasing maximal oxygen uptake (VO_2max), thus enhancing performance at both SL and altitude. [9]

1.1.3. Physiological-induced mechanisms at altitude increasing erythropoietic response

The decreased barometric pressure at high altitude results in reduced oxygen partial pressure and oxygen saturation of Hb in arterial blood. [10] Hypoxemia stimulates ventilation, increases cardiac output, alters the distribution of blood flow, and enhances oxygen extraction from capillary blood to improve tissue oxygen supply. [11] As a defence mechanism to minimize the decrease of

arterial oxygen content at altitude, a decrease in plasma volume will occur within days from ascent to high altitude. [12] Furthermore, the hypoxia inducible factor-1 (HIF-1) is the most important protein regulating the physiological homeostasis against the reduced oxygen supply. [13] HIF-1 regulates the activation of more than one hundred target genes [14,15] out of which the activation mechanism for the production of glycoprotein cytokine EPO is the most known one. Plasma EPO concentration starts to increase after 2 hours of altitude exposure, [16] reaching a plateau 3-4 days after arriving to altitude, starting to fall gradually until reaching pre-altitude values or reaching even lower values. [17,18] Changes in EPO level become non-significant compared to pre-altitude, after the first, [19] second, [20] or third week at altitude. [21] After returning to SL EPO concentration returns to pre-altitude levels immediately, or after a few days, [21,22] or even falls below the initial value. [19,20,23] Red cell volume (RCV) [22,23] and total Hb_{mass} [19,22,24,25] increase relative to pre-altitude after altitude returning. Hemoglobin concentration, Hct, RBC and percent of Ret are also elevated after altitude training. [20,21,22,26,27,28] However, there are studies that report no enhancements of blood markers after altitude training, [29,30,31,32] and also studies that do not show a relation between EPO concentration and blood markers; [30,31,32,33,34] therefore inter-individual variability response must be considered. A relation between the decrease in the partial pressure of oxygen in arterial blood and the increase in EPO concentration has been reported, [16] which highlights a relation between the level of hypoxemia and the stimulation of EPO production. In line with this assumption, there are authors who suggest a threshold for EPO production corresponding to a minimal altitude range of 2100-2500 m. [35]

1.1.4. Optimal sojourn duration for athletes training at altitude

The degree to which RBC volume and Hb_{mass} increase are related to the numbers of hours in hypoxia and there appears to be a minimum threshold of about 12-13 hours per day exposure to altitude/hypoxia needed to increase RBC volume and Hb_{mass} substantially. [36]

Insufficient altitude (< 2000-2200 m) and/or an inadequate period of time spent at altitude (< 3-4 weeks) have been proposed by Rusko et al. [9] as key factors to stimulate an increase in blood markers. In fact, at 4000 m altitude, larger increases in RCV occur after a stage of 4 weeks compared to shorter exposure. [37] Moreover, expected RCV increases vary between 5-9 % after a 4-week altitude camp, while Hb_{mass} gains are of 1.1 % · 100 h^{-1} of hypoxic exposure. [2]

1.1.5. Effects of altitude on during exercise and under resting conditions

A relation between peak heart rate (HR) and the decrease in barometric pressure has been reported, corresponding to a 1 beat · min^{-1} reduction in peak HR for every 7 mmHg decrease in barometric pressure below 530 mmHg (~130 m of altitude gained above 3100 m terrestrial altitude). [38] The opposite trend is observed in resting HR (HR_{rest}) under hypoxic conditions, increasing in chronic hypoxia (after altitude acclimatization) as a result of an enhanced sympathetic activity (sympathoexcitation). [39] Recently, a study with seven healthy lowlanders whose HR_{rest} was monitored at SL and after 15-18 days of exposure to 3454 m terrestrial altitude, demonstrated that a reduction in cardiac parasympathetic activity was considered as the primary mechanism underlying the elevated HR_{rest} associated with 2 weeks of exposure to hypoxia. [40]

1.1.6. Blood pressure and altitude

In a recent study with eight Danish lowlanders who spent 50 days at 4100 m terrestrial altitude, authors reported an increase in mean arterial pressure (MAP) and muscle sympathetic nerve activation on exposure days 10 and 50, compared to SL results. Moreover, an increase in diastolic blood pressure (DP) at altitude compared to SL was observed, being this mechanism associated with a sympathetic response as a way to compensate for hypoxia-induced peripheral vasodilation. Interestingly, these authors did not report significant changes in systolic blood pressure (SP) while at altitude. [41]

1.1.7. Ventilatory response at altitude

On acute exposure to hypoxia (before altitude acclimatization), one of the earliest and most consistent physiological responses is an increase in minute ventilation (V_E) mediated primarily by hypoxic stimulation of the peripheral chemoreceptors. [42] Ventilatory acclimatization to altitude is facilitated by an increased sensitivity of the peripheral chemoreceptors to hypoxia, estimated by the hypoxic ventilatory response (HVR) in humans. [43] The HVR has been reported to correlate with the magnitude of increase in V_E on arrival at altitude, [44] and several studies have demonstrated an increase in the HVR during natural altitude acclimatization. [45,46,47] An enhancement of the HVR during acclimatization is viewed as a positive adaptation, because an increase in V_E improves alveolar oxygen pressure and raises arterial oxygenation while at altitude. [44]

1.1.8. Nutritional strategies and metabolism during altitude sojourns

During altitude acclimatization there is a reduction of intra and extracellular water combined with a decrease in plasma volume, [48,49] which combined with an increase in respiratory water loss, due to greater ventilation [50] along with

urinary water loss that can increase up to 500 mL per day, [51] can result in up to 2 kg of body mass loss. [52] Eating *ad libitum* at altitude is not a proper nutritional strategy, as loss of body fat and fat free mass have been associated to eating *ad libitum* during altitude sojourns. [53,54] Moreover, there are studies concluding that the loss of fat free mass at altitude might increase the risk of injury and illness in extreme environments. [55,56,57] Therefore, strict dietary controls at altitude might attenuate daily energy deficits and mitigate weight loss. [50] Regarding metabolism, glucose oxidation rate seems to be diminished during acute altitude exposured compared to SL, not even reaching normoxic conditions oxidation rates after 21 days of being exposed to 4300 m terrestrial altitude. [58] However, there are studies suggesting that individuals have an increased dependence on glucose as a fuel source at high altitude, especially during exercise. [59,60,61] Moreover, the resting metabolic rate (RMR) defined as "the minimum energy the body requires to perform its basic functions, and which is principally dependent on lean mass", [62] has been observed to remain increased at altitude compared to SL normoxic conditions. [63,64] The reason for an enhancement in RMR is the altitude induced-sympathetic drive [39] which might trigger a subsequent rise in adrenaline levels. [65] In addition, RMR is more pronounced in the acute phase of altitude (i.e., 2-3 days after arrival). [50,66] However, an increase in RMR (≥ 17 %) can persist for up to 21 days after hypoxic exposure. [56] Likewise, there might also be an altitude threshold for increasing RMR, as a study with olympic rowers did not report changes in RMR at 1800 m terrestrial altitude compared to SL, [67] while an increase of ≈ 19 % was observed in middle distance runners training and living at 2100 m terrestrial altitude [63], and a 10 % increase was observed in individuals living and exercising at 3650 m terrestrial altitude. [64] Ultimately, energy expenditure, considered as the amount of energy that a person needs to carry out a biological function (i.e.,

breathing, blood circulation, food digestion) will be elevated at altitude and may be equivalent to a higher intensity exercise conducted at SL. [68]

Considering the aforementioned factors, the primary nutritional target at altitude will be to match the energy intake to the daily expenditure to minimize body mass loss. [69] As observed in cyclists living and training at 4300 terrestrial altitude, a total carbohydrate (CHO) intake of 7.6 $g \cdot kg^{-1}$ of body mass per day did not cover the energetic demands. [70] Up to 70 % of altitude-exposure related weight loss is due to muscle mass loss. [71] However, according to the hypoxic dose theory from Garvican et al., which considers the total duration of exposure in hours (h), and the elevation of exposure in meters (m), being calculated as $km \cdot h^{-1}$ = (m / 1000) x h, [72] an exposure of 5000 $km \cdot h^{-1}$ is the threshold at which muscle loss starts to occur. [73]

At altitude protein synthesis seems to be blunted, as it was reported that protein synthesis after walking at 4559 m terrestrial altitude [74] was much lower than after aerobic low intensity exercise at SL. [75]

1.1.9. Hydration strategies to consider at altitude

Rehydration immediately after session completion must be considered at altitude to compensate for sweat losses. [76] As a usefuel strategy, athletes can consume 100 mL of drink every ten minutes during and after training, reducing the effects of dehydration at a confortable rate. [77,78] Moreover, a target for liquid consumption of 4 to 5 L per day has been recommended while training and living at altitude. [79] Likewise, and considering that altitude environments tend to be located in remote places, non invasive tools for athletes bilogical evaluation are very useful. For their hydration status assessment, urine specific gravity (SG) assessment with stripes, give us information on the dehydration level of the athlete, considering $\leq$1.20 as an optimal hydration status. [80] In addition, an increase in sodium (Na^+) excretion

has been related to acute mountain sickness (AMS), [49] being the most common observed form of acute altitude illness, affecting unacclimatized persons ascending to elevations >2500 m, although it can also be observed in individuals ascending to lower altitudes. [81] There is a lack of consensus in the literature regarding the minimum quantity of Na^+ intake while exercising at altitude. However, the recommendation for endurance disciplines is 0.5 – 0.7 $g \cdot L^{-1}$ [82,83] while training, and an optimum daily range for general population of 1500 to 2300 $mg \cdot d^{-1}$. [84] An enriched Na^+ solution for training has been recommended for several reasons (i.e., stimulate thirst, increased voluntary fluid intake, enhanced glucose and water intestinal absorption, optimized extracellular and intracellular fluid balance, and hyponatremia prevention plasma sodium < 135 $mmol \cdot L^{-1}$). [85,86,87,88] In addition, it seems that greater Na^+ excretion only occurs during acute altitude exposure, as reported in a study with seven trained males at 5050 m terrestrial altitude, in which a significant greater Na^+ excretion compared to SL (166 ± 34 $mEq \cdot d^{-1}$), was observed only 7 days after their arrival to altitude (427 ± 46 $mEq \cdot d^{-1}$), while no differences were observed after 21 days at altitude (257 ± 34 $mEq \cdot d^{-1}$). [89]

1.2. Evaluation of overtraining by the use of psychometric questionnaires

1.2.1. Overtraining and overreaching

Competitive athletes sustain regular training to physically adapt to the demands of competition and improve performance. [90] However, the incorrect administration of the training load (TL) might trigger non-desirable effects on performance because of factors such as excessive fatigue, injury or illness, and therefore demonstrating a need for monitoring TL. [91] Periods of intensified training (overload), in which the TL is increased as a result of an

increase of training intensity and/or duration, are intended to induce some degree of fatigue, which is necessary to promote the physiological adaptations that enhance performance. [90,92,93,94] Fatigue is defined as the inability to complete a task that was achievable within a recent time frame. [95] Moreover, fatigue has been defined as the inability to maintain the same power output during repeated muscular contractions. [96,97] Therefore, training-induced fatigue might result in temporary performance decreament

The European College of Sport Science states that the term 'overtraining' as a process of intensified training that may result in short-term or functional overreaching (FO), non-functional overreaching (NFO) or overtraining syndrome (OTS). [92] In addition, the distinction between FO and NFO is based on the duration of the symptoms until performance returns to normal levels. Recovery of normal performances should incur within days to weeks in FO, whereas in NFO it may take several weeks to months. OTS may last several months or years. [90,98]

Moreover, when prolonged excessive training stresses are applied concurrent with inadequate recovery, performance decrements and chronic maladaptations occur. The OTS afflicts a large percentage of athletes at least once during their careers. [91] Overreaching refers to training that involves a brief period of overload, with inadequate recovery that exceeds the athlete's adaptive capacity. Overtraining exceeds overreaching and results in frank physiological maladaptations and chronically reduced exercise performance. [91] Different signs and symptoms of OTS are: decreased physical performance, general fatigue, loss of vigor, insomnia, change in appetite and mood, loss of bodyweight, loss of motivation, lack of mental concentration and feelings of depression. [94] Moreover, there is no gold standard test to diagnose OTS, the only certain sign is a plateau or decrease in performance during competition and training. [91] In addition, the physical demands of overtraining are not the

only elements in the development of OTS. [91] A complex set of psychological factors are important in the development of OTS, including excessive expectations from a coach and family, competitive stress, personality structure, social environment, relations with family and friends, monotony in training, personal and emotional problems, and school or work related demands. [94] Training programs that are carried out under extreme conditions should guarantee an optimal balance between training and rest to prevent overtraining symptoms, which have been characterized by fatigue, performance decrements, mood changes, irritability, loss of motivation, among others. [99]

1.2.2. POMS questionnaire

The Profile of Mood States (POMS), [100] which reflects the individual's mood on six primary dimensions (i.e., Depression-dejection, Tension-anxiety, Anger-hostility, Vigor-activity, Fatigue-inertia, and Confusion-bewilderment), is widely used in sports to evaluate the psychological state of athletes. High values on the Vigor-activity scale and low values on the remaining scales are desirable for athletic performance. Therefore, when the scores are profiled, a desirable configuration resembles an iceberg. [101] Interestingly, exercise has been suggested to alter mood, depending on the type, volume, duration, intensity, and setting, with low and moderate intensity aerobic activities leading to mood improvements, [102,103] while opposite trends have been associated to high-intensity anaerobic exercises [104] and exhausting competitions. [105] In addition, during periods of overtraining athletes generally report undesirable changes in Total Mood Disturbance (TMD), which represents the sum of the five negative scales of POMS, subtracting vigor score and adding a constant of 100. [106] Ultimately, there seems to be a link between mood states and the TL, as when TL is reduced

during taper periods, athletes have reported improvements in mood as reflected by a return of POMS scores to baseline within 10 days of lowered TL. [107] Moreover, it has been suggested that a 50 % or greater increase in an individual's basal off-season TMD score may be reflective of an overreaching state, as demonstrated in a study with world-class canoeists preparing for the Olympic Games. [108]

1.2.3. Influence of altitude on mood states

Altitudes above 3050 m have been shown to cause adverse changes in mood states. [109,110,111] The severity of these changes are related to both, the level of altitude and the rate of ascent. [111,112] Interestingly, in a recent publication in which the POMS was administered to a 9 people team during a 6000 m ascent of Mount Everest, the authors reported oscillations in fatigue and vigor during the ascent, with no changes in the depression scale. [113] Moreover, mood changes are most severe during the first or second day at altitude and then they gradually decrease over the next 2-4 days. [111] However, longer pre-altitude acclimatization before ascending to higher elevations is not protective for negative mood states when an individual reaches high altitudes. [114]

1.2.4. Rate of perceived exertion

Ratings of Perceived exertion (RPE) during exercise at a fixed power output decrease with physical training, [115] while they can increase significantly following overtraining. [116] The perception of effort at any given moment during physical activity is obviously governed by the exercise intensity, but it is also influenced by other factors such as the individual´s gender, their personality structure, or training status. It is also known that RPE is correlated with various physiological factors such as circulating glucose levels, glycogen depletion, production of creatine kinase, and so on. [117]

According to Borg, changes in RPE for a constant workload or volume might be a more effective predictor of the onset of staleness than would any single physiological variable. [118] Moreover, RPE decreases with rest or reduced TL. [101] As aforementioned, overtraining leads to an increase in anxiety and depression levels, [101] and these psychological states are associated with elevated ratings of perceived exertion for a relative exercise stimulus. RPE correlates highly with physical indicators of fatigue such as HR (r = 0.8 to 0.9), oxygen uptake (VO_2), lactate and ventilation. [119] A validated scale for assessing RPE is the Borg 1-10 scale which links numbers from 1 to 10 with verbal anchors from nothing at all to very, very strong (See Table 1). Borg 1-10 scale allows the subjects to rate the degree of perceived exertion during or after the completion of physical work without any prior knowledge or experience. In addition, as greater the number, as more demanding the effort. [120] Interestingly, studies have consistently found that arm exercise (cranking) elicits higher RPE´s than leg exercise (cycling) for the same absolute power output. [121] Ultimately, RPE has been correlated with depression. [122]

Table 1. Borg scale 1-10.

Value	Description
0	Nothing at all
0.5	Very, very weak (just noticeable)
1	Very weak
2	Weak (light)
3	Moderate
4	Somewhat strong
5	Strong (heavy)
6	
7	Very strong
8	
9	
10	Very, very strong (almost max)

1.2.5. The effect of altitude on RPE

To date there is no agreement on the effects that acute and chronic altitude exposure can have on RPE. In a study in which male soldiers performed a 30-min bout of exercise on a mechanically-braked arm cycle ergometer, maintaining a pedal frequency of 60 rpm in different conditions: 1) at SL, 2) during an acute (<2 hours) exposure to a simulated 4300 m altitude (hypobaric chamber pressure = 445 TORR), 3) on days 1, 8 and 15 at 4300 m terrestrial altitude, and 4) at SL, 48 h after returning from high altitude, authors reported significant greater local RPE (related to sensations from the exercising muscles and joints) at SL and acute exposure to high altitude compared to chronic exposure to high altitude. Moreover, overall RPE did not show differences among conditions. However, a limitation of this study

was that absolute submaximal exercise intensity differed between normoxic (189 W) and hypoxic conditions (140 W acute altitude, 135 W chronic). Therefore, authors concluded that RPE might be more influenced by a particular physiological response (e.g., blood lactate concentration). [123]

1.3. Monitoring stress and fatigue

1.3.1. Different methods for assessing fatigue

As aforementioned, throughout the frame time of a periodization model there will be overload periods (increase of fatigue) and recovery periods (decrease of fatigue) in order to maximize the biological adaptation response to training, aiming to increase performance or peaking in key competitions. Moreover, it has been suggested that measuring fatigue status could lead to a reduction of injury risk, illness and NFO. [124] During the last decade, several publications have suggested to monitor fatigue by selecting a combination of validated tests using smart sensor technology. [125] Nowadays, researchers and coaches are able to use a wide range of tools (i.e., mobile apps) to assess stress and fatigue in sport. [126] In fact, the use of psychometric questionnaires as POMS [107,108], the Total Quality Recovery Scale (TQR) [127] or the Recovery-Stress questionnaire (REST-Q) [128] have been widely used to assess the perceived well-being of athletes. However, one limitation of the use of questionnaires on a daily basis is that they are extensive. Thus, many coaches use shorter validated questionnaires versions. [125,129]

Maximal assessments (i.e., All-Out sprints, maximal voluntary contraction and jumps) have been suggested as a tool to determine the rate of recovery of performance. However, several limitations of these tests stand, as they are volitional exhaustive, therefore injury-risk induced. Moreover, they are difficult to set in the training program schema as they have to be done in completely resting conditions, thus, reducing TL pre-test is a must. An

example of a study using maximal tests (i.e., countermovement jump) to relate with fatigue indices (i.e., average power output during repeated sprint in cycling) is the one recently published by Hughes et al. [130]

The use of biochemical parameters (i.e., hormones, blood markers) is a common tool among coaches and physicians to understand the effect that exercise or several stressful situations (i.e., heat, hypoxic environment) have on status fatigue. In addition, some markers are highly linked to fatigue (i.e., creatine kinase levels increase after extenuating exercise) and provide useful information to modulate TL. [126] However, they are a very expensive instrument and they are time consuming (immediate results are not given). Moreover, these parameters are primarily obtained from invasive tests (i.e., blood samples). Therefore, it is difficult to implement their use on a daily basis, and consequently, that they are a key point in the decision-making process regarding training prescription.

1.3.2. The role of the autonomic nervous system in monitoring fatigue and training adaptation

The autonomic nervous system (ANS) participates in the maintenance of the body´s regulation and, therefore, it plays a key role in the management of the stress-recovery process. [131] ANS regulates involuntary processes such as cardiac regulation, peristaltsis, salivation, etc. [132] Moreover, ANS is divided into two branches with opposite functions: the sympathetic and the parasympathetic or vagal branches. The sympathetic branch mediates the “fight or flight” response, while the parasympathetic branch is predominant during resting situations (Figure 1). Thus, depending on the situation there will be a predominance of one branch over the other.

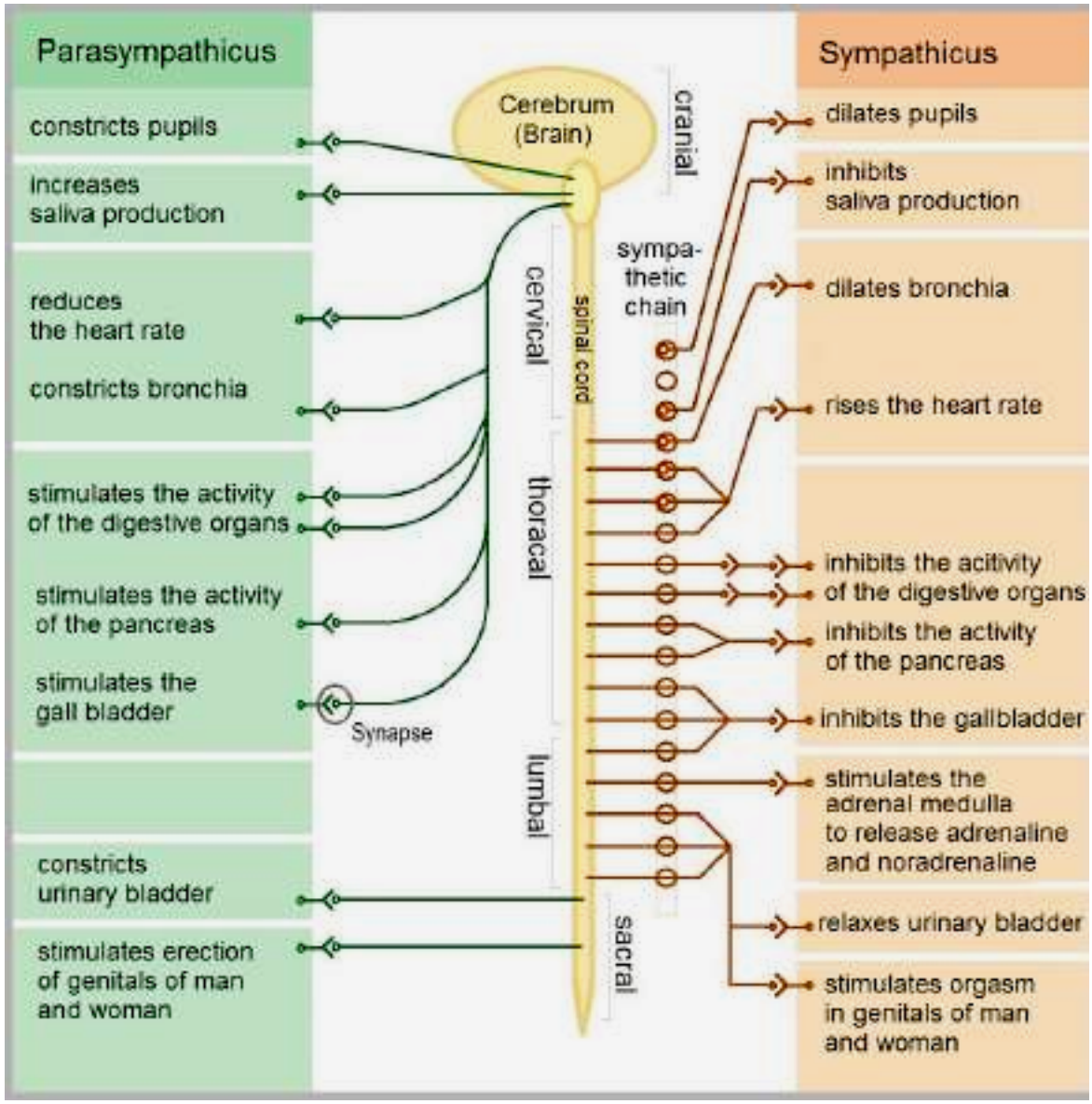

Figure 1. Anatomy of parasympathetic and sympathetic nervous systems and their main functions on different organs. Extract from: https://commons.wikimedia.org/wiki/File:The_ Autonomic _Nervous_System.jpg.

During exercise, there is a predominance of the sympathetic branch, having as a concominant effect an increase of HR. Cardiac parasympathetic activity is initially decreased after exercise [133,134] and returns to pre-exercise levels within 24-72 h [135,136,137,138] or it can rebound above these levels. [138,139] The time-course and amplitude of the post-exercise cardiac-parasympathetic response is primarily dependent on exercise intensity. For example, the time neeed for complete cardiac-autonomic recovery after a single aerobic-based training session is up to 24 h after a low-intensity exercise, 24-48 h after an exercise performed at second ventilatory threshold intensity (VT2), and at least 48 h after an exercise performed over VT2. [140] In contrast, duration of effort does not seem to be determinant in cardiac parasympathetic disturbance, as observed in a study conducted with cross-country skiers who did not show reduced-cardiac parasympathetic activity, ~24 h after finishing a 75 km cross-country ski race. [139] To consider, cardiac autonomic regulation is highly individual and its daily assessment could be used as a tool for organizing microcycle training contents distribution. [140,141,142] Interestingly, several oscillations of cardiac parasympathetic regulation have been linked to FO induced by overload training periods. [143] In addition, different opposite oscillations of cardiac parasympathetic regulations were related to individual positive adaptations to endurance training. [141,142] Mid- and short-term cardiac parasympathetic decreases are related to fatigue, while long-term parasympathetic increases are related to positive adaptations to endurance training due to increases in stroke volume [144,145,146] or hemodynamic changes (i.e., volemia). [147] Therefore, the measurement of the parasympathetic branch allows the management of stress and recovery on an individual basis.

Different markers are used for the assessment of cardiac autonomic regulation. Submaximal HR has been analyzed throughout adaptation/maladaptation process, showing that an increase in fitness and

performance is related to decreases in submaximal HR. However, submaximal HR also decreases in over-reached athletes. [148]

Another variable studied for the assessment of cardiac autonomic regulation is the heart rate recovery (HRR), showing after exercise the adjustment of the body by the sympathetic withdrawal and parasympathetic reactivation. [149] HRR is influenced by fatigue showing decreases after high intensity training. [150] However, a positive adaptation to training not always triggers an acceleration in HRR. [151] Therefore, changes in HRR must be interpreted in accordance with the training context.

Heart rate variability (HRV) has been proposed as a non-invasive tool to assess the cardiac autonomic regulation and it reflects the oscillations in the intervals between successive heartbeats (R-R). [152,153] HRV is defined as the measurement variation between R-R intervals resulting from sinus node depolarization during a continuous electrocardiogram recording. [153] Several variables are derived from this recording by linear (time- and frequency domain) and non-linear methods (Table 2). HRV measurements have been implemented in sport science for different purposes such as the evaluation of short- [139,148] and long-term [154,155] fatigue of athletes, to estimate the ventilatory thresholds, [156,157] to identify FO and NFO, [158] to determine pre-competitive anxiety and cognitive performance, [159] to evaluate training adaptation among soccer players, [160] and to analyze the effect of travelling across new time zones on HRV. [161] The two most used HRV indices to monitor the effects of endurance exercise and fatigue are the high frequency power (HF) and the square root of the mean squared differences of successive R-R intervals (rMSSD). [144] Both variables are vagally-mediated, being commonly measured during rest in standardized conditions. Moreover, the parasympathetic branch reflects the positive and negative adaptation to

training with increases and decreases of those vagal-related indices respectively. [147]

It has been previously demonstrated that HF is able to detect fatigue [158] as well as positive and negative adaptation to training in endurance athletes. [162] However, to obtain valid and reliable recordings it must be taken into account that HF is influenced by breathing frequency [153] and it needs to be controlled properly (i.e., the use of a metronome).

The root mean square of the successive differences can be calculated during a short-term recording, [163] reflects parasympathetic activity [153] and demonstrates greater reliability than other spectral indices. [163] HRV is sometimes used to monitor the training status of endurance athletes. [142,144,164] In fact, rMSSD has been identified as an important recovery marker for day-to-day athletic monitoring and tracking of long term changes in fitness. [160,165] In well-trained athletes, a minimum of three HRV recordings per week must be obtained for a valid assessment of training status. [164] Moreover, rMSSD has been shown to display a coefficient of variation (CV) of 5 – 7 % in elite endurance athletes and ~10 % in recreational runners. [166] Lower day-to-day Ln rMSSD fluctuations (represented by CV) have been associated with more favorable adaptations to training among athletes. [160] In addition, a recent study with rugby players showed that individuals experiencing lower day-to-day fluctuations in rMSSD (represented by the coefficient of variation, rMSSDCV) during intensified training were responding more favorably to the stimulus. [167] For the calculation of rMSSDCV the formula rMSSDCV = (SD/Mean) x 100 is used. [160]

Table 2. Heart rate variability indices.

	Variable	Description
Time-Domain	Mean R-R	Mean R to R interval
	SDNN	Standard deviation of R to R interval
	rMSSD	The square root of the mean squared differences of successive R to R intervals
	pNN50	The mean number of times an hour in which the change in successive normal sinus (NN) intervals exceeds 50ms
Frequency-Domain	VLF	Very low frequency (0.00-0.04 Hz)
	LF	Low frequency (0.04-0.15 Hz)
	HF	High frequency (0.15-0.40 Hz)
	LF/HF	Ratio LF to HF

1.3.3. HRV day-to-day training prescription

HRV assessment allows the possibility of guided training workload distribution to facilitate optimal increases in performance and positive adaptations to training.[1] The hypoxic period examined has been [41,142,168] In this regard, day-to-day training prescription has been previously compared with predefined/prescribed training programs in recreational and moderate training runners, [141,142] in well-trained cyclists[168] and in untrained population. [169]

1.3.4. The effect of altitude on HRV

As aforementioned, sympathoexcitation occurs during acute and chronic altitude. [39] In addition, sympathetic overactivity seems to be a biological

feature in lifelong-highlanders, as reported by Lundby et al. in a study comparing muscle nerve sympathetic activity in lowlanders (Danish population) and Bolivian highlanders, [40] in which authors conclude that lifelong sympathoexcitation persists in highlanders.

Many of the factors that are known to affect HRV (e.g., fatigue, stress, insomnia, hypoxia, and cold) are predominant at high altitude. [170,171,172] Therefore, high altitude exposure is associated with significant changes in HRV compared with SL/low altitude. [173,174,175,176,177] However, the majority of the published data relate to short-term HRV recordings (1–5 min) was obtained in hypoxic chambers during 'simulated' rather than genuine terrestrial high altitude. [178,179,180,181] Moreover, the hypoxic period examined has been generally brief (minutes to <8 h). [182,183]

Non-invasive studies using HRV assessment have shown altered autonomic HR regulation with altitude and endurance training, [184,185] but their results do not agree with the increase in the parasympathetic activity observed at altitude using invasive measures. [186] Cornolo et al. reported an 88 % reduction in HF during the first 2 days at 4350 m altitude, improving only to within 54 % of baseline HF after 6 days. [184] Moreover, in a study with 12 healthy adults, an 11 % decrease in rMSSD at 3619 m terrestrial altitude compared to SL baseline was reported. [175] Interestingly, RPE measured at the end of the previous day will have an impact on HRV when exercising at altitude. [187]

1.3.5. Training program guided by day-to-day HRV oscillations at altitude

Recently, Schmitt et al. published a study with 24 Nordic-skiers divided into three groups, two of them sleeping 15 d (~14 h · d^{-1}) in normobaric hypoxic rooms, simulating 2700 m alitude (FiO_2 = 0.15 %), and one group sleeping in

normoxic conditions. In the HRV-guided group, the TL was adjusted based on the following situations: Maintained or increased HF induced a higher TL than the previous day. Decreased HF equal to or more than 30 % led to lower TL. Two consecutive days of decreased HF prompted a resting day. After a rest day, a low-intensity and medium-volume session was prescribed. While the other hypoxic (H) and normoxic (N) groups followed a predifined training program, in which training structure was set, and no modifications were made througout the camp. This study, demonstrated that the daily individualized adjustment of the TL based on morning HRV measurement blunted the decrease in cardiac parasympathetic in the HRV-guided H group in comparison to the predefined training H group. Moreover, there was a significant improvement in performance and aerobic capacity in HRV-guided and H, without significant differences of change between these groups. [188]

To date, and to the best of our knowledge no studies have been published analyzing a HRV-guided program at terrestrial altitude in endurance disciplines.

1.4. Marathon. Physiology and training

Marathon is one of the events which are part of the Olympic Games, as part of the track and field athletics program. The intensity that marathon elite-athletes can maintain during the ~2 hours (men) and ~2 hours 20 min (women) is close to their VT2. [189] Some of the physiological factors that aknown to be related to marathon and 10000 m performance include: VO_{2max}; running economy (the O_2 cost in $mL \cdot kg^{-1} \cdot min^{-1}$ of running at a certain speed, or the O_2 cost of running a certain distance in $mL \cdot kg^{-1} \cdot km^{-1}$); and the fractional utilization of the VO_{2max}, which is linked to markers of blood lactate accumulation such as lactate threshold and maximal lactate steady state. [190,191] In addition, mathematical modelling indicates three physiological

components as key points of marathon performance: 1) VO_2 at lactate or ventilatory threshold; 2) VO_{2max}; 3) running economy. [192] Elite male distance runners typically have VO_{2max} values of 70–85 mL · kg^{-1} ·min^{-1} while their female counterparts have VO_{2max} values of 60–75 mL · kg^{-1} ·min^{-1}. [192,193,194] These values are ~ 45 % higher than those of age- and sex-matched sedentary individuals. [195] Moreover, the highest VO_{2max} values are found in 5000 m/3000 m steeplechaser specialists, because these athletes are required to run at 94–98 % VO_{2max} during 8-13 min. [196] Marathoners tend to have slightly lower VO_{2max} values compared to 5000 m-3000 m steeplechase specialists probably because their events are run at lower intensities (80-85 % VO_{2max}). [191] Running economy is the most determinant variable of marathon performance. In this regard, former world record-holder in the women´s marathon Paula Radclife (2h:15min:25s), decreased her O_2 cost for running at 16 km · h^{-1} from 1992 (205 mL · kg^{-1} · km^{-1}) to 2003 (175 mL · kg^{-1} · km^{-1}), which represents a 15 % improvement in running economy. In addition, Paula´s running speed at a lactate threshold shifted from 14-15 km · h^{-1} in 1992-1994 to 17.5-18.5 km · h^{-1} in 2000-2003. However, VO_{2max} did not change significantly between 1992 and 2003 (~70 mL · kg^{-1} · min^{-1}). [197] Thus, parameters linked to O_2 consumption or acidosis while running at submaximal speeds seem critical in elite-marathon performance.

Regarding training, case reports with elite-marathoners have been published, in which the average training sessions per week were 13 sessions, averaging 182 km with a peak volume of 231 km · $week^{-1}$. 74 % of all the training mileage was covered at an intensity below or close to VT1, 11 % at lactic threshold, and 15 % close to VO_{2max}. [198] In addition, in a study with top Kenyan athletes, Billat et al. reported that 78 % of the training was based on high-volume, low-intensity training, 4 % was performed close to the lactic threshold and 18 % was performed ≥ lactic threshold. [189] Moreover,

marathoners year periodization is divided into two periods, in which peak moments are in spring (e.g., London, Boston) and fall (Berlin, Chicago, New York). Interestingly, not many East African athletes can take part in three marathons per year (e.g., Dubai in January, London in April and New York in November), however, this is unusual due to the stressful of running mechanics. [199]

1.5. Marathon and wheelchair athletes

Marathon is a very popular event among wheelchair user population. This event, as part of track and field sport, is regulated by the International Paralympic Committee (IPC Athletics), and four categories are differentiated depending of the functional capacity of the athlete: 1) T51, athletes with high spinal cord injury impairment and no or very limited extension arm capacity (i.e., C4-5 spinal cord injuries); 2) T52, athletes with extension arm disfunction, such as athletes with quadriplegia or tetraparesia; 3) T53, athletes without upper limbs affection, but with non-funtionality inback muscles flexors and extensors (i.e., T1 to T7 spinal cord injuries); 4) T54, class athletes without upper limb affection and total functionality of back muscles flexors and extensors (i.e., lower limbs amputee athletes). This classification was created to avoid advantages among individuals within and between classes (i.e., T51, 52, 53, 54), looking for a homogeneous functional motoric capacity, and at the same time to preserve differences built up by training programs. In fact, depending on classes, athletes can develop greater power (e.g., T51 men ~25 W vs. T54 men ~150 W). [200] Wheelchair athletes can maintain average speeds of over 30 km · h^{-1}. And as aforementioned, while able-bodied marathoners are able to compete in two-three marathons per year, wheelchair athletes can race in more than ten marathons per year (e.g., Boston and London marathons within 6 d of difference). The reason for

this, might be the lower mechanical stress on joints, as body mass does not fall on the propulsion joints as in running, [199,200] despite of the fact that wheelchair´s handrim contact time while propulsing the chair in a case conducted with a world recordholder, [201] was shown to be similar (~210 ms) to ground contact time from elite-african athletes, but greater than the contact time of Elite-European athletes (~200 ms). [202] However, significant differences related to step frequency in elite-runners (180 to 200 steps · min^{-1}) [202] versus stroke frequency in elite-wheelchair athletes (111 strokes · min^{-1}) have been reported. [203] The scarce literature on wheelchair marathon, makes it difficult to analyze the physiology of this discipline. However, a study from Asayama et al. [204] reported similar cardiovascular load and lower O_2 uptakes in elite wheelchair marathoners when compared to able-bodied marathoners [189,197]. The reason might be in the lower muscle mass involved in wheelchair athletes compared to runners. [205] The participants from the Asayama study maintained an average HR throughout the marathon of 171.6 ± 20.5 beats · min^{-1}. [204] In contrast, an elite runner with a personal best marathon time of ≤ 2 hours and 11 minutes had an average HR of 167 ± 5 beats · min-^{1} during a 10 km test at marathon pace. [189]

1.6. Charcot Marie Tooth Disease and exercise

Charcot Marie Tooth disease (CMT) is the most common hereditary peripheral neuropathy, affecting up to 30 per 100000 people worldwide. CMT totally affects distal muscle function and partially affects proximal function. [206] In addition, there are many forms of CMT disease, and specifically type CMT II individuals might be wheelchair users due to their inability to walk. [206] Different studies have shown the benefits that interval training [207,208] and resistance [209,210] training have over CMT population.

However, no studies on CMT and demanding training programs have been published.

To date, only one study has analyzed the physiological and performance effect of a LHTH camp at high altitude (4000 m) in a group of well-trained collegiate runners. This study was conducted 53 years ago by Buskirk and colleages. [211] Moreover, no studies on altitude training among Paralympians have been conducted.

Therefore, the first aim of this research was to analyze the physiological, psychological and the performance effects of a 5-week 3860-4090 altitude training camp in an elite-wheelchair athlete marathoner with CMT, previous to his participation in the Boston and London Marathons (studies 1 and 2). The second aim was to analyze the physiological response during and after an international wheelchair marathon (study 3) in same athlete, who has a double role in this study as participant and researcher. Full details of aims and purposes are given in chapter 2: Objectives and hypothesis of the research.

2. OBJECTIVES AND HYPOTHESIS OF THE RESEARCH

2. OBJECTIVES AND HYPOTHESIS OF THE RESEARCH

2.1. Objectives

1) To compare the effects on performance (power output, 3000 m time trial) after returning to SL relative to pre-altitude of two different training programs: i) a predifined training program (Inflexible planning or IP) and ii) a HRV-guided training program (Flexible planning or FP) conducted during two consecutive years, in the specific preparatory period of the Boston and London marathons, during 5-weeks at 3860-4090 m terrestrial altitude in the Peruvian Altiplano (Puno, Peru) on a professional wheelchair marathoner with CMT.
2) To compare the effects on performance of 2000 m repetitions at VT2 at altitude relative to SL in IP and FP.
3) To compare the effects of IP and FP on cardiac autonomic activity (rMSSD and rMSSDCV), oxygen saturation (SO_2), systolic blood pressure (SP), diastolic blood pressure (DP), resting HR (HR_{rest}), conducted during two consecutive years, in the specific preparatory period of the Boston and London marathons, during 5-weeks at 3860-4090 m terrestrial altitude on a professional wheelchair marathoner with CMT.
4) To analyze changes occurred in red blood cell parameters (erythrocytes, Hb, Ret, Hct, EPO) during a 5-week HRV-guided training program conducted at 3860-4090 m terrestrial altitude and after returning to SL, in the base general training period, three months prior to his participation in the Boston and London

marathons, during 5-weeks at 3860-4090 m terrestrial altitude in a professional wheelchair marathoner with CMT.

5) To analyze changes occurred in resting breathing frequency (BF) during and after returning to SL after a 5-week HRV-guided training program conducted at 3860-4090 m terrestrial altitude, in the specific preparatory period of the Boston and London marathons, during 5-weeks at 3860-4090 m terrestrial altitude on a professional wheelchair marathoner with CMT.
6) To examine the psychological response (mood states) of an elite-wheelchair marathoner with CMT during a TL imposed throughout 7-weeks of training at SL using the POMS. This response would be considered as baseline. Moreover, to determine if any changes in mood states occurring at 3860-4090m terrestrial altitude would return to baseline when training resumed at SL under a reduced TL.
7) To examine the impact of a TL imposed at 3860-4090 m terrestrial altitude by an HRV-guided program on the mood states in a professional wheelchair marathoner with CMT.
8) To examine the influence of altitude on mood states when considering SO2, SP and DP as covariates, assessed at 3860-4090 m terrestrial altitude in a professional wheelchair marathoner with CMT.
9) To determine if any changes in mood states occurring at 3860-4090m terrestrial altitude would return back to baseline when training resumed at SL under a reduced TL in a professional wheelchair marathoner with CMT.
10) To assess the effectiveness of an evidence based individualized nutrition program applied to an elite wheelchair marathoner with

CMT during a 5-week HRV-guided training camp conducted at 3860-4090 m terrestrial altitude.

11) To evaluate an individualized hydration intervention, and its effects on the athlete's body mass, diuresis, and hydration status in a professional wheelchair marathoner with CMT prior to and during a 5-week training camp at 3860-4090 m terrestrial altitude, and also after returning to SL.
12) To analyze HRV oscillations before and after a marathon which involved trans-meridian air travel and substantial time zone differences in a professional wheelchair marathoner with CMT.

2.2. Hypothesis

1) The FP model will produce greater increases in performance than the IP model. [142,168]
2) No improvement in performance (relative to both models) will be observed after returning to SL relative to pre-altitude. [211]
3) The IP model will produce greater disturbance in the autonomic cardiac vagal activity (greater rMSSDCV) [142,160] than the FP model. [188,212]
4) Performance of 2000 m repetitions at VT2 at altitude in both models will decrease by ~20 % compared to SL. [211]
5) A lower SO_2 [10] and a greater HR_{rest} [40] will show at altitude compared to SL in both models.
6) Plasma EPO concentration in a FP model will significantly increase during acute altitude exposure, [16] decreasing to below pre-altitude values at chronic altitude exposure. [21] In addition, EPO concentration will be lower after returning from altitude compared to pre-altitude. [21,22]

7) A significant increase will be observed at altitude (FP model) in erythrocytes, Hb, Ret, and Hct compared to pre-altitude. [2] In addition, peak values in erythrocytes, Hb, and Hct will be observed after the altitude camp completion. [37] Moreover, after returning to SL, erythrocytes, Ret and Hb will remain elevated compared to pre-altitude. [20,21,22,26,27,28]
8) An enhancement in resting BF will be observed after arrival to altitude in an FP model. [45,46,47]
9) An increase in TMD will be observed when TL increases at SL and vice-versa in an FP model. [108]
10) A lower TL imposed at altitude (FP model) will have a greater TMD compared to SL.[111]
11) Despite a significant increase in daily energy intake at altitude (FP model) compared to SL, body mass will decrease (2-5 %) at altitude compared to SL as a consequence of a greater amount of carbohydrates and protein daily intake, [52] the greatest magnitude of change appearing during altitude acclimatization. [48,49,50,51] Moreover, body mass will return to pre-altitude values immediately after returning to SL after altitude sojourn. [52]
12) To maintain an optimal hydration status (SG $\leq$ 1.020), a significant greater fluid intake will be observed throughout the entire altitude sojourn (FP model) compared to sea level. [50,51,79] In addition, the greatest magnitude of change will be observed during acute altitude exposure. [50,51] As a consequence of greater fluid intake, diuresis will be greater at altitude compared to SL. [51]
13) There will be a significant decrease in cardiac autonomic vagal markers (i.e., LnrMSSD), and an increase in LnrMSSDCV, the day after arriving to the new timetable zone compared to baseline,

calculated with the data from the six days prior to the overseas flight. [161]

14) A slight decrease in rMSSD compared to baseline will be observed the morning of the marathon, as a consequence of precompetitive anxiety [213] and pre-race tapering. [164]

15) The greatest reduction in rMSSD [139,214,215,216] and the greatest rMSSDCV [154] compared to baseline will be observed the day after the marathon completion.

3. SUMMARY OF THE METHOD

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3.1. Participant

The study participant was a 36-year-old professional wheelchair racer, class T52 (upper limb involvement category). Some of his awards include winning two silver medals and two bronze at the Sydney 2000 and Athens 2004 Paralympic Games, as 107 victories in international road events, including a victory at the 2016 Boston Marathon, ten weeks after returning to SL from Los Andes. Our participant's Height = 1.76 m; Body mass = 52.6 ± 0.4 kg; Power output at second lactic threshold = 62 W; training 8000 km per year; number of season sessions ~450; former world record-holder in his division (T52, quadriplegics) in 800 m (1min:56s) and 1500 m (3min:36s); world record-holder in 5000m (12min:37s), half marathon (50min:28s) and fourth best ever time in marathon (1h:42min:05s). Additionally, he has more than ten years of altitude training experience, with training camps performed in Boulder, CO, USA (1655 m), Navacerrada, Spain (1858 m), Flagstaff, AZ, USA (2106 m), Sierra Nevada, Spain (2320 m), Keystone, CO, USA (2796 m) and Breckenridge, CO, USA (2926 m), performing both altitude models: LHTH and LHTL, and has been exposed more than 8000 hours to normobaric-hypoxia. Training mileage from the last five-seasons were 7124 km, 8150 km, 8600 km, 8410 km and 9173 km. In the last five seasons he performed 410, 456, 451, 440 and 502 sessions; while days spent at moderate terrestrial altitude (from 1655 to 2926 m) for the last five seasons were: 78, 82, 101, 79 and 62 days. The study was part of the participant´s doctoral thesis research project. The study was approved by the Ethical Committee of the Miguel Hernandez University, Elche, Spain and it was conducted conforming to the recommendations of the Declaration of Helsinki.

3.2. Experimental design

3.2.1. Inflexible planning study, IP (January-February 2015)

The week before the altitude sojourn (B_N), the athlete completed exactly same training as the first week at altitude (B_H), and the week after returning from altitude (Post). All sessions performed at B_N and Post were done at 16 m terrestrial altitude. Sessions performed at B_H were at 3860 m terrestrial altitude. Moreover, the first 2 days of B_H involved passive rest to minimize jet lag and acute mountain sickness symptoms caused by the long trip from Spain to Peru (6-hour time difference) and the change in altitude. Two daily training sessions were performed from Wednesday to Friday at an intensity below the aerobic threshold (i.e., "easy push"). The morning and afternoon sessions were 20 and 16 km workouts. At $B_{N,}$ Saturday afternoon and Sunday was overseas trip (Alicante – Madrid – Lima – Juliaca – Puno). An HRV reference value (RV) in B_H was calculated to perform specific sessions, but it was not used in the IP model. RV was calculated as one standard deviation below the mean of rMSSD throughout B_H. [142] Weeks 2 to 5 at altitude ($W_{1,2,3,4}$) included specific predefined training. 12 sessions per week (200 km every 6 consecutive days) were performed, and Sundays were reserved for passive rest. Two resistance sessions were performed on Mondays and Thursdays, and 2 interval sessions were performed on a plateau at 4090 m terrestrial altitude on Tuesdays (20 x 400 m, recovery time between repetitions: 75 seconds) and Fridays (6 x 2 km, recovery time between repetitions: 120 seconds). Two-hour sessions at the aerobic threshold were performed on Wednesdays and Saturdays. Flight back home was on Monday at Post week, arriving to Spain on Tuesday (Table 3).

3.2.2. Incidences occurred throughout IP

In W_2 and W_4 on Friday and Saturday, the athlete was not able to complete

the afternoon session (12 km <VT1) due to his feeling of exhaustion.

In W_3 on Friday morning the athlete was forced to modify his session (8 km <VT1 + technique drills + 5 x 80 m + 6 x 2000m + 2 km <VT1) due to heavy rain. The athlete performed 12 km <VT1.

In W_4, the afternoon session on Monday was canceled due to heavy rain.

Table 3. Training program schema throughout IP.

	B_N	B_H	$W_{1,2,3,4}$	Post
Monday AM	Rest	Rest	10 km <VT1 + Strength session 4 x 8 repetitions (80 % RM) (3860 m altitude)	Flight
Monday PM	Rest	Rest	16 km <VT1 (3860 m altitude)	Flight
Tuesday AM	Rest	Rest	8 Km <VT1 + technique drills + 5 x 80 m + 20 x 400m VT2; recovery time between repetitions 75 seconds + 2 km <VT1 (3860-4090 m altitude)	Flight
Tuesday PM	Rest	Rest	16 km <VT1 (3860 m altitude)	Arrival
Wednesday AM	20 km <VT1	20 km <VT1	2 hours VT1 (3860-4090 m altitude)	20 km <VT1
Wednesday PM	16 km <VT1	16 km <VT1	12 km <VT1 (3860 m altitude)	16 km <VT1
Thursday AM	20 km <VT1	20 km <VT1	Same as Monday AM	20 km <VT1
Thursday PM	16 km <VT1	16 km <VT1	Same as Monday PM	16 km <VT1
Friday AM	20 km <VT1	20 km <VT1	8 Km <VT1 + technique drills + 5 x 80 m + 6 x 2000m VT2; recovery time between	20 km <VT1

			repetitions 120 seconds + 2 km <VT1 (3860-4090 m altitude)	
Friday PM	16 km <VT1	16 km <VT1	12 km <VT1 (3860 m altitude)	16 km <VT1
Saturday AM	Rest	Rest	Same as Wednesday AM	Rest
Saturday PM	Flight	Rest	Same as Wednesday PM	Rest
Sunday AM	Flight	Rest	Rest	Rest
Sunday PM	Arrival	Rest	Rest	Rest

3.2.3. Flexible planning study, FP (January-February 2016)

Almost the same training structure was followed in FP than IP in $B_{N,}$ B_H, and Post with the exception of a 20 km <VT1 workout performed on Saturday morning, due to a later schedule flight to Peru on Saturday. All the sessions performed at B_N and Post were done at 16 m terrestrial altitude. Sessions performed at B_H were at 3860 m terrestrial altitude. Moreover, from W_1 to $W_{4,}$ the specific sessions were performed only if the HRV RV was reached after awakening HRV assessment. Specific sessions at altitude were: A (20 x 400 m; recovery time between repetitions 75s); B (2 hours at the aerobic threshold or VT1); C (6 x 2000m; recovery time between repetitions 120s). The order to perform specific sessions was always in the same sequence: A, B, C, and the sequence order would not be affected by the end of a week. In case of not reaching RV, two workouts were performed: (20 km <VT1 in the morning and 16 km <VT1 in the afternoon). The resistance sessions with same structure as in IP were performed from W_1 to W_4 on Mondays and Thursdays afternoon: however, they were not preceded by a 10 km <VT1 workout. Moreover, a 16 km < VT1 was carried out on Mondays and Thursdays morning (Table 4).

3.2.4. Incidences occurred throughout FP

At W_4 on Monday, heavy rain forced the athlete to cancel sessions.

At W_4 on Saturday, the athlete was not able to complete the afternoon session (16 km <VT1) due to his feeling of exhaustion.

3.2.5. HRV and HR_{rest} assessment

Heart rate variability was recorded daily in supine position after waking, bladder emptying, and in a fasted state. A metronome was used during HRV recordings to control the respiratory rate (15 Breaths · min^{-1}). A HR monitor (Polar RSCX 800; Kempele, Finland) was used to record R-R intervals. Filtering, correction, and detrending were applied to avoid ectopic beats, using specific software (Polar Protrainer 5.0, Polar Electro, Kempele, Finland). The HRV parameters were calculated with Kubios HRV 2.0 (Kuopio, Finland, 2008) analyzing the last 5 minutes of a 10-minute recording. The rMSSD was selected as the main index to assess HRV. The weekly CV of rMSSD was calculated for W_1–W_4, following formula: rMSSDCV = (SD/mean) x 100. [160]

The HR_{rest} was calculated as the HR average of the last 5 minutes of the R-R interval recording.

Table 4. Training program schema throughout FP.

	B_N	B_H	$W_{1,2,3,4}$	Post
Monday AM	Rest	Rest	16 km <VT1 (3860 m altitude)	Flight
Monday PM	Rest	Rest	Strength session 4 x 8 repetitions (80 % RM) (3860 m altitude)	Flight
Tuesday AM	Rest	Rest	HRV	Flight
Tuesday PM	Rest	Rest	HRV	Arrival
Wednesday AM	20 km <VT1	20 km <VT1	HRV	20 km <VT1
Wednesday PM	16 km <VT1	16 km <VT1	HRV	16 km <VT1
Thursday AM	20 km <VT1	20 km <VT1	Same as Monday AM	20 km <VT1
Thursday PM	16 km <VT1	16 km <VT1	Same as Monday PM	16 km <VT1
Friday AM	20 km <VT1	20 km <VT1	HRV	20 km <VT1
Friday PM	16 km <VT1	16 km <VT1	HRV	16 km <VT1
Saturday AM	20 km <VT1	20 km <VT1	HRV	20 km <VT1
Saturday PM	Flight	Rest	HRV	Rest
Sunday AM	Flight	Rest	Rest	Rest
Sunday PM	Arrival	Rest	Rest	Rest

3.2.6. Oxygen saturation assessment

The SO_2 was measured with a finger pulse oximeter (Colson 650 2100;

Frouard, France) in a seated position on awaking.

3.2.7. Blood pressure assessment

The brachial blood pressure was measured (only in FP) in a seated position, with a validated (Omron HEM- 705CP) oscillometric sphygmomanometer. Measurements were made in triplicate and averaged. Both SP and DP were recorded.

3.2.8. Laboratory test

Four days before B_N (Pre_{-4}) and 11 days after the altitude camp ($Post_{11}$), an incremental test was performed on a specific wheelchair ergometer, in which steady conditions were maintained (temperature 22–24^o C, humidity 73–75 %). The protocol (as described by Polo-Rubio) [217] included a 20-minute warm-up period at constant power (20 W). Then, the athlete started an incremental test at a brake power of 6 W, maintaining a stroke frequency between 90 and 100 strokes · min^{-1} and increasing the power by 3 W every 60 seconds until the athlete's heart rate passed 170 b · min^{-1} (just over his marathon pace intensity). Power output was considered as the ergometer braking power during the last completed step of the test. A HR monitor was included to measure HR. VO_2 was not assessed due to the invasiveness of wearing a gas analyzer device during wheelchair propulsion.

3.2.9. 3000 m Test

Three days before B_N (Pre_{-3}) and 12 days after the altitude camp ($Post_{12}$), a 3000 m test was performed on a 200-m indoor track. The test started from a static position after a 6 kilometer plus 80 m strides warm-up, Conditions for all track tests were: temperature = 18.3 ± 2.1° C and humidity = 74–79 %. The reason for choosing $Post_{12}$ as the day to perform the 3000-m test was because the athlete won at the Oita Marathon (1 hour 43 minutes 46 seconds)

and the Chicago Marathon (1 hour 46 minutes and 13 seconds), both still quadriplegic division course records and both set in 2007 12 days after his arrival from altitude, after completing camps at 2320 m terrestrial altitude.

3.2.10. Blood markers and resting BF assessment

Blood markers and resting BF were only analyzed in FP due to funding constraints. Moreover, attending to reviewers suggestions in the review process of this manuscript, acronyms of phase time periods from the experimental design were shifted from B_N to W_{-1}, B_H to W_1, W_1 to $W_{2,}$ W_2 to $W_{3,}$ W_3 to $W_{4,}$ W_4 to W_5 and Post to W_{+1}.

Blood tests were conducted, under fasting conditions, after a day of rest. Blood was withdrawn: i) the first day of the season or 7 weeks before the altitude camp ($T_{-7\ weeks}$); ii) 7 days before the altitude camp ($T_{-7\ days}$); iii) at the altitude camp on days eight, fifteen, twenty-one, twenty-eight and thirty-five ($T_{8,15,21,28,35\ days}$); iv) 6 days after returning from altitude ($T_{+6\ days}$). Plasma EPO was assessed: i) 7 days before the altitude camp ($T_{-7\ days}$); ii) 35 days after altitude arrival (T_{35h}); iii) on the 21st day at the altitude camp ($T_{21\ days}$); iv) 16 days after returning from altitude ($T_{+16\ days}$). Hb and erythrocytes count were measured with a Coulter T 840 Counter (Coulter Electronics, Krefeld, Germany). Hct was determined with microcentrifugation. Ret were measured with flow cytometry (Epics XL, Beckmann). For serum EPO, a 7 mL blood sample was centrifuged (3000 rpm, 10min, 4 degrees Celsius) and measured (Erythropoietin ELISA, IBL, Hamburg, Germany). Ferritin was measured photometrically (EIAgen Ferritin Kit, Adaltis, Freiburg, Germany) (Figure 2).

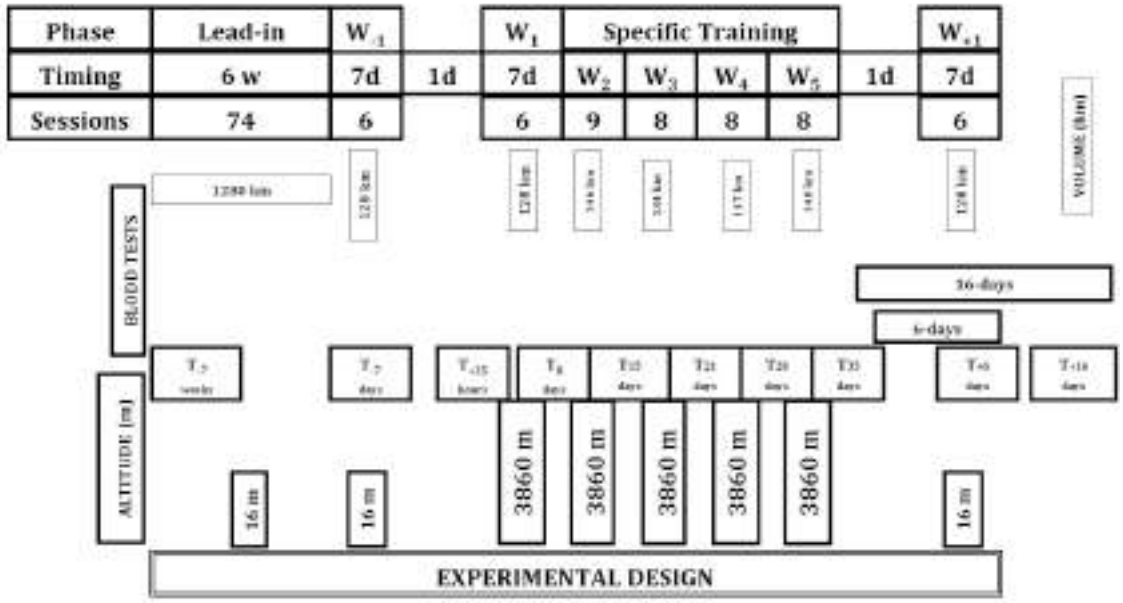

Figure 2. Blood tests schedule throughout the FP model.

An Hexoskin wearable body metrics shirt (Hexoskin, Carré Technologies Inc. San Francisco, CA), which was found to be valid and reliable, [218] was used to assess resting BF. Measurements were obtained upon awaking in supine position during 5 min, with the average value for the last 60 s retained for final analysis.

3.2.11. Mood states assessment

Mood states were assessed using the Spanish short form version of the Profile of Mood States self-report questionnaire (POMS). This version consists of 58 adjectives, each rated on a five-point scale. [219] Seven weeks before the altitude camp (starting season week), every Sunday (rest day) after breakfast, the athlete responded to the question of how he had felt during the previous week including that day. The first week results constituted baseline. Week 7 (B_N) data was not included for analysis because it coincided with the overseas travel. The timing sequence periods for this study were: W1 to W7

pre-altitude weeks; W8 to W12 altitude sojourn; W13 week after returning from altitude.

3.2.12. Training load estimation and RPE assessment

Physical TL was defined with Foster´s equation [220] as the product of the rating of perceived effort (RPE), using Borg scale 1-10 [120] multiplied by the active time of the session duration. RPE was recorded thirty minutes after the morning and afternoon sessions and it was calculated based on the morning sessions (one-day training) or as the average of the morning and afternoon sessions (two session training day). Daily TL was calculated by adding the Fosters values for morning and afternoon sessions. Weekly TL was calculated as the addition of the daily values from Monday to Saturday.

3.2.13. Body mass assessment

Throughout the experiment, basal body mass was daily recorded in fasting conditions, naked, after waking up, with a digital scale (Tanita BC-601®, TANITA Corporation, Tokyo, Japan).

3.2.14. Daily intake recording

A nutritional diary was maintained by the participant using a food recording system [221] to record the daily intake, which included main meals (breakfast, lunch and dinner), two small snacks and all training activities that occurred post-intake.

Total energy (kcal), carbohydrates, proteins and fats ($g \cdot kg^{-1}$ body mass) were estimated according to a nutritional composition database supported by the Spanish Ministry of Science and Innovation (BEDCA). [222]

3.2.15. Diuresis and SG assessment

Attending to the reviewers suggestions in the review process of this manuscript, acronyms of phase time periods from experimental design were

shifted from B_N to W_{-1}, B_H to W_1, W_1 to W_2, W_2 to W_3, W_3 to W_4, W_4 to W_5 and Post to W_{+1}.

Urine was collected throughout the day (resting and training hours) with a 2000 mL recipient with 100 mL reference marks. Urine was weighed (Tanita kd-321®, TANITA Corporation, Tokyo, Japan), while liquid intake (Fluid) was recorded by weighing liquid consumed/leftover in bottles used for training and resting hours. Urine specific gravity (SG) was measured upon waking (AM SG) (Mission® U500, ACON Laboratories, San Diego, California) and two hours after dinner (PM SG).

3.2.16. Hydric balance estimation

To estimate Hydric Balance (HB) after each session, we used the formula: HB = (ingested fluid volume) - (water loss); [223] in which ingested fluid volume corresponds to the liquid intake during a session. Fluid volume intake for morning session and afternoon session were identified as Fluid Vol.1 and Fluid Vol.2, respectively. If volume suggested (VS) for training was not consumed completely, leftover liquid (LL) was weighed and the formula fluid volume = VS – LL was applied. Water loss was quantified as the difference between pre- and post-training body mass.

3.2.17. Sodium estimation

Na^+ from solid intake was estimated according to the nutritional composition established by BEDCA. [222] Moreover, Na^+ from liquid intake was estimated according to the nutritional composition labeled on both, sport drinks and gels consumed by the athlete.

3.2.18. Ambient temperature and humidity recording

Ambient temperature and relative humidity were recorded with a portable device (Tenmars TM-183®, Taipei, Taiwan) attached to the racing

wheelchair. Average temperature and humidity were recorded daily prior to the training sessions.

3.2.19. Hydration plan

The sport drink used for workout routines was Isolin Isotonic (AMIX). It was recommended that the athlete drink ~700 mL solution for workouts at <VT1 and a solution of 1250 mL plus a 70 mL CHO gel for specific sessions. Recommended drinking rate was 100 mL every 10 min as previously reported.[77] Water was consumed *ad libitum* during resistance training and a minimum of 400 mL was consumed immediately after the resistance session as a rehydration strategy. Overall, the daily liquid consumption target was 4 to 5 L. [79]

3.2.20. Nutritional program

The athlete was instructed by a nutritionist to prepare all his meals. This included weighing ingredients prior to cooking them and the left overs prior to their disposal. On the days when the athlete ate at restaurants, which occurred on four occasions, he was instructed to send pictures of these meals to the research team. [224] A personal chef was contacted to buy and cook all foods/ingredients for the athlete on a daily basis according to the athlete´s instructions while the weighing and cooking process occurred under the athlete's supervision. Additionally, the athlete was instructed to prepare all training drinks and post-training recovery solutions. At SL the athlete cooked all his meals at home.

Daily energy intake was increased ~20 % from pre-altitude (B_N), to arrival at altitude (B_H) to avoid body mass loss from increased RMR which, as aforementioned is common while living and training at higher altitudes (i.e., 2200 m [63] and 3650 m [64]). Moreover, main meals were designed according to the type of training session performed. In addition, main meals were

accompanied by two rich-carbohydrate snacks, based on reports that the inclusion of several rich CHO snacks, covers increased energy requirements better than three standalone main meals. [76] Furthermore, regarding proteins, a minimum intake of 2.4 g · kg^{-1} body mass was targeted in the current nutritional design to avoid loss of lean mass. [225]

To avoid gastrointestinal issues (GI) and fullness, [226] a low protein/fat intake was provided for the breakfast and PM sessions; however, the percentage of lipids at lunch was lower than at dinner. Protein intake at lunch and dinner were ≈ 1 g · kg^{-1}, given that the specific and most physiological demanding sessions (A, B, C) were performed in the morning, and muscle tissue repair was a main meal target. [225] The ingestion of lipids was set at a minimum of 1 g · kg^{-1} body mass throughout SL and altitude camps, as fat cells increase their sensitivity to hormonal stimulation after training, resulting in a greater mobilization of fatty acids. [227]

3.2.21. Nutritional intake during and immediately after training

A sport drink (Iso-Lyn Isotonic, AMIX) was used for workouts <VT1 which were shorter than 65 min (20 and 16 km). The athlete was instructed to drink a 750 mL water and 56.4 g of CHO solution, while a 1250 ml with 80 g of CHO solution was recommended for specific sessions. The CHO rate was 0.5 to 1 g · kg^{-1} body mass per hour. [228] Moreover, the athlete drank a 42 g CHO (Glucose + Fructose) Iso-Gel carbo snack (AMIX) during specific session workouts. [229] Gels were consumed in the A session after fourteen 400 m rep, in the B session 90 min after starting and in the C session after four 2000 m rep. Both types of carbs used in the solution and gels were multiple transportable carbohydrates, as directed by Jeukendrup. [230]

Water was consumed *ad libitum* during the gym sessions and immediately

after the gym sessions the athlete co-ingested a rich leucine whey protein (23.6 g) (Whey Fussion, AMIX) dissolved in 400 mL of water and a CHO gel (Iso-Gel Recovery, AMIX) (37.6 g maltodextrin + fructose + Vitargo ®) as directed for speeding up to 25 % glycogen synthesis. [231] For refueling purposes, carbohydrate guidelines [228] suggest aiming for post-exercise rapid recovery of muscle glycogen deposits, with 1 g · kg^{-1} body mass of CHO, repeated every 2-3 hours. After specific sessions, a carbohydrate shake was taken with a carbohydrate gel, providing 1.4 g · kg^{-1} body mass. In the hour immediately after the 16 km and the 20 km <VT1 workouts, the athlete drank a carbohydrate solution (Carbojet Gain, AMIX) (34 g CHO, 7.5 g prot, 1.8 g fat) dissolved in 400 mL of water, and after the specific sessions he ingested a combination of the same drink plus an Iso-Gel Recovery. To consider, 2.4 g · kg^{-1} body mass CHO were consumed at lunch which occurred approximately two hours post-exercise meal, in order to achieve 3.1 g · kg^{-1} body mass of CHO 3h post-exercise for our athlete vs. 3 g · kg^{-1} body mass as suggested by Burke et al. [228]

On specific session days, rest took place in the evenings along with a snack at 5:30 PM, to meet increased energy requirements. [76] This snack included two 30 g cereal bars (Tri-Fit Bar, AMIX) (34.9 g CHO, 3.9 g prot, and 10.1 g fat).

3.2.22. Nutritional supplements

To try to avoid loss of body mass [232] and enhance muscle protein synthesis [233] the athlete consumed (2.5 g leucine, 1.5 g isoleucine, and 1.5 g valine) immediately after each session (BCAA Elite Rate, AMIX). Before bedtime, 30 g of casein protein (Micellar Casein, AMIX) (1.7 g CHO, 24 g prot, 0.6 g fat) was ingested as suggested by Snijders et al. [234]

Finally, the athlete maintained iron levels with a daily intake of 105 mg of ferrous sulphate (Ferogradumet®, Ross, Abbott Científica), as ferrous

sulphate intake has been related to the production of Hb and RBC. [235,236] To comply with World Anti-Doping Agency (WADA) regulations, none of the aforementioned supplements contained any prohibited substance.

3.2.23. Physiological assessment before, during and after international marathon (November 2015)

Ten days before the race date (RD) an aforementioned incremental test [217] was performed on a specific wheelchair ergometer in which steady conditions were maintained (temperature 22-24° C, humidity 73-75 %). Power output was considered as the ergometer braking power during the last completed step of the test. [217] The same HR monitor used in the marathon was used to register HR and a telemetry system (K4 b2, COSMED, Rome, Italy) was used during wheelchair propulsion to measure O_2 uptake and CO_2 production. The recommendations by Chicharro et al. were followed to calculate Vt2. [237]

The day-to- day HRV upon awakening in supine position and after bladder-emptying were measured six days before ($RD_{-6,-5,-4,-3,-2,-1}$) the marathon day in Oita, Japan and two days after racing ($RD_{+1,+2}$). A metronome was used to ensure breathing was consistent (15 Breaths $\cdot$ min $^{-1}$) for the HR recordings (Polar RSCX 800, Kempele, Finland). Detection and correction were applied to the R-R intervals to remove ectopic beats. [238] The rMSSD was calculated using Kubios HRV 2.0 (Kuopio, Finland, 2008), analysing the last five minutes of each 10-min recording. [238] Only natural logarithm (Ln) values were used in the statistical analyses. The rMSSD were chosen as the main vagal HRV index.

The same monitor for morning HRV recordings was used for HR recording during exercise. During the race, a GPS (Polar, G3 GPS sensor, Polar ElectroOy, Kempele, Finland) was used to measure speed and record split

times every 5000 m (0-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30- 35, 35-40) and the last 2195 m (40-42.2).

The athlete flew from Spain (GMT + 1) to Japan (GMT +9) immediately after the morning HRV assessment on RD_{-3}, so no measurements were taken on RD_{-2} because the trip took almost thirty hours.

The Japanese Association of Athletics Federations officially approved the marathon course in Oita city. Race conditions were dry with temperatures ranging between 24-26° C, 77% humidity and 1.39 $m \cdot s^{-1}$ winds. The course was almost completely flat, with starting and finishing lines at the same altitude, so the only up and down course oscillations were short bridges.

Some nutritional recommendations were followed to avoid a performance decline and to optimize HR response. [239] A solution of 1250 mL with 70 g of CHO was consumed at a comfortable rate, adhering to the minimum rate of 100 mL · 10 min^{-1} to avoid dehydration. [77,78] Therefore, the athlete´s drinking-rate was 116 mL · 10 min^{-1}. Moreover, at the 31 km mark he consumed a 42 g CHO gel (Glucose + Fructose). [229] The total CHO rate was 1.03 $g \cdot min^{-1}$ which was within the recommended range. [228]

3.3. Statistical analysis

Statistical analyses were performed using SPSS version 22.0 (SPSS, Inc., Chicago, IL, USA) software and Statgraphics (STSC, Inc., Rockville, MD, USA) version 16.1.17.

3.3.1. Analysis from physiological comparison between IP and FM models

The distribution of each variable was examined using the Kolmogorov-Smirnov normality test. Natural logarithm transformations (Ln) were applied to rMSSD. All data (except SO_2, SP, and DP) were normalized in

percentages with reference to mean value estimated at B_N (Δ), and presented as a mean ± SD. A repeated-measures ANOVA was performed for rMSSD, SO_2, HR_{rest} including the factor TIME with levels B_N, B_H, W_1, W_2, W_3, W_4, and Post. A post hoc least significant difference (LSD) multiple range test determined differences between factor levels. Pearson's correlation coefficients were calculated for the rMSSD, SO_2, HR_{rest}, SP, and DP variables. Statistical significance was set at alpha = 0.05.

3.3.2. Analysis for blood markers and resting BF assessed during FP model

Blood parameters and ferritin data are presented as raw values, while BF data are presented as mean ± SD (Table 2). A ΔEPO was calculated with reference to $T_{-7\ days}$ which was assigned the value 100%. Δerythrocytes, ΔRet, ΔHb, ΔHct and Δferritin were calculated with reference to $T_{-7\ weeks}$ and they were assigned the value 100%. Repeated-measures ANOVA was carried out for BF with level W_{-1}, W_{1-5}, and Post. Effect size (d) associated with change in BF were calculated using Cohen's d (difference in mean scores over time divided by pooled SD) and they were interpreted as trivial (≤0.19), small (0.20–0.49), medium (0.50–0.79), and large (≥0.8). [240] An alpha level of 0.05 was stated for statistical significance.

3.3.3. Analysis for POMS dimensions in the FP model

Kolmogorov-Smirnov was used to assess data normality. A repeated measures ANOVA was performed for POMS dimensions with TIME at two levels (SL and altitude). SO_2, SP and DP were added as covariates. A post hoc least significant difference (LSD) multiple range test determined the differences between factor levels. Pearson's correlation coefficients were calculated for tension, fatigue, vigor, anger, depression, confusion, TMD, weekly Foster, SO_2, SP and DP. Effect size (d) associated with change in

every POMS dimension was calculated using Cohen's d and it was interpreted as trivial (≤0.19), small (0.20–0.49), medium (0.50–0.79), and large (≥0.80). Statistical significance was set at alpha = 0.05.

3.3.4. Analysis for nutritional intervention in the FP model

Kolmogorov-Smirnov was used to assess data normality. All data are presented as mean ± SD. A repeated-measures ANOVA was carried out for body mass, daily intake, CHO, proteins and fat, including the factor TIME with levels B_N, B_H, W_1, W_2, W_3, W_4 and Post. A post hoc LSD multiple-range test was performed to determine differences between the factor levels. Effect size (d) associated with change in body mass was calculated using Cohen's d with its 95 % confidence limits (CL), [241] and they were interpreted as trivial (≤0.19), small (0.20-0.49), medium (0.50-0.79), and large (≥0.80). An alpha level of 0.05 was stated for statistical significance.

3.3.5. Analysis for hydration status assessment in the FP model

All data are presented as mean ± SD. Data were screened for normality with a Kolmogorov Smirnov test. A repeated measures ANOVA was used for SG AM, SG PM, Fluid, Na^+, urine excretion, Fluid Vol.1, Fluid Vol.2, including the factor TIME with levels W_{-1}, W_1, W_2, W_3, W_4, W_5 and W_{+1}. A post hoc LSD multiple range test was performed to examine differences between factor levels. Effect size (d) associated with change in body mass, Fluid, and urine were calculated using Cohen's d (difference in mean scores over time divided by pooled SD) and they were interpreted as trivial (d≤0.19), small (0.20-0.49), medium (0.50-0.79), and large (d≥0.80). Statistical significance was set at an Alpha level of 0.05.

3.3.6. Analysis for physiological assessment pre-, during, and post-marathon

Ln rMSSD was averaged from RD_{-6} to RD_{-2} to serve as baseline. The smallest worthwhile change (SWC) in Ln rMSSD was determined as ± 0.5 of the baseline standard deviation. [242,243] Thus, all Ln rMSSD values obtained post-travel were compared to the baseline thresholds.

The distribution of marathon-derived exercise HR was examined using the Kolmogorov-Smirnov normality test. A repeated measures ANOVA was carried out for HR variable, including the factor RACE SPLIT into levels 0-5, 5- 10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40 and 40-42.2. A post hoc LSD multiple range test determined differences between factor levels. An alpha level of 0.05 was stated for statistical significance.

4. SUMMARY OF THE RESULTS

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This chapter summarizes the main results obtained in the studies presented in this research.

4.1. Results from IP and FP models

4.1.1. Differences between training programs at altitude between models

Training distance from W_1 to W_4 in IP was 33.1 % greater than in FP (806 vs. 539.7 km). Moreover, during specific training weeks the difference of sessions was 25 % greater in IP (44 in IP vs. 33 in FP). In addition, the number of sessions at VT1 were 50 % greater in IP (8) than in FP (4). As a consequence, the distance covered at VT1 was 55.3 % greater in IP (266 km) compared to FP (118.8 km). The same number (8) of interval sessions (A, C) were performed in both models; however, in FP the number distance covered at VT2 was 5 % greater (80 vs. 76 km).

4.1.2. Estimation of the HRV RV

The SD of the mean of rMSSD upon wakening throughout B_H in IP was 27.0 ms, while in IP it was 19.55 ms.

4.1.3. HRV in IP and FP

Normalized rMSSD was greater in both ($p = 0.001$) IP and FP at SL compared to altitude. No differences were found between B_N (100 %) and Post at both IP (88.94 ± 25.34 %) and FP (104.47 ±35.80 %). In IP, rMSSD remained > 10 % below at Post (88.94 ± 25.34 %) compared to B_N.

4.1.4. HRV within-models analysis

During FP, normalized rMSSD was significantly greater than IP at B_H (72.94 ± 11.59 vs. 57.30 ± 2.38 %, p = 0.004), W_2 (81.65 ± 8.87 vs. 63.99 ± 10.32 %, p = 0.005), and W_4 (59.35 ± 6.81 vs. 46.11 ± 8.61 %, p = 0.008).

4.1.5. rMSSDCV during specific training weeks (W_1 to W_4) during IP and FP

The athlete demonstrated consistently larger reductions in rMSSD relative to baseline and thus a higher rMSSDCV during each training week at altitude during IP. CV percentage fluctuations in IP from W_1 to W_4 were 7.6; 4.6; 5.9; 7.3 % while fluctuations in FP from W_1 to W_4 were 3.9; 3.3; 3.7; 4.0 %.

4.1.6. SO_2 in IP and FP

In IP, greater hypoxemia (p = 0.001) was observed in B_H compared to B_N (86.29 ± 0.53 vs. 98.86 ± 0.12 %). From B_H (86.29 ± 0.53 %) to W_4 (92.06 ± 1.35 %), SO_2 values increased each week. Greater hypoxemia was shown in B_H with SO_2 values being significantly lower (p = 0.001) than in B_N (88.31 ± 2.46 vs. 98.64 ± 0.14 %) in FP. From B_H (88.31 ± 2.46 %) to W_4 (92.64 ± 1.12 %), SO_2 values increased each week, the same as in IP.

4.1.7. SO_2 within-models analysis

SO_2 was higher in FP at B_H (88.31 ± 2.46 vs. 86.29 ± 0.53 %, p = 0.049), W_1 (91.19 ± 0.76 vs. 88.74 ± 0.90 %, p = 0.001), and W2 (91.92 ± 0.82 vs. 90.33 ± 0.53 %, p = 0.001), suggesting a faster normalization at altitude of this variable.

4.1.8. HR_{rest} in IP and FP

In IP, increasing normalized HR_{rest} was observed after exposure to altitude (B_N vs. B_H) (130.49 ± 8.10 %, p = 0.001). HR_{rest} at Post (96.20 ± 4.23 %) was

significantly lower (p = 0.001) than at altitude values. A negative correlation was found between HR_{rest} and SO_2 (r = 20.43; p = 0.0188). No differences were observed within any altitude period. In FP, normalized HR_{rest} increased significantly (p = 0.001) from B_N to B_H (130.17 ± 5.93 %). HR_{rest} at Post (96.64 ± 8.99 %) was significantly lower (p = 0.001) than at altitude values. A negative correlation was found between HR_{rest} and SO_2 (r = 20.83; p = 0.001). We did not observe differences in the within-models analysis.

4.1.9. Brachial blood pressure in FP

We observed increased DP (p = 0.001) comparing all altitude periods (range 80.4 to 77.7 mmHg) in normoxic conditions (B_N, 68.4 ± 3.8 mmHg; Post, 73.7 ± 4.7 mmHg). Likewise, SP was significantly higher at altitude (B_H to W_4) (126.0 ± 5.1 mmHg in B_H and 124.9 ± 3.5 mmHg in W_4) compared to pre-altitude (B_N) (111.0 ± 3.3 mmHg) and to Post (111.3 ± 7.6 mmHg).

4.1.10. Power output test in IP and FP

The ergometer test in IP showed relative changes (p = 0.001) in power output (46 W at Pre_{-4} vs. 49 W at $Post_{11}$), whereas in FP, change was 44 W at Pre_{-4} vs. 50 W at $Post_{11}$ (p = 0.001).

4.1.11. 3000 m test in IP and FP

Both models reduced time set in the 3000 m test; however, due to the magnitude of the change, [242] it cannot be considered a significant improvement (IP Pre_{-3} = 470 seconds vs. IP $Post_{12}$ = 463 seconds; FP Pre_{-3} = 472 seconds vs. FP $Post_{12}$ = 456 seconds).

4.1.12. Blood parameters at SL and altitude in FP

A remarkable increase in erythrocytes, Hb and Hct was observed from the first day of the season ($T_{-7\ days}$) to the last day of the altitude sojourn ($T_{35\ days}$). Moreover, Ret decreased by 38.3 % from $T_{-7\ days}$ to $T_{35\ days}$, and by 42.7 %

from $T_{35\ days}$ to $T_{+6\ days}$. At $T_{+6\ days}$ erythrocytes, Hb and Hct were enhanced by 8 %, 8.8 % and 12.1 % respectively, compared to $T_{-7\ days}$. However, erythrocytes at $T_{+6\ days}$ were 1 % lower compared to $T_{-7\ weeks}$. Compared to $T_{-7\ days}$, EPO increased by 259 % at T_{+35h}, while lower values were observed at $T_{21\ days}$ (-29 %) and $T_{+16\ days}$ (-84 %) (Table 5).

4.1.13. BF at SL and altitude in FP

Compared to W_{-1} (5.1 ± 0.4 Breaths · min^{-1}) and Post (5.5 ± 0.8 Breaths · min^{-1}), resting BF was elevated ($p < 0.001$) during the 5-week altitude training camp (9.1 ± 1.6 Breaths · min^{-1} in W_1; 9.3 ± 2.1 Breaths · min^{-1} in W_2; 7.0 ± 0.8 Breaths · min^{-1} in W_3; 6.4 ± 0.8 Breaths · min^{-1} in W_4; 6.6 ± 0.8 Breaths · min^{-1} in W_5).

Table 5. Blood markers pre-, during, and post-altitude.

	T_{-7w}	T_{-7d}	T_{+35h}	T_{8days}	T_{15days}	T_{21days}	T_{28days}	T_{35days}	T_{+6d}	T_{+16d}
	SEA LEVEL				3860 m ALTITUDE				SEA LEVEL	
Erythrocytes	5.04	4.62		5.06	5.17	5.04	5.28	5.45	4.99	
(x 10^6 · mm^3)		-8.3 %		+0.4 %	+2.6 %	0 %	+4.8 %	+8.1 %	-1.0 %	
Hb	14.3	13.7		15.3	15.6	15.3	16.1	16.6	14.9	
(g · dL^{-1})		-4.2 %		+7.0 %	+9.1 %	+7.0 %	+12.6%	+16 %	+4.2 %	
Ret	12.4	16.7		15.0	12.6	12.4	12.0	10.3	5.9	
(%)		+25.8%		+21.0%	+1.6 %	0 %	-3.2 %	-16.9 %	-52.4 %	
Hct	42.3	40.4		46.0	47.0	46.0	48.0	50.0	45.3	
(%)		-4.5 %		+8.7 %	+11.1%	+8.7 %	+13.5 %	+18.2%	+7.1 %	
Ferritin	284	247		110	126	132	122	150	217	
(ng · mL^{-1})		-13.0 %		-61.3 %	-55.6 %	-53.5 %	-57.1 %	-47.2 %	-23.6 %	
EPO		12.2	31.6			8.7				1.9
(U · L^{-1})			+259%			-29 %				-84 %

4.1.14. Ferritin values at SL and altitude in FP

Compared to $T_{-7\ days}$, ferritin values were 56 % lower at $T_{8\ days}$ (247 vs. 110 ng · mL^{-1}) but 36.4 % higher (150 ng · mL^{-1}) at $T_{35\ days}$ and they remained 12.2 % lower at $T_{+6\ days}$ (Table 5).

4.1.15. POMS evaluation at SL and altitude in FP linked to TL

Considering SO_2 and SP as covariates, we observed significant differences between SL and altitude in fatigue and vigor. Fatigue was significantly higher at altitude compared to SL (97.66 ± 18.92 vs. 17.39 ± 13.71, p = 0.0362, d = 6); also, vigor was significantly lower at altitude (73.23 ± 8.62 vs. 26.48 ± 11.89, p = 0.0484, d = 6.1). No significant differences were observed between SL and altitude in any POMS dimension when DP was considered as the covariate (Figure 3).

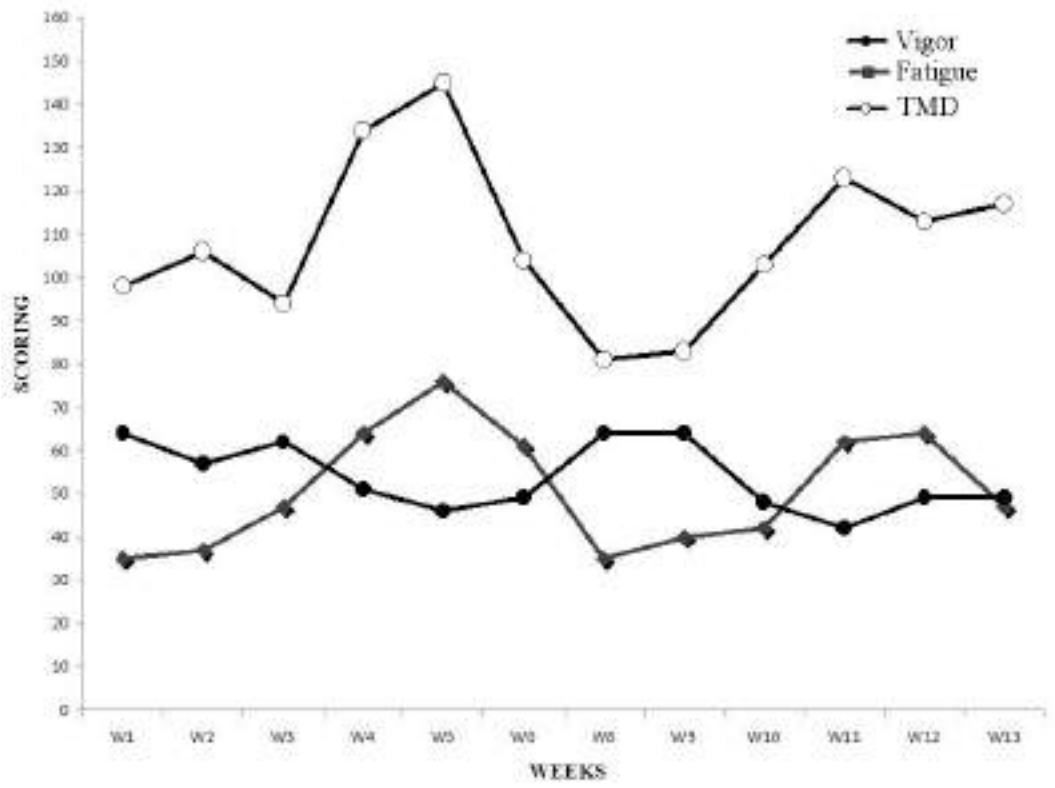

Figure 3. Weekly POMS vigor, POMS fatigue, and TMD from the start of the season (W1) to the week after returning to SL (W13).

The greatest values in TMD were found in week 5 of the season at SL (145), followed by week 4 at SL (134), considering that mean TMD during the 6 weeks preceding altitude was (113.5 ± 20.9). Moreover, TMD increased at altitude from the end of the second specific training week (83 in W9 and 103 in W10), reaching the greatest values at altitude in W11 (123), and remaining high after returning from altitude at W13 (117). To consider, weekly Foster training load score reached its greatest level (7406.86 AU in week 5 and 5916.1 AU in week 4). Indeed, a strong correlation was observed between TMD and weekly Foster training load score ($r = 0.66$; $p = 0.0258$). A strong correlation was observed between TMD and weekly Foster training load

score (r = 0.66; p = 0.0258) in week 5. In week 5, when SO_2 remained high at SL, value for fatigue reached the greatest level (76 AU), and so did anger (56 AU), not showing a relation between those dimensions and SO_2, SP and DP when they were considered as covariates. Furthermore, we found a strong correlation between fatigue and anger (r = 0.70; p = 0.0109). Both, depression and confusion, remained similarly stable throughout the whole experiment. However, in the last week, confusion increased 38.7 % and the magnitude of change compared to the last altitude week was large (d = 3.7). Moreover, we found a strong correlation between depression and confusion (r = 0.60; p = 0.0367). Finally, tension was greater pre-altitude and a strong correlation was found between tension and weekly mileage (r = 0.67; p = 0.0244).

4.1.16. Body mass evolution at pre-, altitude, and post-altitude in FP

A significant reduction in body mass was observed from B_N to B_H (52.6 ± 0.4 vs. 50.7 ± 0.5 kg, p < 0.001, d = 4.16). However, body mass returned to near pre-altitude values at Post (52.1 ± 0.5 kg). No differences were observed from specific training weeks at altitude, except between W_1 and W_4 (50.6 ± 0.2 vs. 51.2 ± 0.3, p < 0.001, d = 2.35).

4.1.17. Daily energy intake at pre-, altitude, and post-altitude in FP

Energy intake in B_N was greater than at altitude acclimatization B_H (2397 ± 242 vs. 2899 ± 270 kcal, p < 0.01, d = 0.96); W_1 (3037 ± 490 kcal, p < 0.01, d = 1.61); W_2 (3116 ± 170 kcal, p < 0.01, d = 3.49); W_3 (3101 ± 385 kcal, p < 0.01, d = 2.15). The same differences were observed within B_H, $W_{1,2,3}$ and Post (2411 ± 137 kcal, p < 0.01, d = 0.97; 1.68; 4.52; 2.31). No differences were observed between B_N, W_4 and Post.

4.1.18. Macronutrients intake at SL and altitude in FP

The amount of CHO was greater in $W_{1,2,3}$ compared to B_N. (9.6 ± 2.1 vs. 7.1 ± 1.2 g · kg^{-1}, $p < 0.001$, d = 1.43; 9.9 ± 1.2 vs. 7.1 ± 1.2 g · kg^{-1}, $p < 0.001$, d = 2.33; 9.6 ± 1.2 vs. 7.1 ± 1.2 g · kg^{-1}, $p < 0.001$, d = 2.08). In addition, we observed differences between $W_{1,2,3}$ and Post (6.3 ± 0.8 g · kg^{-1}, $p < 0.001$, d = 2.01; 3.47; 3.18).

The intake of proteins was greater than in B_N (2.9 ± 0.5; 2.7 ± 0.5; 2.6 ± 0.4; 2.7 ± 0.5; 2.5 ± 0.3 vs. 1.9 ± 0.2 g · kg^{-1}, p 0.001, d = 2.54; 2.03; 2.16; 2.03; 2.31) during all altitude periods (B_H to W_4). The same differences were observed between all altitude periods and Post (1.9 ± 0.2 g · kg^{-1}, $p < 0.001$, d = 2.38; 1.90: 1.96; 1.90; 2.00). No differences were found in lipid intake within any period range (1.0 ± 0.2 to1.4 ± 0.5 g · kg^{-1}).

4.1.19. Daily total fluid intake at SL and altitude in FP

We observed an increase in Fluid from pre-altitude (W_{-1}) to the first week at altitude (W_1) (4280.8 ± 723.0 vs. 5552.2 ± 1302.6 mL, $p < 0.05$, d = -1.21). However, overall results at altitude showed a decreasing trend in Fluid, as differences from W_1 to W_4 (4257.1 ± 499.1 mL, $p < 0.05$, d = 1.31), and from W_1 to W_5 (4213.1 ± 460.4 mL, $p < 0.05$, d = 1.37) were observed. As expected, Fluid diminished after altitude sojourn (W_{+1}) (3763.6 ± 1321.9 mL, $p < 0.05$, d = 0.49). In addition, there was a reduction in Fluid from W_2 to W_{+1} (4628.8 ± 839.6 vs. 3763.6 ± 1321.9 mL, $p < 0.05$, d = 4.19). Moreover, no differences were observed among the first three weeks at altitude ($W_{1,2,3}$). As expected, Fluid showed a strong correlation with urine production (r = 0.67; p = 0.001).

4.1.20. Diuresis at SL and altitude in FP

An increase in diuresis was observed from W_{-1} to W_1 (3504.3 ± 652.4 vs. 4448.0 ± 444.3 mL, $p < 0.001$, d = 1.69). However, diuresis decreased from

W_1 to W_2 (3815.0 ± 382.9 mL, $p < 0.001$, $d = 1.53$), W_3 (3610.7 ± 476.1 mL, $p < 0.001$, $d = 1.82$), W_4 (3141.4 ± 471.0 mL, $p < 0.001$, $d = 2.85$) and W_5 (3206.4 ± 518.1 mL, $p < 0.001$, $d = 2.57$). In addition, we observed lowest diuresis after returning to SL at W_{+1} (2526.0 ± 517.3 mL, $p < 0.001$, $d \geq 1.24$).

4.1.21. SG AM and SG PM at altitude and SL in FP

AM SG was lower in W_1 compared to W_{-1} (1.010 ± 0.006 vs. 1.020 ± 0.002, $p < 0.05$, $d = 2.23$), W_2 (1.019 ± 0.002, $d = -2.01$) and W_{+1} (1.023 ± 0.006, $d = -2.17$). No differences throughout the altitude sojourn were observed in AM SG. Furthermore, lower or equal PM SG values (range 1.008 ± 0.003 to 1.017 ± 0.006) compared to AM SG were observed, reflecting a better hydration status by the end of each day and beyond an optimal PM SG (< 1.020) week by week. In regard to this observation, PM SG was significantly lower in W_2 compared to W_{-1} (1.008 ± 0.003 vs. 1.014 ± 0.004, $p < 0.05$, $d = -1.69$). Moreover, it was significant higher in $W_{4,5}$ compared to W_1 ($d = -2.66$; -1.98, respectively). Interestingly, we observed a greater AM SG compared to PM SG at W_2 (1.019 ± 0.002 vs. 1.008 ± 0.003, $p < 0.05$, $d = 4.31$) and W_3 (1.018 ± 0.004 vs. 1.012 ± 0.004, $p < 0.05$, $d = 1.50$).

4.1.22. Sodium daily intake at SL and altitude in FP

No time interaction ($W_{-1,1,2,3,4,5,+1}$), nor any condition interaction (altitude vs. SL) was observed in the mean Na^+ daily intake (range 1601 ± 244 to 2379 ± 750 mg); however participant met suggested optimal quantities. [84]

4.1.23. Sodium intake while exercising at SL and altitude in FP

Sodium did not reach the minimum quantity recommended (0.5 to 0.7 g · L^{-1}) [82,83] during the workouts at <VT1 (0.13 g · L^{-1}) and at specific sessions (0.17 g · L^{-1}).

4.1.24. Hydric balance after training at SL and altitude in FP

A positive HB was observed in every training session with the exception of a strength session performed at the gym (HB = -1000 mL), in which temperature was higher than usual (21° C, humidity 51 %). Moreover, a negative moderate correlation was found between ambient temperature and HB (r = - 0.37; p = 0.006); a moderate correlation was also found between HB and Fluid (r = 0.53; p = 0.001). In addition, a negative moderate correlation was found between water loss and HB (r = - 0.59; p = 0.001).

4.1.25. Fluid intake rate average related to the type of session in FP

Fluid intake was different according to type and duration of session. We observed these results: 1) For 20 km <VT1 586.8 ± 102.7 mL · h^{-1}; 2) For 16 km <VT1 749 ± 86.5 mL · h^{-1}; 3) For resistance sessions 495.4 ± 175.6 mL · h^{-1}; 4) For A sessions 834.4 ± 17.9 mL · h^{-1}; 5) For B sessions 704.9 ± 130 mL · h^{-1}; 6) For C sessions 642.2 ± 34.7 mL · h^{-1}.

4.1.26. Fluid Vol.1 and Fluid Vol.2 at SL and altitude in FP

Fluid Vol.1 was the same in W_{-1} and W_{+1} (700.0 mL), but the same training program was done in pre- and post-altitude weeks. Moreover, in the acclimatization week (W_1), almost the same fluid intake was observed (678.8 ± 219.4 mL), considering some sessions were programed in $W_{-1,1,+1.}$ In addition, due to a lower number of specific sessions (#1) in W_5, a significant

lower fluid intake was observed in the last week of the altitude sojourn (780.0 ± 270.7 mL). No differences were observed among specific training weeks ($W_{2,3,4}$) in Fluid Vol.1 when mostly all A, B, C sessions were done. Fluid Vol.1 range was 1089.2 ± 340.5 to 1199.0 ± 324.8 mL in $W_{2,3,4.}$

Fluid Vol.2 was significantly lower at SL in W_{+1} compared to W_{-1} (500.0 vs. 700 mL, $p < 0.05$) after arriving from altitude. At altitude, we found significant ($p < 0.05$) lower fluid intake (321.0 ± 21.7 mL) In W_3 compared to W_{-1}, W_1 (d = -16.91), W_2 (d = -3.61), W_4 (d = -1.49), W_5 (d = -2.47), W_{+1}.

4.2. Results from physiological assessment pre-, during, post-marathon

4.2.1. Marathon splits

The average speed of the race was 6.51 m · s^{-1}, while the 5 km segment average of time was 770 s, being both very steady throughout the race. The 25 to 30 km segment was the slowest (6.37 m · s^{-1}), while the fastest was from km 5 to km 10 (6.84 m · s^{-1}). Moreover, the first half of the marathon time was 3202 s, while the second half of the marathon time was 77 s slower compared to the first half (3279 s).

4.2.2. Heart rate during the marathon

The average HR during the second half of the marathon (21096 to 42195 m) was higher than the HR of the first half of the marathon (start line to 21096 m), (163 ± 6 vs. 167 ± 6 beats · min^{-1}, $p < 0.001$, d = -0.66). Moreover, we did not find significant differences between the first half of the marathon HR and the overall race HR (163 ± 6 vs.165 ± 7 beats · min^{-1}, d = -0.31). In addition, the second half of the marathon HR was slightly higher ($p > 0.05$) than the overall race HR (167 ± 6 vs.165 ± 7 beats · min^{-1}, d = 0.31).

4.2.3. Intensity of the race related to incremental laboratory test

In the incremental test performed ten days before the RD, the estimated HR at VT2 was 166 beats · min^{-1}. Oxygen uptake (VO_2) at VT2 was 51 ml · kg · min^{-1}. At VO_{2max} HR was 176 beats · min^{-1} and VO_{2max} was 57 ml · kg · min^{-1}. From the start of the race to the 30 km mark, the athlete raced at an intensity slightly below the HR at VT2 (166 beats · min^{-1}), (0-5 km 161 ± 5 beats · min^{-1}; 5-10 km 164 ± 6 beats · min^{-1}; 10-15 km 162 ± 6 beats · min^{-1}; 15-20 km 165 ± 6 beats · min^{-1}; 20-25 km 163 ± 5 beats · min^{-1}; 25-30 km 163 ± 7 beats · min^{-1}), while from the 30 km mark to the finish line he raced at an intensity slightly higher than the HR at VT2 (30-35 km 169 ± 7 beats · min^{-1}; 35-40 km 170 ± 5 beats · min^{-1}; 40-42.2 km 170 ± 4 beats · min^{-1}).

4.2.4. HRV oscillations pre-RD and post-RD

Ln rMSSD was averaged across all pre-travel days (RD_{-6} – RD_{-2}) to serve as BL. The smallest worthwhile change (SWC) in Ln rMSSD was determined as ± 0.5 of the BL standard deviation. Results for BL were 3.77 ms, as SWC was 3.85 to 3.69 ms. Compared to BL, Ln rMSSD exceeded the SWC (-4.66 %) on the first day post-travel (RD_{-1}), and on RD (-3.8 %). The greatest Ln rMSSD reduction was observed the day after the marathon (RD_{+1}) (-23.1 %), while LnrMSSD returned to within BL (inside SWC) two days after the race (RD_{+2}) (3.71 ms).

5. SUMMARY OF THE DISCUSSION

5. SUMMARY OF THE DISCUSSION

The studies comprised in this research aimed to determine the following in a professional wheelchair marathoner: 1) the influence of a 5-week HRV-guided training program (FP) conducted at a 3860-4090 m terrestrial altitude on performance at SL after returning from altitude, and on the physiological disturbance (i.e., rMSSDCV) comparing the concominant effects with the same altitude exposure carried out a year before with a predefined training program (IP); 2) The effects of both TL and hypoxic/normoxic environment on several blood markers from the beginning of the season to several time points during the altitude sojourn, and at SL after returning; 3) the psychological response assessed with the POMS to a TL imposed during the 7-week leading training at SL pre-altitude, 5-week at altitude, and post-altitude at FP model; 4) The effects of a nutritional intervention planned for FP model on body mass; 5) The hydration status and diuresis during SL and altitude (FP) according to a specific hydration intervention set for both, resting and training hours, considering the specificity of hypoxic environment and the type of session (intensity and duration). As previously described in the summary of methods (chapter 3), different evaluations were performed in order to analyze the aforementioned variables.

The major findings were that the FP model results in a lower degree of cardiac autonomic vagal disturbance than IP. Moreover, IP resulted in a greater cardiac vagal activity suppression compared to FP. In addition, a slightly greater change in performance (power output test and 3000 m test) after altitude return was observed in FP. A progressive increase in blood markers, except Ret were observed throughout the altitude camp in FP, while EPO reached its greatest level 35-hours after hypoxic exposure, showing a suppression at chronic altitude and lower values than pre-altitude ones after returning to SL. Interestingly, BF increase significantly at altitude, being

almost two-fold greater during acute altitude exposure. Despite a specific nutrition and hydration intervention, body mass decreased significantly at altitude; however, a significant increase in body mass was observed from B_H to W_4. The body mass decrease could be related to the greater diuresis observed at altitude, especially during the first week of exposure.

As we mentioned previously, to the best of our knowledge this is the first study in Paralympic sport conducted under altitude conditions, and the second in history in which an athlete is exposed to ~4000 m terrestrial altitude. There are studies conducted at similar terrestrial altitude, but in different sports.

In this research we also include a study which aimed to analyze the physiological response pre-, during and post-marathon in the same professional wheelchair athlete. The main findings in this study where: 1) The athlete was able to race during the whole marathon at an intensity very close to his VT2, being the intensity greater than VT2 from 30 km to 42.2 km; 2) Greater cardiac vagal autonomic disturbance was observed the morning after the marathon; 3) The overseas trip triggered cardiac vagal autonomic activity reduction compared to pre-travel values (BL), which was also observed on RD, this was maybe due to the pre-race anxiety or pre-race tapering; 4) Autonomic cardiac vagal activity returned to BL values two days after RD.

The main purpose of this chapter is to present a summary of the discussion. Anyway, the results presented in the three studies are deeply discussed in the specific discussion section in chapters 7, 8, 9, 10, 11 and 12.

5.1. HRV, SO_2, HR_{rest} in both, IP and FP

The significant reductions in rMSSD observed at B_H in IP (42.7 %) and FP (27.1 %) relative to B_N cannot be interpreted as a reduction in vagal activity

because an increase in parasympathetic activity using a vagal blockade [186] and sympathoexctitation with peroneal microneurography have been previously observed. [41] Likewise, Lundby et al. did not find differences in Danish-lowlanders in muscle sympathetic nerve activity in acute altitude (FiO_2 = 0.12 %) compared to SL. [41] Interestingly, the athlete rMSSD reduction at B_H was almost two-fold in IP compared to FP, despite the same training performed in B_N in both models. The reason might be explained by a different physiological response to overseas travel. [161] Moreover, the greater suppression of rMSSD observed at FP (e.g., 53.9 % rMSSD reduction at W_4 in IP vs. 40.7 % reduction in IP) might be the result of 40 % greater TL at IP than FP. In line with this observation, increased TL has caused greater reductions in rMSSD along with greater rMSSDCV, both being linked to greater RPE and worse muscle soreness. [155] As a consequence of a greater reduction in rMSSD and a greater rMSSDCV in IP, the athlete was unable to complete prescribed the afternoon sessions due to his feeling of exhaustion. Finally, greater cardiac autonomic vagal restoration was observed after returning from altitude at Post in FP, as rMSSD were ~4 % greater compared to pre-altitude in FL. However, ~11 % lower values compared to B_N were shown in IP, which may reflect persisting effects of fatigue after returning to SL.

An impairment in VO_{2max} was indirectly confirmed in both IP and FP, as induced-VO_{2max} reduction has been associated with hypoxemia below 95 %. [244] In line with this observation, a greater drop in SO_2 was observed at B_H in IP (12.6 %) than in FP (10.3 %) relative to B_N. In fact, the 2.6 % difference might induce lower HVR in IP, which has been associated to AMS. [245] In addition, SO_2 was restored faster in FP, as observed in W_1 (88.74 ± 0.90 % in IP vs. 91.19 ± 0.76 % in FP), which combined with the lower SO_2 values until W_2 observed in IP compared to FP might reflect a greater impairment of

the cardiorespiratory function, as well as a lower ability to adapt to the hypoxic environment in IP. [246] However, we must consider the progressive increase observed in IP until W_3 and until W_4 in FP, which might reflect positive altitude acclimatization, [245] considering that the decrease in IP from W_3 to W_4 was insignificant (0.69 %). Moreover, and in relation with rMSSD suppression observed in both models, sympathoexcitation (decrease in Ln rMSSD and increase in Ln LF to HF ratio) has been associated to a decrease in SO_2 at normobaric altitude (FiO_2 = 0.096 %). [247]

The HR_{rest} increased similarly (~17 beats · min^{-1}) in IP and FP, as previously reported at an altitude of 3452 m. [40] Interestingly, the greater reduction observed in IP might be explained by the greater TL compared to FP (~200 km vs. ~140 km per week), and also by the greater number of VT1 sessions (8 vs. 4). [248]

5.2. Brachial blood pressure in FP

The significant increase in SP and DP observed at altitude are not in line with the observations from Lundby et al. who did not observe an increase in SP at altitude compared to SL. [41] However, the more extenuating circumstances in our experiment (trekking vs. marathon training) might be the reason for a greater increase in this sympathetic marker. This argument is supported by the greatest value in SP observed in W_2 (second specific training week at altitude), in which more demanding sessions and less recovery time between them was observed. The increase in DP considered as a sympathetic response, observed throughout the altitude sojourn relative to SL, might be due to an induced-vasodilation in hypoxia. [41]

5.3. Performance after altitude training in IP and FP

The greater improvement in FP (not statistically significant) [242] observed in

both, laboratory and 3000 m test, goes in line with a study in which Nordic skiers guided by HRV improved roller-ski performance (-2.7 ± 3.6 %) 21 days after a 15-day LHTL intervention (FiO_2 = 0.15 %) compared to two predefined-training groups (Nordic and Alpine skiers). [188] Moreover, and in contrast with our findings, after more than 40-days living and training at 4000 m, Buskirk et al. did not find improvements (3 to 7 days) and (10 to 15 days) after returning to SL, in middle-distance athletes who were tested pre- and post-altitude in the following distances (400 m, 800 m, 1609 m and 3218 m). [211]

5.4. Decrease of performance at altitude in FP

Our athlete was able to perform 2000 m repetitions at 4090 m terrestrial altitude at an average time of 310 seconds, decreasing his performance compared with SL by around 3 %. At the same altitude, able-bodied athletes decreased performance (20 to 24 %) in distances ranging from 1609 to 3218 m, respectively. [211]

5.5. Blood markers pre-altitude, during altitude sojourn and after returning to SL in FP

The 259 % increase in EPO, 35-hours after altitude arrival goes in line with previous observations. [16,21] Moreover, 21 days after arrival to altitude (T_{21days}), we observed lower values (-29 %) compared to pre-altitude. In line with our observations, a study performed with 15 male elite-biathletes who stayed 3-weeks at 2015 m, and did base training at 3000m four times per week plus three interval sessions per week at 1000 m terrestrial altitude did not find significant changes in EPO from the third week of exposure onwards (6.4 vs. 8.8 $U \cdot L^{-1}$, $p > 0.005$). [21] In this study, researchers found a 75 % EPO enhancement after two-weeks at altitude relative to pre-altitude.

Regarding the greatest EPO suppression (-84 %) observed in our study 16-days after returning to SL ($T_{+16days}$) relative to 7-days to pre-altitude goes in line with a study carried out with elite-biathletes conducted at 2050 m terrestrial altitude, [31] which compared EPO values 16-days after returning to SL with pre-altitude (1-day before exposure); however, the authors do not provide data for post-value. Different studies have shown lower EPO values post-altitude relative to pre-altitude assessment. [19,20,23] Interestingly, and as a result of endurance training, [249] a 25.8 % increase in Ret was observed 7-days before the altitude sojourn, and 7-weeks after the start of the season, in which the athlete completed 74 training sessions and 1280 km. In addition, the 38.3 % decrease of Ret at altitude might go in relation with the aforementioned EPO suppression at altitude. The same EPO suppression observed at T_{21days} and $T_{+16days}$ might also explain the 8.5 % decrease in erythrocytes from T_{35days} to T_{+6days}; however, we must be cautious with this assumption. [30,34] The greatest erythrocytes and Hb were observed at altitude week completion, as observed in a meta-analysis conducted by Rasmussen et al., who state that 4-weeks of exposure at 4000 m will trigger the maximum increment in RCV. [37] It must also be considered that it was preceded by the week (W_5) with lower number of specific training sessions (#1). In regard to this assumption, the excessive number of specific training sessions (#4) performed in W_3 might be the reason for the decrease of erythrocytes, Hb, and Hct, as there seems to be a negative correlation between demanding training and blood markers. [250,251] The most important observation regarding blood markers, considering the main target of this training camp, was the increase in Hb, erythrocytes and Hct at T_{+6} days relative to T_{-7days}, which goes in line with previous studies. [20,21,22,26,27,28,31]

5.6. Resting BF at altitude and SL in FP

The increase in BF observed at altitude might be the consequence of induced-sympathoextitation [39] and also as a response to the decrease in SO_2 described above. [246] The reduction in BF observed in W_3 might be the consequence of a 12-14 % increase in erythrocytes and Hb in W_3 relative to W_{-1} and the aforementioned increase in SO_2. In line with our results, Saugy et al. reported an increase in BF during days 1 to 18 at altitude relative to the two nights prior to the altitude camp (15.5 ± 1.5 vs. 13.9 ± 1.5 Breaths · min^{-1}, $p < 0.005$, $d = 1.07$) [3] in a study with well-trained triathletes who trained 3-weeks at 1150 m and lived at 2250 m terrestrial altitude. Furthermore, these authors observed a descent in BF relative to the mean of the first eighteen days of exposure, as we found in our athlete at the completion of the camp (days 19 to 21) (15.1 ± 1.3 vs. 15.5 ± 1.5 Breaths · min^{-1}, $p < 0.005$, $d = 0.28$). However, and as our athlete experienced, triathlete's BF remained greater than at SL after camp completion (15.1 ± 1.3 vs. 13.9 ± 1.5 Breaths · min^{-1}, $p < 0.005$, $d = 0.85$).

5.7. Ferritin descent observed at altitude

When erythropoiesis is activated, iron demand increases, considering that erythropoiesis stimulation leads to a reduction of the production of hepcidin, [252] a peptide hormone regulating body iron homeostasis, resulting in an enhanced iron absorption and a release of iron stores. [253] As a consequence, if iron stores are insufficient or a diet at altitude is inadequate for the nutritional demands, [254] a hematological response will not be elicited. [253] In addition, ferrous sulphate intake has been related to the production of Hb and RBC; [235,236] however intravenous iron administration has not shown additional benefits in terms of the magnitude of the erythropoietic response (e.g., Hb_{mass}) compared to oral supplementation. Therefore, the daily intake of 105

mg per day of ferrous sulphate might help our athlete to maintain the ferritin levels within a normal range (12 to 40 $\mu g \cdot L^{-1}$) [255,256,257,258,259,260,261,262] at altitude, avoiding anaemia and iron deficit as previously described. [263] However, differences were observed between SL and altitude in our athlete, reaching more than a two-fold descent (-56 %), which has been reported in elite-biathletes training during 3-weeks at 2050 m terrestrial altitude, [31] but the magnitude of the change was quite remarkably smaller (-15 %). Although differences in magnitude of exposure must be considered (3860 vs. 2050 m). In line with our results, Govus et al. found a serum ferritin decrease (-33.2 %) relative to pre-altitude in athletes training at different altitudes (1350 to 3000 m). [264] Interestingly, the 13 % reduction of serum ferritin observed at SL seven-weeks after the season began might be related to the aforementioned high TL imposed on the athlete, as a (~21 %) reduction in serum ferritin in training periods has been observed in elite-road cyclists in which both, intensity and volume were greater. [251]

5.8. POMS and TL at altitude and SL in FP

We found increased fatigue (97.66 ± 18.92 vs. 17.39 ± 13.71, p = 0.0362, d = 6) and decreased vigor (73.23 ± 8.62 vs. 26.48 ± 11.89, p = 0.0484, d = 6.1) at altitude, when considering SO_2 and SP as covariates, as previously reported in studies with able-bodied participants in altitudes ranging from 3080 to 6000 m terrestrial altitude. [111,113,114] Recently, a study in which 12 men and 6 women were acutely exposed to 3800 m terrestrial altitude found an increasing trend in fatigue (p = 0.052) and TMD (p = 0.063) at altitude compared to SL. [265] In this study, the confusion POMS dimension increased at altitude; however, in our athlete the two weeks in which confusion was greater were related to the trip weeks (W7 = 60 and W13 = 43), considering that mean confusion for the whole experiment was (34.8 ± 8.2). Moreover,

we observed than when TL was peaking at W5 (7406.85 AU) it had a negative effect on fatigue (76) and TMD (145), both reaching the greatest values of the thirteen-week assessment period. Moreover, TMD increased significantly from the second week of the specific training at altitude (W10) taking into consideration that it was the week in which less time of recovery between specific sessions was observed. Therefore, a relation between TMD and intra-sessions recovery might exist. Moreover, the 35.6 % increase in POMS confusion observed after returning from altitude, might trigger the remaining high TMD after returning to SL. In addition, vigor reached its second lowest value (46), only surpassed by the fourth week at altitude (W11) (42). This altogether confirms that excessive TL might be detrimental for positive POMS dimension as vigor and for negative dimensions as TMD and fatigue, which goes in line with observations among elite judoists. [266] However, a study with cyclists did not find differences in mood states between cyclists diagnosed with acute fatigue and cyclists diagnosed with FO, [267] although this study was not performed under hypoxic conditions. [111,113,114,268,269] Moreover, it seems that vigor, fatigue and TMD are immediately sensitive to increases in TL, as observed in a group of well-trained swimmers who showed a 2/3 increase in TL in a 3-day overload microcycle. [270] An interesting observation was the greater Foster in W5 compared to W4 (7406.85 vs. 5916.1), despite the fact that similar training distance was covered in both weeks (196.4 vs. 211.6 km). Thus, this might suggest a Foster´s threshold which might cause an increase in TMD. In fact, a TMD 48 % greater than BL may reflect overreaching symptoms. [108] Regarding this fact, in W5 at SL, TMD was 54 % greater than the week at SL where lower TMD was observed (W3; 145 vs. 94). In our study we found lower TMD at altitude compared to SL; however, training volume at altitude was 1/3 lower. Moreover, we found inverted iceberg profile at W5 at SL and

at W12 at altitude unveiling overreaching symptoms. [101] Despite a strong positive correlation between tension and weekly volume ($r = 0.67$; $p = 0.0244$), greater tension scores were observed in W1 and W2, reflecting stress imposed by the equipment preparation for the season. Moreover, greater anger, tension and fatigue were associated with overloading, as previously reported. [266] Furthermore, a strong correlation between weekly Foster and TMD was found ($r = 0.66$; $p = 0.0258$). Thus, considering altitude as a stressful environment, coaches should consider decreasing TL to avoid an increase in TMD. Moreover, as a consequence of a 42.7 % reduction in TL after returning to SL, fatigue returned to BL in W13. We found similar results in the literature in a case report in which a Master track athlete decreased his POMS fatigue 10 days after TL was lowered 41 %. [271]

5.9. Nutritional intervention during FP

Nutrional program design was based on the existing literature for able-bodied athletes due to the lack of publications related to individuals with CMT or wheelchair-athlete nutritional interventions. Moreover, the main target for such a nutritional program were: 1) to maintain the athlete´s body mass during the altitude sojourn; 2) to minimize performance decrements at altitude compared to SL (e.g., the athlete worsened the time in 2000 m repetitions at altitude by ~3 % relative to SL vs. the 20 to 24 % worsened times observed in able-bodied athletes performing 1609 and 3218 meters time trials, respectively); 3) to facilitate inter-session glycogen restoration, especially when demanding sessions (i.e., A, B, C sessions) were performed in consecutive days; 4) to maintain quality training sessions at altitude.

5.10 Body mass loss at altitude during FP

Despite a significantly greater energy intake (~500 kcal · d^{-1}), CHO intake (2 g · kg · d^{-1}), and protein intake (0.8g · kg · d^{-1}) at altitude compared to SL, athlete body mass loss at B_H ~2 kg, [52] might be explained due to the decrease in plasma volume after altitude arrival, [12] water loss by greater diuresis (~500 ml · d^{-1}), [51] and increase in ventilation. [50] However, body mass returned within pre-altitude values at Post, and it increased significantly from altitude arrival to altitude completion. In addition, we tried to anticipate a loss of body mass induced by an increase in RMR, especially in the acclimatization phase.[50,66] Therefore, and considering the same training performed in B_H and B_N, the athlete increased his energy intake during the first week of the altitude camp in 502 kcal, as CHO and protein intake were 1 g · kg · d^{-1} greater than B_N. In addition, at W_4 and despite a ~1 g · kg · d^{-1} less CHO intake compared to the three previous specific training weeks ($W_{1,2,3}$), we observed a significant increase in body mass relative to the first week at altitude that might be explained by a ~16 % lower training volume and a lower number of specific sessions: 2 in W_1, 3 in W_2, 2 in W_3 and 1 in W_4. The reason why we did not find differences in energy intake between B_H and specific training weeks is because once a specific training was done in the morning, it was followed by a resting afternoon; however, during the acclimatization period, two daily sessions were performed with a daily mileage of 36 km. Furthermore, three hours before training sessions, a rich CHO meal was consumed, as it has been proved to increase glycogen availability. [228] In regard to the previous assumption, we refused the hypothesis of a lower exogenous glucose oxidation at acute and chronic altitude, [272] as it has been recently reported that it might vary between individuals exercising in a post-prandial compared to individuals exercising in fasted state. [273] In addition, a shift in substrate use to favor greater

dependency on glucose in response to hypoxia has been reported. [59,61,274,275,276]

To avoid loss of lean body mass, high protein foods were spread across all meals, while whey [277] and casein [232] protein were consumed to ensure a minimum of 2.4 g · kg · d^{-1}. [225] However, the the hypoxic dose [72] of this training camp was 3300 km · h^{-1}, and the cut-off point, where muscle mass loss begins (5000 km · h^{-1}) [73] was not reached. In regard to protein supplementation, Cintineo et al. afirm *"protein intake plays a potentially useful role in optimizing physical performance and positively influencing the subsequent recovery processes"*. [277]

5.11 Hydration status at altitude and SL in FP

From acclimatization, AM SG, an indicator of the hydration status, remained a bit lower than at pre-altitude (W_{-1}), and inside the optimal range. [80] Moreover, and except in one session, HB, considered as the difference between fluid intake minus water loss, [223] was always positive and helped to maintain body mass close to the ± 2% recommened [78] to avoid dehydration which will trigger heat storage by reducing sweating rate and skin blood flow response for a given core temperature, [278] and also to avoid an excessive body mass gain as a consequence of overhydratation which might trigger hyponatremia. [279,280,281,282,283] In this regard, we observed nine sessions in which HB exceded the +2 % body mass (Table 6).

Table 6. Sessions in which HB exceded the +2 % suggested to avoid overhydration.

Date	**Body Mass (kg)**	**Phase**	**Session**	**Temperature (°C)**	**Humidity (%)**	**HB (mL)**	**% relative to Body Mass**
19-01-16	50.5	W_2	A	4	46	1300	2.57
22-01-16	50.6	W_2	B	2	44	1300	2.57
23-01-16	51.0	W_2	C	4	45	1100	2.16
26-01-16	50.8	W_3	A	5	51	1200	2.36
29-01-16	51.1	W_3	C	7	51	1100	2.15
30-01-16	51.3	W_3	A	5	43	1400	2.73
2-02-16	51.2	W_4	B	10	48	1100	2.15
6-02-16	51.1	W_4	B	8	44	1250	2.45
12/02/16	51.3	W_5	C	7	49	1050	2.05

In addition, the sessions in which overhydration occured were all specific sessions (3 times A, 3 times B, 3 times C). Moreover, when HB was > 2.5 % of body mass, temperature was equal or below 5 celsius degress and relative humidity was below or equal to 46 %. However, it is still not clear if changes in relative humidity will enhance/diminish sweating rate, as it has been

reported in a study with well-trained runners that increases in relative humidity from 23 to 71 %, did not elicite a greater sweat loss. [284] Moreover, in the observations of > 2 % body mass in HB from our study, the range of relative humidity was very narrow (46 to 51 %). In addition, Périard et al. observed sweat production increased significantly in hot conditions (35º C) during self-pace exercise in eight endurance trained-cyclists compared to a thermoneutral trial (20º C) (1.8 ± 0.5 vs. 1.1 ± 0.4 L · h^{-1}, p < 0.05, d = 1.55), leading to a greater Fluid intake in hot condition (1486.5 ± 675.4 vs. 650.1 ± 472.6 mL). [285] In line with Périard et al study, Maughan and Shireffs affirm that *"during exercise in the cold, fluid replacemet may not be necessary as sweat rate will be low, but there is still a need to supply additional glucose to the exercising muscles"*. [286] Moreover, Galloway and Maughan found a double sweat rate at (31º C) in eight males riding at 70 % of their VO_{2max} compared to the same exercise performed under cold conditions (4º C) (1.15 vs. 0.55 L · h^{-1}). [287] Therefore, lower ambient temperature should be accompanied with a reduction in Fluid rate. In this regard, in the three A sessions in which >2 % of body gain was observed, the mean fluid rate was 841.5 mL · h^{-1}. Therefore, a reduction of ~200 mL per hour would have guaranteed the minimum rate suggested. [77] In the rest of sessions in which > 2 % of body mass was observed, the fluid intake rate ranged from (589.5 to 679.8 mL · h^{-1}).

As a strategy to obtain an optimal SG PM, assessed two hours after dinner, the athlete was encouraged to avoid feeling thirsty, and reach the 4 L of daily fluid consumption at altitude. [79] No significant differences were observed between SG AM and SG PM. However, a lower trend was observed in SG PM, displaying optimal hydration status throughout altitude and SL. Furthermore, the athlete did not suffer headaches, and therefore he did not exhibit AMS symptoms, [81] reflecting positive altitude acclimatization which

might have been eased by an increase in diuresis in W_1 as water retention has been related to AMS. [49,288] Interestingly, if we estimate the ratio between Fluid and urine excretion, similar values are observed among the different time-phases of the entire experiment (Table 7). However, the relative increase observed in W_1, might be influenced by the inability to attend restroom on the overseas flight, as reported verbally by the athlete, who was also forced to decrease Fluid intake during ~24 hours after returning from the altitude trip.

Table 7. Ratio Fluid intake and urine excretion

Phase	**Fluid (mL)**	**Urine (mL)**	**Fluid to Urine ratio**
W_{-1}	4280.8 ± 723.0	3504.3 ± 652.4	1.22
W_1	5552.2 ± 1302.6	4448.0 ± 444.3	1.24
W_2	4834.7 ± 850.7	3815.0 ± 382.9	1.27
W_3	4628.8 ± 839.6	3610.7 ± 476.1	1.28
W_4	4257.1 ± 499.9	3141.4 ± 471.0	1.36
W_5	4213.1 ± 460.4	3206.4 ± 518.1	1.31
W_{+1}	3763.6 ± 1321.9	2526.0 ± 517.3	1.49

As SL sessions were performed at a relative confortable intensity (<VT1) and

Fluid Vol.1 and Fluid Vol.2 were inside or close the recommended fluid rate, [77] it helped to maintain body mass inside ± 2 %, except in one session in which the athlete experienced a 2 % body mass loss in a 20 km <VT1 session in which the duration was ~70 min and the temperature was higher than usual (16º C ambient temperature, 61 % relative humidity). However, the loss of body mass was far less than reports of able-bodied runners who displayed up to an 8 % loss after running a marathon. [78] Thereafter, the differences in exercise intensity (<VT1 vs. VT2), and also in muscle mass involvement might help us to understand the greater magnitude in body mass loss observed in able-body athletes compared to our athlete. In addition, and regarding lower muscle mass involvement, our athlete was able to maintain an optimal HB during W_{+1} in sessions in which fluid rate was lower than 600 $mL \cdot h^{-1}$ (range 503.1 to 599.4 $mL \cdot h^{-1}$). [77]

Sodium did not reach the minimum quantity recommended during workouts at <VT1 (0.13 $g \cdot L^{-1}$) and specific sessions (0.17 $g \cdot L^{-1}$). [82] However, we think that Na^+ requirements were reached, as lower diuresis induced by hyponatremia was not observed. In fact, a relation between increased Na^+ loss accompanied by a reduced urine production ($r = -0.478$; $p = 0.0447$) was reported in male endurance athletes. [88] Moreover, the lower diuresis in W_{+1} was consequence as a reduction in Fluid intake, which might be partially explained by the incapacity of attending restroom during the return overseas flight. Furthermore, our results cannot be compared with the study by Zaccaria et al.as in the acclimatization phase in which they found greater urine Na^+ concentration, our athlete was performing low intensities workouts (<VT1), while in Zaccaria´s study, participants were performing exhaustive exercise. [89] Finally, daily intake of Na^+ (1500 to 2300 $mg \cdot d^{-1}$) met suggested quantities. [84]

5.12 Heart rate during marathon

Our athlete was able to maintain a HR which exceeded the HR estimated at VT2 from the 30 km mark to the finish line. We might think that incorrect hydration [77,78] might have enhanced HR to regulate core temperature, [278] as event conditions were warm (24º C ambient temperature). However, the athlete´s fluid rate (116 mL · 10 min^{-1}) was inside suggested fluids amounts, [77] leading us to think that an excessive increase in core temperature induced by dehydration did not influence HR in our athlete, as it has been demonstrated. [239] In line with our observations, no differences are observed between HR maintained during a marathon in elite wheelchair-athletes [204] and able-bodied marathoners. [189] In addition, our athlete´s VO_2 at VT2 assessed in the laboratory (51 mL · kg · min^{-1}) was significantly higher than reported from wheelchair-marathoners without upper-limb affection (e.g. paraplegics). [204] However, it remained significantly lower than in elite able-bodied marathoners. [189,197,198]

5.13 Speed and time in different segments from the marathon

The speed throughout the entire race was quite homogeneous with a mean race speed of 6.51 m · s^{-1}. The second segment (5-10 km) was the fastest (6.84 m · s^{-1}) whilst the slowest segment (6.37 m · s^{-1}) was the sixth segment (25-30 km). These results go in line with Haney and Mercer who suggest that slower marathoners show greater variability of pace compared to faster athletes. [289] To consider, the softer surface from the 600 m stadium compared to tarmac might have a negative influence on rolling resistance, decreasing the speed and increasing the time in the last segment of the race. [290]

5.14 Pre-race and post-marathon HRV

The coefficient of variation of BL Ln rMSSD (5 %) was similar to that observed among elite-endurance athletes during tapering. [166] Moreover, the decrease observed in Ln rMSSD relative to BL the morning after arriving to Japan has been observed in an elite male decathlete following eastward travel across 6-times zones, [291] and also in junior rowers after travelling across 5-times zones. [161] In regard to HRV response after overseas travels, it seems that individual responses in HRV to air travel are mediated by fitness and body composition. [292] This is the reason why our highly-trained and fit athlete only experienced small reductions in Ln rMSSD in response to relocation.

The athlete in the current study only performed one workout (8 km <VT1) in the three days before RD because of travel and relocation, which might lead to tapering physiological mechanisms (i.e., hemodynamic perturbation). [164] However, it is difficult to determine if reduction in Ln rMSSD observed at RD_{-1} and RD was due to relocation to new timetable, [161,291] pre-race anxiety [213], or due to hemodynamic response induced by tapering. [164] However, since the result of the marathon was outstanding, the cardiac autonomic response was more related to optimal condition to race [293] rather than fatigue-related. [294]

A reduction in Ln rMSSD (23 %) was observed the morning after the marathon (RD_{+1}), reflecting a reduction in cardiac parasympathetic activity, as previously reported in cross-country skiers the day after completing a 75-km cross-country ski race, [143] and also the night after amateur-athletes complete a marathon. [294] Moreover, it has been found that recreational marathon athletes show a decrease in vagally-related markers when an internal load model (i.e., TRIMP) reached the highest values. [216] In line with these observations, a study with seven middle-distance runners found a 41 % decrease in cardiac-vagal-autonomic activity during overload training

periods. [214]

Overall, it seems that the cardiac-autonomic-activity after a marathon from our wheelchair-athlete was similar to that reported by able-bodied athletes. [139,140,293]

6. CONCLUSIONS / CONCLUSIONES

6 CONCLUSIONS / CONCLUSIONES

6.1 General conclusions

The objectives stated in these research work were mostly ratified, and were contrasted with previous literature, however some observations need to be confirmed with similar athletes featuring CMT.

6.2 Conclusions of results

The main conclusions of this research are summarized in the following points.

1) FP displayed greater performance improvement despite administration of lower TL. Therefore, hypothesis #1 was met.
2) Both models showed an increasing trend in performance (incremental ergometer test and 3000 m time trial) relative to pre-altitude. Therefore, hypothesis #2 was not met.
3) A day-to-day HRV-guided training program (FP) induced less suppression and faster restoration in rMSSD and lower rMSSDCV at 3860 m terrestrial altitude compared to a predefined training program (IP). Therefore, hypothesis #3 was met.
4) We observed almost the same times (~3 % impairment) in intense bouts of 2000 m at 4090 m altitude compared to pre-altitude. Therefore, hypothesis #4 was not met.
5) We observed lower SO_2 and greater HR_{rest} at altitude in both models. Therefore, hypothesis #5 was met. However, SO_2 was higher in FP at B_H, W_1, and W_2, suggesting a faster normalization at altitude of this variable. Regarding HR_{rest}, no differences within models were observed.

6) Plasma EPO was ~250 % greater during acute altitude, however it decreased to below pre-altitude values at chroninc altitude, and the lowest values were reached after returning to SL. Therefore, hypothesis #6 was met. Moreover, as previously reported in the literature, we did nod find a relation between EPO suppression observed at chronic altitude and at camp completion, and the peak values observed 35-days after arriving to altitude in erythrocytes, Hb and Hct.
7) A 5-week 3860-4090 m altitude training camp guided by HRV increased blood markers significantly, except Ret. Therefore, hypothesis #7 was not met.
8) Resting BF was significantly enhanced at altitude (FP model) as a positive acclimatization response. Therefore, hypothesis #8 was met. Moreover, resting BF might be considered as a non-invasive and easy to assess tool to establish if athletes cope well with the stress of hypoxia when training in remote mountains areas in which it is difficult to assess red blood cell parameters.
9) Total mood disturbance might be more dependent on greater TL than environment (SL vs. altitude), as observed by TMD peak in W5 compared to W8. Moreover, a volume threshold might trigger an exponential increase in TMD. Therefore, hypothesis #9 was met.
10) POMS was sensitive to detect worsening patterns in fatigue and vigor at altitude (FP model) when physiological variables as SO_2 and SP were considered as covariates. However, we did not find differences in TMD when comparing SL and altitude. Therefore, hypothesis #10 was not met.

11) The nutritional program designed for the FP model helped our athlete to maintain body mass throughout the altitude sojourn, despite: 1) a ~2 % body mass loss during acute altitude due to an increase in water loss as a result of increased diuresis and increased respiration and a significant decrease in plasma volume; 2) an increase in RMR as a sympathoexcitation response at altitude. Therefore, hypothesis #11 was met.
12) We found significant greater fluid intake and diuresis at altitude compared to SL. Moreover, the magnitude of change was greater during early altitude acclimatization compared to chronic altitude. Therefore, hypothesis #12 was met.
13) We observed a decrease in LnrMSSD and an increase in rMSSDCV the day after arriving to the new timetable zone compared to BL. Therefore, hypothesis #13 was met.
14) Slightly lower Ln rMSSD values cope well with positive performance in endurance events, as observed in our athlete and reported previously in endurance disciplines athletes. Therefore, hypothesis #14 was met.
15) Marathon imposes a vagally-mediated marker suppression 24-hours after the event. Therefore, hypothesis #15 was met. Moreover, the rebound in Ln rMSSD reaching BL 48-hours after marathon values, led us to consider that coaches must be cautious if they use HRV as a tool to predefine training program, as this vagally-mediated marker enhancement might be induced by hemodynamic changes triggered by marathon or similar physiological stimulus sessions (i.e., tempo session).

6.3 Conclusiones generales

Se han alcanzado casi todos los objetivos propuestos en este trabajo de investigación, además de ser contrastados con la literatura existente, sin embargo, algunas observaciones necesitan ser confirmadas con atletas de similares características con CMT.

6.4 Conclusiones de resultados

A continuación, se exponen las principales conclusiones de este trabajo de investigación.

1) FP mostró una mayor mejora en el rendimiento, a pesar de una menor TL administrada, por tanto, la hipótesis #1 se cumple.
2) Ambos modelos (IP y FP) mostraron un incremento en el rendimiento (test incremental en ergómetro y test de 3000m contrarreloj), comparado a resultados previos a la estancia en altitud, por tanto, la hipótesis #2 no se cumple.
3) Un entrenamiento guiado por fluctuaciones diarias de la HRV (FP) indujo menos supresión y una restauración más rápida de la rMSSD, así como un menor rMSSDCV at 3860 m de altitud terrestre, comparado a un modelo de entrenamiento predefinido (IP), por tanto, la hipótesis #3 se cumple.
4) Observamos prácticamente los mismos tiempos (~3 % aumento) en repeticiones de 2000 m a alta intensidad a 4090 m de altitud, en comparación a SL, por tanto, la hipótesis #4 no se cumple.
5) Observamos una menor SO_2 y mayor HR_{rest} en altitud en ambos modelos, por tanto, la hipótesis #5 se cumple, sin embargo, la SO_2 fue mayor en FP en B_H, W_1 y W_2, sugiriendo una mayor

normalización en altitud de esta variable. En relación a HR_{rest} no se observaron diferencias entre modelos.

6) La concentración de EPO plasmática fue un ~250 % más elevada durante la fase de aclimatación a altitud, sin embargo, en la fase de altitud crónica disminuyó por debajo de los valores obtenidos previos a altitud y los valores más bajos se alcanzaron al regresar de altitud, por tanto la hipótesis #6 se cumple, además y como previamente expuesto en la literatura, no encontramos una relación entre la supresión de EPO observada en exposición crónica y al final de la concentración y los valores más elevados de eritrocitos, Hb y Hct 35 días después de la llegada a altitud.
7) Cinco semanas de entrenamiento a 3860-4090 m de altitud, guiado por la HRV, incremento de manera significativa parámetros sanguíneos, excepto los Ret, por tanto, la hipótesis #7 no se cumple
8) La frecuencia respiratoria en reposo aumentó significativamente en altitud (modelo FP), como respuesta adaptativa positiva, por tanto, la hipótesis #8 se cumple. De hecho, la BF en reposo, debería ser considerada como una herramienta no invasiva y fácil de medir para determinar si los atletas lidian bien con el estrés hipóxico cuando entrenan en áreas montañosas remotas, donde es difícil medir parámetros sanguíneos.
9) La alteración total del estado de ánimo (TMD) podría depender más de mayores cargas de entrenamiento que del contexto medioambiental (SL vs. altitud), como observamos con el máximo valor de TMD en W5 comparado a W8. Además, podría haber un umbral del volumen de carga que desencadene un incremento exponencial de la TMD, por tanto, la hipótesis #9 se cumple.

10) El cuestionario POMS fue sensible para detectar patrones adversos en fatiga y vigor en altitud (modelo FP) cuando las variables fisiológicas SO_2 y SP fueron consideradas covariables, sin embargo, no encontramos diferencias en TMD cuando comparamos SL y altitud, por tanto, la hipótesis #10 no se cumple.
11) El programa nutricional diseñado para el modelo FP ayudó a nuestro atleta a mantener la masa corporal durante la estancia en altitud, a pesar de: 1) una pérdida del ~2 % de la masa corporal en la fase temprana de exposición debido a un incremento de pérdida de agua por un incremento de la diuresis, un aumento del ritmo respiratorio y una disminución del volumen plasmático; 2) un incremento de la tasa metabólica basal como respuesta a un incremento de la actividad simpática en altitud, por tanto la hipótesis #11 se cumple.
12) Encontramos una mayor ingesta hídrica y diuresis en altitud comparada a SL, además de que la magnitud del cambio fue mayor durante la fase de aclimatación comparada a la altitud crónica, por tanto, la hipótesis #12 se cumple.
13) Observamos una disminución del Ln rMSSD y un amento del rMSSDCV el día después de llegar a la nueva zona de huso horario comparado a BL, por tanto, la hipótesis #13 se cumple.
14) Disminuciones leves del Ln rMSSD están relacionadas con rendimientos positivos en eventos de resistencia, tal y como observamos en nuestro atleta y ha sido reportado previamente en deportistas de disciplinas de resistencia, por tanto, la hipótesis #14 se cumple.

15) La maratón inflige una supresión de marcadores vagales de control cardíaco 24 horas después del evento, por tanto, la hipótesis #15 se cumple. Además, el rebote observado en el Ln rMSSD 48 horas después de la prueba, alcanzando los niveles del BL, nos hacen considerar que los entrenadores deben ser precavidos si usan la HRV como una herramienta para definir la estructura, volumen e intensidad del programa de entrenamiento, ya que el incremento de este marcador vagal podría ser resultado de cambios hemodinámicos provocados por la maratón o entrenamientos de estímulo fisiológico similar (carrera continua de alta intensidad).

6.5 Research limitations and future directions

The studies that make up this research show some limitations that must be mentioned:

The main limitation from FP model HRV study is that we did not modify HRV RV throughout the 5-week altitude sojourn, as it is recommended to update the RV as resting HRV can increase as a positive adaptation to training (i.e., the athlete was able to reach HRV RV even less than 24 hours after performing A, B, C sessions. Regarding this issue, Vesterinen et al. estimate after 4-weeks of intense training the HRV RV. [142] However, our study was shorter (7 vs. 12 weeks) and also HRV tend to increase faster as a positive adaptation to training in untrained and moderately trained athletes. [165, 295,297,297,298]

Another limitation was the assessment of SO_2 under resting conditions, so in future studies should be consider the assessment while exercising.

We did not estimate HR at VT1, VT2 and VO_{2max} at altitude, and as aforementioned [38] they differ from SL, so in future studies it should be consider, as we observe during 2000 m repetitions ~20 % decrease in HR during bouts.

We were not able to estimate Hb_{mass}, RCV and hemodynamics parameters (i.e., blood volume and plasma volume), as we were not able to use the carbon monoxide (CO) rebreathing technique. [299,300,301] Despite we have shown optimal hydration status in our athlete, the dehydration has a concominant decrease in plasma volume, so some blood markers results could be influenced by hypovolemia. Therefore, in future research we suggest the use of CO-rebreathing technique for haematological parameters assessment. In addition, we only assessed EPO 35-hours after altitude arrival,

so more assessment time-points (e.g., 4-h, 12-h, 24-h, 48-h) would allow us to know the peaking time-point of EPO.

Other possible limitations might be the influence of loneliness on mood states at weeks four and five at altitude, however, the athlete feedback on social relations with the hotel's workers and area coaches was quite positive.

Regarding nutritional intervention study, the absence of outcomes like upper body skinfolds, and upper arm circumference measurements, which could help us to know if body fat percentage and loss of muscle mass occurred in our athlete. Thus, these outcomes should be considered in future research. Moreover, RMR was not assessed, what could lead nutritionist to design a more adjusted training program, as RMR may vary throughout altitude sojourn. Moreover, the use of pictures on four occasions to record restaurant meal consumption must be considered as a limitation. However, this methodology has been supported by exercise nutritionists as a useful strategy, particularly when research teams are not present. [222] In addition, the absence of muscular biopsies did not allow us to measure glycogen and protein muscle content.

One methodological limitation in the hydration status study was the absence of urine Na^+ assessment, so we were not able to compare results with studies reporting an increase in sodium excretion during acute altitude. In addition, as athlete was not able to collect urine throughout training sessions, it was quite uncomfortable to avoid urinate, especially in long sessions where ambient temperature was low. Moreover, in future studies with movement restrictions, there should be consider do not include as part of statistical analysis the time-points, where some variables might be determined by movement restrictions (i.e., overseas flights).

A terminological limitation of this study is the term water loss, as we only assessed body mass difference between pre- and post-session, however we

discriminate water loss occurred by hyperventilation, which is exacerbated while exercising at altitude.

As a reliable altitude acclimatization tool, we did not use the Lake Louise Score questionnaire to assess the AMS, so it is highly recommended for future studies.

The main limitation of the current study was evident in our inability to find current literature with similar experimental designs among elite marathoner populations. Indeed, only one study with middle distance collegiate athletes was conducted at a similar altitude (4000 m). Moreover, no studies at altitude with wheelchair-athletes population have been conducted, so we encourage researchers and coaches to consider our experimental design to replicate and obtain useful data for scientific community.

Regarding the marathon study we might consider longer data time-point assessment post-marathon, being equal as BL (6-days). Moreover, we did not assess muscle tissue oxygenation (i.e., latissimus dorsi) with a non-invasive monitoring technique such as Near Infrared Spectroscopy (NIRS), which is reliable and cost-effective tool, and allows investigation of changes in local tissue oxygenation. [302]

To consider as practical applications for future directions or coaches training athletes with similar features:

1) Same number of interval sessions were performed in both models (#8), however long sessions at VT1 were the double in IP compared to FP (#8 vs. #4). Moreover, and considering marathon performance is more depending in VO_{2max} and VO_2 at VT2, we think that athlete performance was not impaired with lower number of VT1 sessions in IP compared to VT2, however, it should be considered for training camps set in the

general base training period, as development of VT1 is main target.

2) The increase in rMSSDCV from W_2 to W_3 and diminution of blood markers observed in FP the week after more number of interval sessions, and lower inter-specific sessions recovery (W_2), led us to think that a minimum of 48 to 72 hours recovery must be determine to accomplish a new specific training, rather than performing it according to a HRV RV, as HRV is high-dependent on hemodynamics, and plasma volume might increase after intense long sessions, being accompanied by an increase in rMSSD, so we propose a mixed model based on HRV but respecting a recovery window between A, B, C sessions.
3) A 40 % decrease in TL once coaches observe significant decrease in vigor or significant increases in fatigue, anger or TMD might be an strategy for avoiding NFO when athletes train at both SL and altitude.
4) Our nutritional program helped to minimize performance perturbations (i.e., minimal worsening in VT2 intensity bouts), facilitate overall recovery immediately after sessions (i.e., accelerate protein synthesis and glycogen resynthesis), and enhance athlete´s performance post-altitude, therefore could be used for wheelchair athletes to complete sojourns at very high altitude.
5) Our nutritional intervention can help to develop a unique model for athletes with lower limbs affection training at altitude, as their differences with able-bodied athletes (i.e., runners maintain their center of masses against gravity while

moving) might affect the selection of micro and macro-nutrients pre- and during training.

6) Individualized hydration strategies to optimize hydration and re-hydration of wheelchair marathoners training at altitude should consider: 1) type and length of session; 2) hydration markers which can be non-invasively assessed as SG and Na^+; 3) diuresis assessment to determine if liquid retention exists, as it has been associated to AMS; 4) Guarantee a Fluid intake of 600 mL · h^{-1} during training and 4 L per day; 5) Estimation of HB after training and compare with body mass to avoid (± 2 %) body mass oscillations; 6) personalize Fluid rate to x mL · h^{-1} if ± 2 % in body mass after training is observed.
7) Ln rMSSD oscillations the day after arriving to a new geographic zone with significant timetable change (i.e., 3 hours) is a reliable indicator of positive/negative relocation, so it should be used as a non-invasive tool to determine the degree of readaptation to new zone timetable.
8) Cardiovascular response (i.e., HR) during a marathon in a world-class tetraparesic athlete seems similar to the reported in previous literature among elite able-bodied marathoners, so it might be use as a practical tool for monitoring the relation between HR and racing performance in a time course.

Chapter II:

Published and under review manuscripts

7 PUBLISHED MANUSCRIPT I

The study was published as:

Sanz-Quinto S, López-Grueso R, Brizuela G, Flatt AA, Moya-Ramón M. Influence of Training Models at 3,900-m Altitude on the Physiological Response and Performance of a Professional Wheelchair Athlete: A Case Study. *J Strength Cond Res.* 2019;33(6):1714-1722. DOI: 10.1519/JSC.0000000000002667.

The journal is indexed in the Journal Citation Reports with an impact factor of 3.017 (2018) and is ranked 13 out of 83 in the category of sports sciences.

Abstract

This case study compared the effects of two training camps using flexible planning (FP) vs. inflexible planning (IP) at 3,860-m altitude on the physiological and performance responses of an elite marathon wheelchair athlete with Charcot-Marie-Tooth disease (CMT). During IP, the athlete completed preplanned training sessions. During FP, training was adjusted based on vagally mediated heart rate variability (HRV) with specific sessions being performed when a reference HRV value was attained. The camp phases were baseline in normoxia (B_N), baseline in hypoxia (B_H), specific training weeks 1–4 (W_1, W_2, W_3, W_4), and Post-camp (Post). Outcome measures included the root mean square of successive R-R interval differences (rMSSD), resting heart rate (HR_{rest}), oxygen saturation (SO_2), diastolic blood pressure and systolic blood pressure, power output and a 3,000-m test. A greater impairment of normalized rMSSD (B_N) was shown in IP during B_H (57.30 ± 2.38 % vs. 72.94 ± 11.59 %, p = 0.004), W_2 (63.99 ± 10.32 % vs. 81.65 ± 8.87 %, p = 0.005), and W_4 (46.11 ± 8.61 % vs. 59.35 ± 6.81 %, p = 0.008). At Post, only in FP was rMSSD restored (104.47 ± 35.80 %).

Relative changes were shown in power output (+3 W in IP vs. +6 W in FP) and 3,000-m test (-7s in IP vs. -16s in FP). This case study demonstrated that FP resulted in less suppression and faster restoration of rMSSD and more positive changes in performance than IP in an elite wheelchair marathoner with CMT.

Keywords: Hypoxia, Heart Rate Variability, Autonomic Nervous System, Paralympic, Marathon

Introduction

It was first demonstrated in 2003 through peroneal microneurography that during acclimatization to high-altitude hypoxia, sympathetic overactivity occurs in lowlanders. [39] More recently, Lundby et al. reported the same sympathoexcitation in lifelong high- landers at 4,100 m and that their muscle sympathetic nerve activity burst frequency was twice the level recorded in a group of highlanders at sea level. [41] Slightly lower activity was shown in highlanders compared with lowlanders after 50 days of exposure (chronic hypoxia). Noninvasive studies have shown altered autonomic heart rate regulation with altitude and endurance training [184,185] but their results are not in agreement with the increase in parasympathetic activity observed at altitude using invasive measures. [186]

Higher resting heart rate variability (HRV) has been associated with better fitness [303] and increased exercise performance. [304] In addition, successful adaptation to an endurance training program is reflected in an increase in several time- and frequency-domain indices of HRV [215] along with less day-to-day fluctuations (represented by the coefficient of variation, CV). [160] By contrast, lower HRV values have been related to maladaptive training responses. [143,305] Recently, to maximize adaptations and avoid the risk of overreaching, a new training approach, using a flexible training program model (FP) has emerged in which daily training loads are adjusted based on a reference value (RV) of postwaking, vagally mediated HRV. [141,142] For example, a group of recreational endurance runners after an FP based on HRV improved 3,000-m running performance, whereas no improvement was reported for the group after an inflexible planning (IP) (preplanned) program. [142] Although HRV has been evaluated in athletes during periods of standardized training under hypoxic conditions, [306] it remains unclear whether HRV-guided training at altitude would offer any advantages over

standardized training. In a recent study with elite Nordic skiers who slept in normobaric hypoxia (FiO_2 = 0.15 %), only the HRV-guided training group improved roller-ski performance, whereas oxygen uptake was improved by both hypoxic groups after the intervention. [188]

Increased diastolic blood pressure (DP) at altitude has been associated with a sympathetic response as a way to compensate for hypoxia-induced peripheral vasodilation. [41] However, the same research did not report changes in systolic blood pressure (SP). It is unknown whether marathon wheelchair athletes demonstrate a faster acclimatization to a hypoxic environment than able-bodied marathoners. Wheelchair athletes have less active muscles during propulsion [307] and the energy cost of running depends on anthropometric features such as the length of the muscles involved. [205] Moreover, despite a similar heart rate in elite wheelchair marathoners [204] and elite able-bodied marathoners, [189] oxygen uptake is quite lower in wheelchair athletes. [204] Thus, oxygen status may be differently affected among individuals with a higher level of muscular atrophy in upper extremities, as in the current case study in which the participant was diagnosed with Charcot-Marie-Tooth disease (CMT). Charcot-Marie-Tooth disease is the most common hereditary peripheral neuropathy, affecting up to 30 per 100,000 people worldwide. [206] Charcot-Marie-Tooth disease totally affects distal muscle function and partially affects proximal function. Although muscle atrophy is associated with CMT, respiratory and cardiac system responses are not disturbed. Only one study has evaluated the effect of endurance training on HRV indices among individuals with CMT, finding that 12 weeks of interval training increased cardiac-parasympathetic activity. [208]

With increasing altitude, there is a progressive decrease in peak heart rate, [38] as it has been shown in 5 sea level lowlanders who performed maximal efforts under hypobaric hypoxia conditions, corresponding to altitudes of

3,300, 4,300, 5,300, and 6,300 m above sea level. Reduction in peak heart rate is approximately 1 b · min^{-1} for every 7 mm Hg decrease in barometric pressure below 530 mm Hg. Although mechanical stress in wheelchair racing seems to be lower than in running, cardiovascular strain is higher when comparing with an arm leg exercise, [308] whereas oxygen uptake is lower. [204] Furthermore, resting heart rate range in sedentary individuals with CMT population seems to be the same as in the general population. [208]

This case study compared the physiological and performance effects of 2 different training programs (IP and FP) conducted in the Peruvian Andes (3,860 m), over 2 consecutive years in an elite wheelchair marathoner with CMT. The primary objective was to determine which model facilitated better physiological and performance adaptations.

Methods

Experimental approach to the problem

A single-subject case study featuring an elite wheelchair marathoner with CMT was conducted to determine the effects of 2 different training models (i.e., IP vs. FP) at altitude on physiological and performance responses.

Training camps at altitude were repeated on consecutive years at the same time and location in preparation for the competitive season. Cardiac-autonomic activity (vagally mediated HRV and resting heart rate, HR_{rest}), oxygen saturation (SO_2), and blood pressure were measured daily throughout the following periods: sea-level baseline, altitude baseline, 4 weeks of training at altitude, and 1-week post-training at sea level. Performance tests to evaluate power output on an ergometer and aerobic power in a 3,000 m test were conducted before and after the training camps. Changes in physiological and performance parameters were assessed within and between camps. Relationships between physiological parameters were quantified.

Subjects

One professional male wheelchair athlete with CMT (mean ± SD: age = 36 years; height = 1.76 m; body mass = 50.0 ± 0.81 kg; power output at second lactate threshold = 62 W) participated in this case study. This athlete was a silver medalist at the 2000 and 2004 Paralympic Games; a former world record-holder in his division (T52, quadriplegics) in 800 m (116 seconds) and 1,500 m (216 seconds); a world record-holder in 5,000 m (757 seconds) and half marathon (3,028 seconds); he possesses the fourth best ever time in marathon in his division (6,125 seconds); and holds a record of 107 victories in road events. He has accumulated more than 10 years of altitude training experience, has performed both altitude models, Live High-Train High and Live High-Train-Low [2] and had been exposed to more than 8,000 hours of normobaric hypoxia.

After being informed of the requirements and risks associated with his involvement in this study, the participant provided written informed consent to be a research subject in this case study. All procedures were approved by the Ethics Research Committee of the Miguel Hernández University (Elche, Spain).

Procedures

The 2 training camps (IP and FP) lasted 5 weeks in duration each and were completed over 2 successive years (January and February 2015–2016) during the spring marathon preparatory cycle (Figures 4 and 5).

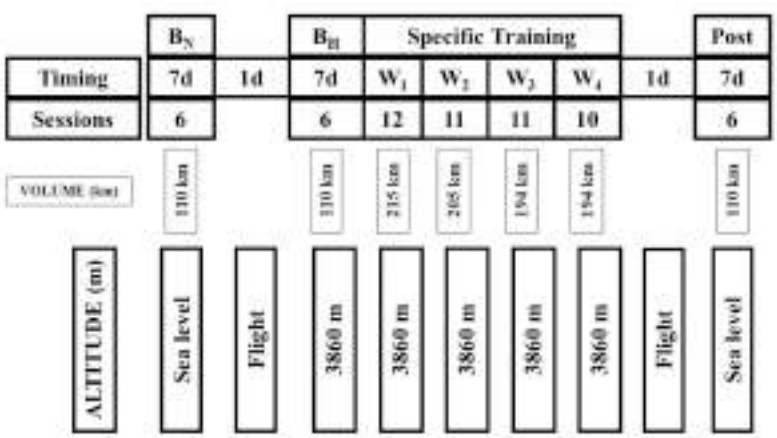

Figure 4. Inflexible planning (IP) experimental design.

B_N = baseline in normoxia; B_H = baseline in hypoxia; $W_{(1,2,3,4)}$ = first, second, third, and fourth weeks, in which specific sessions were performed after the acclimatization phase; Post = returning sea level week. In W_2, PM session on Friday was canceled due to his feeling of exhaustion (5 days after day off). In W_3, PM session on Monday was canceled due to heavy rain. In W_4, PM sessions on Friday and Saturday were canceled due to his feeling of exhaustion (5 and 6 days after the day off). In W_4, PM session on Monday was canceled due to heavy rain. Number of interval sessions: W_1 #2–W_2 #2–W_3 #2–W_4 #2. Number of sessions ~65 % VO_{2max} ~2-hour workouts W_1 #2–W_2 #2–W_3 #2–W_4 #2.

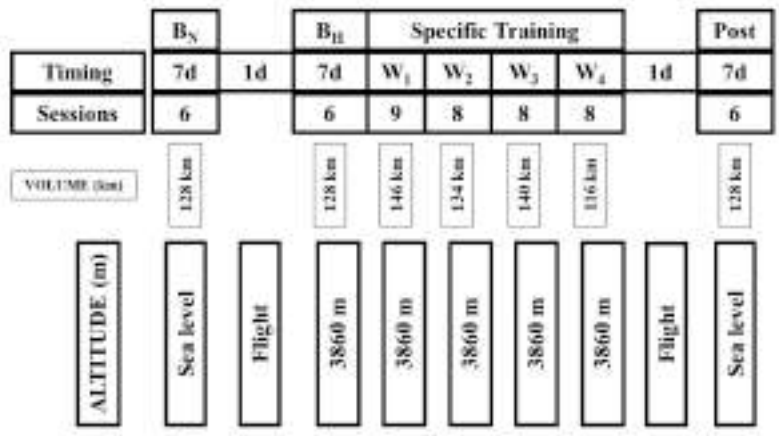

Figure 5. Flexible planning (FP) experimental design.

B_N = baseline in normoxia; B_H = baseline in hypoxia; $W_{(1,2,3,4)}$ = first, second, third, and fourth weeks, in which specific sessions were performed after acclimatization phase; Post = returning sea level week. In W_2, PM session on Friday was canceled due to his feeling of exhaustion (5 days after the day off). In W_3, PM session on Monday was canceled due to heavy rain. In W4, PM session on Monday was canceled due to heavy rain. Number of interval sessions: W_1 #2 (1 x "A"+1 x "C"), W_2 #3 (2 x "A"+1 x "C"), W_3 #2 (1 x "C"+1 x "A"), and W_4 #2 (1 x "C"). Number of sessions ~65 % VO_{2max} ~2-hour workouts W_1 #1 (1 x "B"), W_2 #1 (1 x "B"), W_3 #2 (2 x "B"), and W_4 #0.

Inflexible Model. In the IP camp, the participant completed every preplanned workout. Camps were divided into 4 periods: 1-week at sea level as baseline in normoxia (B_N), a 1-week acclimatization phase as baseline in hypoxia (B_H), 4 specific training weeks (W_1, W_2, W_3 and W_4), and 1 week back at sea level (Post). Heart rate variability, SO_2, and resting HR_{rest} were collected daily.

B_N training was similar during B_H for both IP and FP camps. The first 2 days of B_H involved passive rest to minimize jet lag and acute mountain sickness symptoms caused by the long trip from Spain to Peru (time difference of 6 hours) and the change in altitude. Two daily training sessions were performed from Wednesday to Friday at an intensity < the aerobic threshold (i.e., "easy push"). The morning and afternoon sessions were 20 and 16 km, respectively, with a 20-km easy push also performed on Saturday morning. 12 sessions per week (≈200 km every 6 consecutive days) were performed from W_1 to W_4 during IP, whereas Sundays were reserved for passive rest. (Table 8). Two resistance sessions were performed on Mondays and Thursdays (Figure 4), and 2 interval sessions were performed on a plateau at 4,090 m altitude on Tuesdays (20 x 400 m, recovery repetitions: 75 seconds) and Fridays (6 x 2 km, recovery repetitions: 120 seconds). Two-hour sessions at the aerobic threshold were performed on Wednesdays and Saturdays.

Table 8. Weekly mileage and strength volume and intensity in FP and IP.*

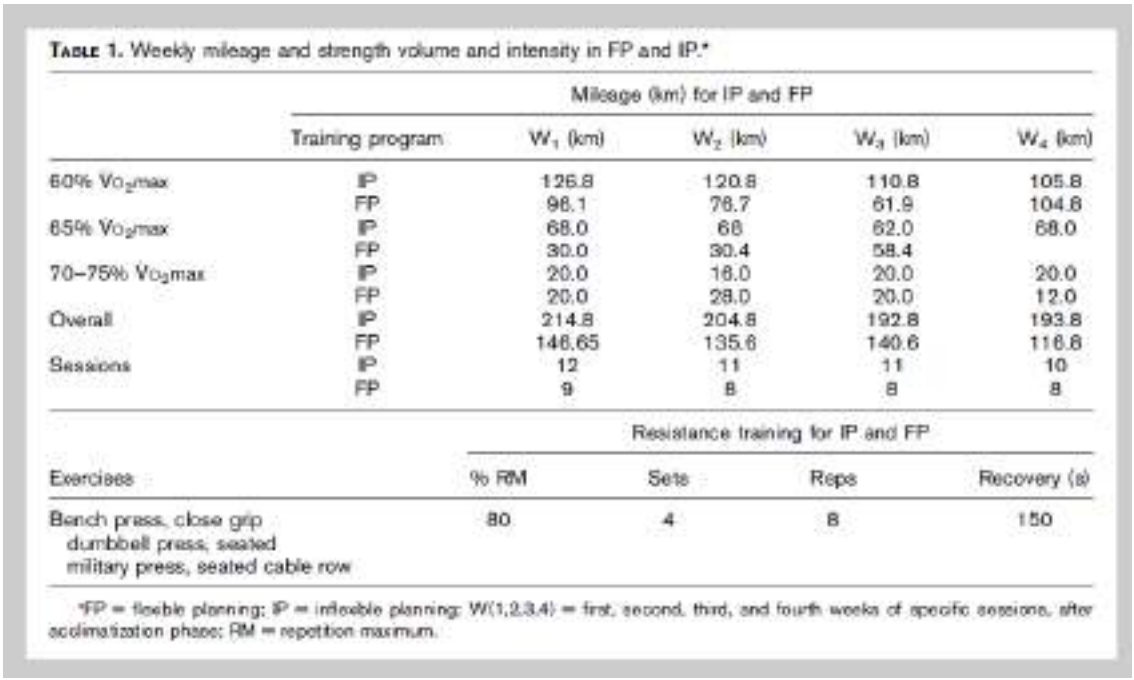

TABLE 1. Weekly mileage and strength volume and intensity in FP and IP.*

		Mileage (km) for IP and FP			
	Training program	W_1 (km)	W_2 (km)	W_3 (km)	W_4 (km)
60% Vo_2max	IP	126.8	120.8	110.8	105.8
	FP	96.1	78.7	61.9	104.8
65% Vo_2max	IP	68.0	68	62.0	68.0
	FP	30.0	30.4	58.4	
70–75% Vo_2max	IP	20.0	16.0	20.0	20.0
	FP	20.0	28.0	20.0	12.0
Overall	IP	214.8	204.8	192.8	193.8
	FP	146.65	135.6	140.6	116.8
Sessions	IP	12	11	11	10
	FP	9	8	8	8

	Resistance training for IP and FP			
Exercises	% RM	Sets	Reps	Recovery (s)
Bench press, close grip dumbbell press, seated military press, seated cable row	80	4	8	150

*FP = flexible planning; IP = inflexible planning; W(1,2,3,4) = first, second, third, and fourth weeks of specific sessions, after acclimatization phase; RM = repetition maximum.

FP = flexible planning; IP = inflexible planning; $W_{(1,2,3,4)}$ = first, second, third, and fourth weeks of specific sessions, after acclimatization phase; RM = repetition maximum.

Flexible Planning. During B_H, the HRV reference value was determined in both models but it was only used to guide specific sessions [142] in FP throughout W_1–W_4. One SD below the mean of the root mean square of successive differences (rMSSD) throughout B_H was chosen as the RV. [142] Accordingly, the RV calculated for IP was 27.0 ms and it was 19.55 ms for FP.

From W_1 to W_4, the training was fixed on Mondays and Thursdays, with morning sessions involving a 16-km easy push and the afternoon sessions involving resistance training (Table 8). Sundays were passive rest.

If the RV was reached (rMSSD ≥ 19.55 ms), a specific session was performed in the morning, followed by an evening off. The specific sessions were: A (20 x 400 m on a plateau at 4,090 m, recovery repetitions: 75 seconds), B (2 hours at the aerobic threshold), and C (6 x 2 km on a plateau at 4,090 m, recovery repetitions: 120 seconds). The order to perform the specific sessions was always in sequence (e.g., A, B, C). If the RV was < than 19.55 ms, 2 easy workouts were performed (morning, 20-km "jog" and afternoon, 16-km "jog"). The specific sequence of the sessions would not be affected by the end of a week. For details of daily training in IP and FP, see Figures 6 and 7.

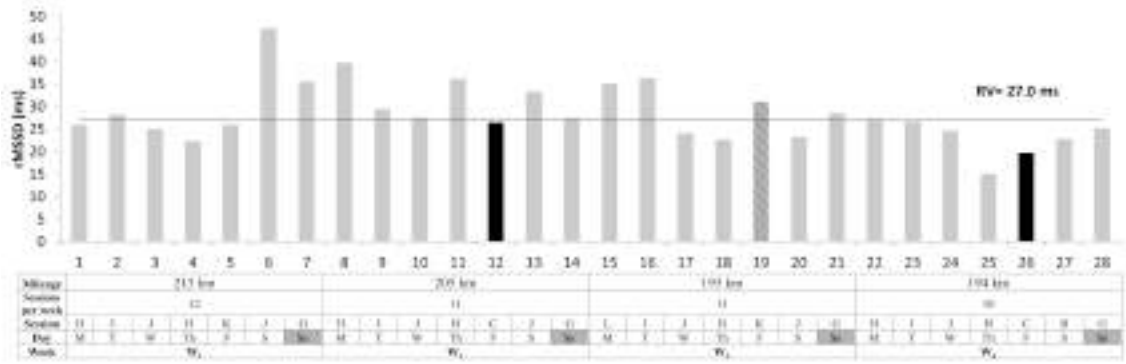

Figure 6. Heart rate variability during specific training weeks at 3,900 meters in IP.

$W_{1,2,3,4}$ = specific training weeks in hypoxia; rMSSD = square root of the mean of the squares of the successive differences between adjacent NNs; RV = rMSSD value chosen to perform specific sessions in FP; Session B = 2 hours at the aerobic threshold; Session C = 6 x 2,000 m recovery repetitions 120; Session G = rest; Session H = 10 km below aerobic threshold + gym session +16 km below aerobic threshold in the afternoon; Session I = 20 x 400 m recovery repetitions 75 seconds + 16 km below aerobic threshold in the afternoon; Session J = 2 hours at the aerobic threshold +12 km below the aerobic threshold in the afternoon; Session K = 6 x 2,000 m recovery repetitions 120 seconds + 12 km below the aerobic threshold in the afternoon; FP = flexible planning; IP = inflexible planning. Columns in bold represent days in which the athlete was not able to complete the afternoon session due to his

feeling of exhaustion in IP. Columns with bars represent days where heavy rain forced the athlete to cancel or modify a session.

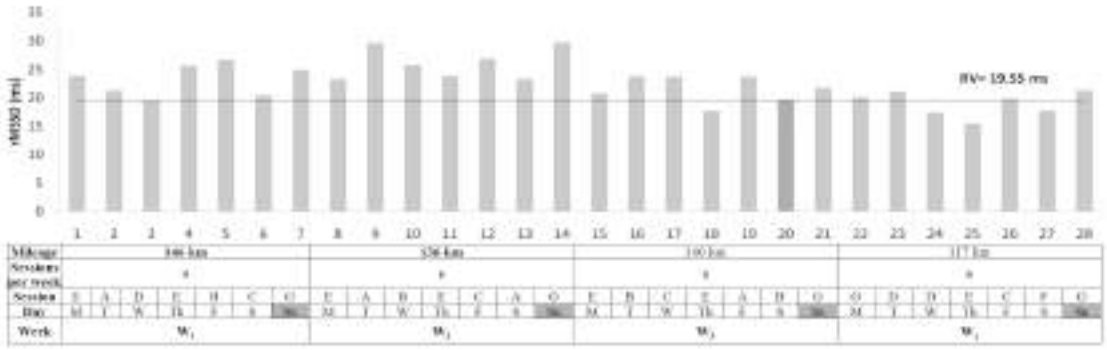

Figure 7. Heart rate variability during specific training weeks at 3,900 meters in FP.

$W_{1,2,3,4}$ = specific training weeks in hypoxia; rMSSD = square root of the mean of the squares of the successive differences between adjacent NNs; RV = rMSSD value chosen to perform specific sessions in FP; Session A = 20 x 400 m recovery repetitions 75 seconds; Session B = 2 hours at the aerobic threshold; Session C = 6 x 2,000 m recovery repetitions 120; Session D = 20 km below aerobic threshold in the morning +16 km below the aerobic threshold in the afternoon; Session E = 16 km below aerobic threshold in the morning + gym session in the afternoon; Session F = 20 km below aerobic threshold in the morning; Session G = rest; FP = flexible planning. Columns with bars represent days in which heavy rain force athlete to cancel or modify a session.

Resistance training structure and exercises are described in Table 8. Heart rate variability was recorded daily in the supine position after waking, bladder emptying, and in a fasted state. A metronome was used during HRV recordings to control for respiration rate (15 Breaths · min^{-1}). A heart rate monitor (Polar RSCX 800; Kempele, Finland) was used to record R-R intervals. Filtering, correction, and detrending were applied to avoid ectopic beats. The HRV parameters were calculated with Kubios HRV 2.0 (Kuopio, Finland, 2008) analyzing the last 5 minutes of a 10-minute recording. [238,309]

The rMSSD was selected as the main index to assess HRV. [165,310] Raw rMSSD values expressed in ms can be seen in Figures 6 and 7. The weekly CV of rMSSD was calculated for W_1–W_4 (Figure 8) as an indicator of training adaptation. [160] The HR_{rest} was calculated as the HR average of the last 5 minutes of the R-R interval recording.

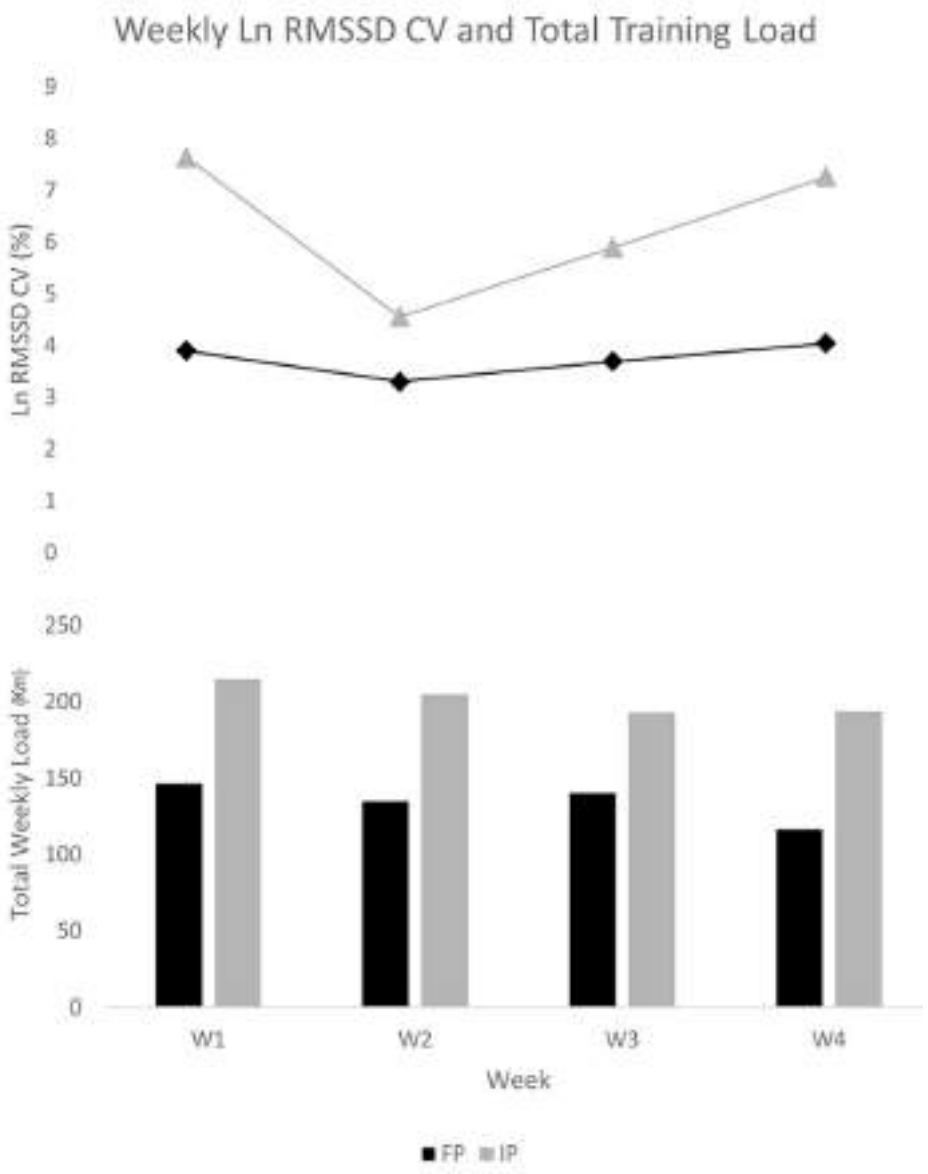

Figure 8. Weekly CV RMSSD and total training load for specific training weeks.

Ln RMSSD CV = change in the CV of log-transformed root mean square of successive R-R intervals from FP (gray line and bars) and IP (black line and bars). Total weekly volume in kilometers from FP and IP. FP = flexible planning; IP = inflexible planning.

Laboratory Test. Four days before B_N (Pre_{-4}) and 11 days after the altitude camp ($Post_{11}$), an incremental test was performed on a specific wheelchair ergometer, in which steady conditions were maintained (temperature 22–24º C, humidity 73–75 %). The protocol (as described by Polo- Rubio) [217] included a 20-minute warm-up period at constant power (20 W). Then, the athlete started an incremental test at a brake power of 6 W, maintaining a stroke frequency between 90 and 100 strokes · min^{-1} and increasing the power by 3 W every 60 seconds until the athlete's heart rate passed 170 b · min^{-1} (just over his marathon pace intensity). Power output was considered as the ergometer braking power during the last completed step of the test. A heart rate monitor was included to measure heart rate. Due to the invasiveness of wearing a gas analyzer device during wheelchair propulsion, oxygen consumption (VO_2) was not assessed.

3,000 m Test. Three days before B_N (Pre_{-3}) and 12 days after the altitude camp ($Post_{12}$), a 3,000 m test was performed on a 200-m indoor track. After a warm-up of 6 kilometers plus 80 m strides, the test started from a static position. Conditions for all track tests were: temperature = 18.3 ± 2.1º C and humidity = 74–79 %. The reason for choosing the $Post_{12}$ as the day to perform the 3,000-m test was because the athlete had his greatest marathon performance (Oita Marathon, 1 hour 43 minutes 46 seconds and Chicago Marathon, 1 hour 46 minutes and 13 seconds, (both still quadriplegic division course records and both set in 2007) 12 days after arrival from altitude, after completing camps at 2,320-m altitude.

Statistical Analysis

The distribution of each variable was examined using the Kolmogorov-Smirnov normality test. Natural logarithm transformations (Ln) were applied to rMSSD. All data (except SO_2, SP, and DP) were normalized in

percentages with reference to the baseline in normoxia (Δ) and presented as a mean ± SD. A repeated-measures ANOVA was performed for all the variables, including the factor TIME with levels B_N, B_H, W_1, W_2, W_3, W_4, and Post. A post hoc least significant difference (LSD) multiple range test determined differences between factor levels. Pearson's correlation coefficients were calculated for the rMSSD, SO_2, HR_{rest}, SP, and DP variables. Statistical significance was set at alpha = 0.05. Statistical analyses were performed using SPSS version 22.0 (SPSS, Inc., Chicago, IL, USA) software.

Results

Results for rMSSD, SO_2, HR_{rest}, SP, and DP can be viewed in Table 9. In IP, B_N rMSSD was significantly greater than all altitude periods but not Post. Greater hypoxemia was observed in B_H ($p = 0.001$) compared with B_N. From B_H to Post, SO_2 values increased each week.

Increasing HR_{rest} was observed after exposure to altitude ($p = 0.001$). A negative correlation was found between HR_{rest} and SO_2 ($r = 20.43$; $p = 0.0188$).

In FP, B_N rMSSD was signficantly greater than in all altitude periods but it was not different from Post (Table 9). A strong correlation was found between rMSSD and SO_2 ($r = 0.54$; $p = 0.001$).

Greater hypoxemia was shown in B_H with SO_2 values being significantly lower ($p = 0.001$) than in B_N (Table 9). From B_H to Post, SO_2 values increased each week. HR_{rest} increased significantly ($p = 0.001$) from B_N to B_H. Post HR_{rest} was significantly lower ($p = 0.001$) than at altitude values. A negative correlation was found between HR_{rest} and SO_2 ($r = 20.83$; $p = 0.001$).

We observed increased DP comparing all altitude periods with normoxic

conditions ($p = 0.001$), whereas SP did not differ from pre-altitude to acclimatization ($p > 0.05$).

Between-camp analysis revealed that rMSSD during FP was significantly greater than IP at B_H ($p = 0.004$), W_2 ($p = 0.005$), and W_4 ($p = 0.008$).

SO_2 was higher in FP at B_H ($p = 0.049$), W_1 ($p = 0.001$), and W_2 ($p = 0.001$), suggesting a faster recovery of this variable.

The ergometer test in IP showed relative changes in power output 46 W at Pre_{-4} vs. 49 W at $Post_{11}$, whereas in FP, change was 44 W at Pre_{-4} vs. 50 W at $Post_{11}$ ($p = 0.001$).

Both models reduced time set in the 3,000-m test; however, due to the magnitude of the change, it cannot be considered a significant improvement. (IP Pre_{-3} = 470 seconds vs. IP $Post_{12}$ = 463 seconds; FP Pre_{-3} = 472 seconds vs. FP $Post_{12}$ = 456 seconds).

Table 9. Time- and frequency-domain indices of HRV, SaO_2 and HR_{rest} before, during, and after both training programmes. [*†]

Training program	Variable (mean ± SD)	B_N 1	B_H 2	W_1 3	W_2 4	W_3 5	W_4 6	Post 7
IP	Δ rMSSD	100	57.30 ± 2.38[‡]	61.14 ± 17.65[‡§]	63.99 ± 10.32[‡§]	58.43 ± 11.54[‡§]	46.11 ± 8.61[‡§¶#]	88.94 ± 25.34
FP		100	72.94 ± 11.59[††]	72.58 ± 8.74	81.65 ± 8.87[††]	67.51 ± 7.38[‡]	59.35 ± 6.81[‡§¶††]	104.47 ±35.80[**]
IP	SO_2 (%)	98.86 ± 0.12	86.29 ± 0.53	88.74 ± 0.90	90.33 ± 0.53[§‖]	92.78 ± 1.07[§‖]	92.06 ± 1.35[§‖]	98.17±0.32[§‖¶#**]
FP		98.64 ± 0.14	88.31 ± 2.46	91.19 ± 0.76[§††]	91.92 ± 0.82[§††]	92.35 ± 1.14[§‖]	92.64 ± 1.12[§]	98.08±0.26[§‖¶#**]
IP	Δ HR_{rest}	100	130.49 ± 8.10[‡]	131.83 ± 6.98[‡]	128.12 ± 7.58[‡]	124.64 ± 6.71[‡]	123.80 ± 5.93[‡]	96.20±4.23[§‖¶#**]
FP		100	130.17 ± 5.93[‡]	134.07 ± 3.92[‡]	130.06 ± 3.18[‡]	128.70 ± 6.84[‡]	127.87 ± 6.51[‡]	96.64±8.99[§‖¶#**]

[*]HRV= heart rate variability; B_N = baseline in normoxia at 16 m; B_H = baseline in hypoxia at 3,860 m; W_1 = first week of specific training; W_2 = second week of specific training; W_3 = third week of specific training; W_4 = fourth week of specific training; Post = values after altitude training camp at 16m altitude; IP = inflexible planning; FP = flexible planning; rMSSD = root mean square of successive R-R interval differences.

[†] The values (less SO_2) are normalized in percentages (Δ) with reference to the baseline in normoxia (B_N) at 16 m altitude.

[‡] Differences from B_N ($p < 0.01$).

[§] Differences from B_H ($p < 0.01$).

‖ Differences from W_1 ($p < 0.01$).

¶ Differences from W_2 ($p < 0.01$.

Differences from W_3 ($p < 0.01$).

** Differences from W_4 ($p < 0.01$).

††Differences between groups ($p < 0.01$).

Discussion

This case study compared the effects of 2 different training models (IP and FP) on cardiac-autonomic activity and the performance responses of an elite marathon wheelchair athlete with CMT. The primary objectives were to determine which model facilitated more desirable physiological and performance responses.

During IP, significant reductions in rMSSD (42.7 %) were observed at B_H relative to B_N; however, it cannot be interpreted as a reduction in vagal activity because it has been reported that in acclimatization, there is an increase in both parasympathetic [186] using a vagal blockade and sympathetic activity [41] with peroneal microneurography. In line with our findings, one study demonstrated an 88 % reduction in high frequency (HF) power (a frequency-domain index of HRV) during the first 2 days at 4,350 m altitude, improving only to within 54 % of baseline HF after 6 days. [184] However, we must be cautious with these results as Lundby et al. did not report differences in muscle sympathetic nerve activity in acute exposure to hypobaric hypoxia (FiO_2 = 0.12 %) in lowlanders compared with sea level. [41] The rMSSD remained >10 % below B_N values at Post, suggesting that the alteration of HRV remained after altitude exposure.

The athlete may have experienced greater suppression of rMSSD during IP than FP for several reasons. For example, total training loads at altitude were ~40 % greater during IP compared with FP. Increased training loads have been shown to cause larger reductions along with greater daily fluctuation in

rMSSD (i.e., higher rMSSDcv) concurrent with decrements in perceived fatigue and muscle soreness. [155] The participant demonstrated consistently larger reductions in rMSSD relative to baseline (Table 9) and thus a higher rMSSDcv during each training week at altitude during IP (Figure 8). Moreover, the athlete was unable to complete prescribed sessions on at least 2 occasions during IP due to his feeling of exhaustion (Figure 4). Finally, rMSSD remained >10 % below B_N in IP when training loads were reduced at near-sea level (i.e., Post), which may reflect persisting effects of fatigue and inadequate recovery.

A significant decrease in SO_2 (14.71 %) was shown in B_H. Hypoxemia below 95 % SO_2 has been associated with impaired VO_{2max}. [244] A slight increase until W_3 was shown, reflecting acclimatization to altitude. [142]

Similar drops in SO_2 were shown in FP and IP; however, it was restored faster in FP. Until W_2, there could be a greater impairment of the cardiorespiratory function in IP, as well as a greater impairment of the ability to adapt to the acute hypoxia phase. [246]

Lower SO_2 in B_H in IP could be induced by a lower ventilatory response, [245] a phenomenon related to acute mountain sickness. Sympathoexcitation due to a decrease in SO_2 has been observed under hypoxic conditions. [247]

The HR_{rest} showed similar increases in FP and IP (~17 b · min-^{1}) from normoxia to hypoxia, a response reported recently at 3,454 m.[40] There was a greater decreasing pattern in IP that might be explained by the greater training loads in IP (200 km per week) compared with FP (140 km per week) and more sessions at the aerobic threshold (8 vs. 4).

We found significant increases in both SP and DP. Although Lundby et al. did not find differences in SP, [41] the more extenuating circumstances in our experiment might be the reason for a greater increase in this sympathetic marker. In fact, once specific training began, we found SP to be significantly

higher than normoxic conditions (not observed in acclimatization). A phenomenon defined as a sympathetic response to hypoxia is an increase in DP, [41] which was observed in the current case study, possibly to increase vasodilation. [41]

Both training camps generated positive relative changes in power output and 3,000 m times. The greater improvement in FP is in agreement with a recent study from Schmitt et al., [188] in which only Nordic skiers guided by HRV improved roller-ski performance (22.7 ± 3.6 %) 21 days after hypobaric hypoxia intervention. Our results conflict with those of Buskirk et al., [211] in which well-trained runners did not improve endurance performance after more than 40-days training at 4,000 m altitude. Our athlete was able to perform 2,000 m repetitions at 4,090 m in an average of 310 seconds, decreasing his performance compared with sea level by around 3 %. At the same altitude, well-trained runners decreased performance 20–24 % in 1,609 m or 3,218 m, respectively.

This study showed that an FP model guided by HRV induced less suppression and faster restoration of rMSSD and lower $rMSSD_{CV}$ compared with the IP training model. Both models showed an increase in performance after altitude exposure, but greater enhancement was observed after the FP model, despite administration of lower training loads. HRV-guided FP may therefore be a useful training method for maintaining training loads within the recovery capacity of the athlete at altitude.

Practical Applications

This case study suggests that HRV is a convenient, non- invasive, physiological marker that can be used to help autoregulate training loads in wheelchair marathoners. Individuals may be able to limit the magnitude of the autonomic nervous system imbalance associated with living and training

at altitude by using HRV-guided training in favor of inflexible, preplanned training. This method may facilitate smaller reductions and less fluctuations in indices such as rMSSD and $rMSSD_{CV}$ in addition to inducing less fatigue and greater endurance performance improvements from a lower training load.

Acknowledgements

The authors thank the athlete who volunteered for this case study.

7.1 Manuscript I post-published rectifications

The following issues were observed while reading this published article and should be considered as rectifications.

- Nordic-skiers from Schmitt et al. study were exposed to normobaric hypoxia not hypobaric hypoxia.
- In the sentence *"a slight increase until W_3 was shown, reflecting acclimatization to altitude"* the reference was: Schoene RB, Lahiri S, Hackett PH, Peters RM, Milledge JS, Pizzo CJ et al. Relationship of hypoxic ventilatory response to exercise performance on Mount Everest. *J Appl Physiol.* (1984);56(6):1478–1483, however we wrote a wrong reference regarding this assumption: Vesterinen V, Nummela A, Heikura I, Laine T, Hynynen E, Botella J et al. Individual endurance training pre- scription with heart rate variability. *Med Sci Sports Exerc*. (2016);48(7):1347-1354.
- We found SP significantly greater in B_H compared to B_N ($p < 0.0001$) compared to pre-altitude (B_N) (126.0 ± 5.1 vs.111.0 ± 3.3 mmHg, $p < 0.0001$, $d = -3.49$).
- In Figure 6 the type of session L refers to H.
- The athlete´s greatest ever time in marathon is 1 hour 42 minutes 05 seconds (Beijing, Paralympic Games 2008) and not Oita International Wheelchair Marathon 2007 edition, which is second best ever time for the studied athlete and still being record in T52 division in this international event.

8 MANUSCRIPT II (UNDER REVIEW)

Hematological and ventilatory responses to 3860 m altitude training in a wheelchair marathoner.

Abstract

Purpose: To assess red blood cell parameters and resting breathing frequency (BF) before, during the course and after a 5-week live high - train high camp at 3860 m terrestrial altitude in a 36-year old professional wheelchair marathoner. **Methods:** Erythropoietin concentration (EPO), hemoglobin (Hb), reticulocytes count (Ret), erythrocytes and hematocrit (Hct) were measured. Resting BF was assessed using a smart T-shirt. **Results:** EPO increased up to 259 % (31.6 $U \cdot L^{-1}$) 35 h after altitude arrival, and decreased below pre-altitude level (12.2 $U \cdot L^{-1}$) on the 21st day of the camp (8.7 $U \cdot L^{-1}$), reaching the lowest values 16 days after returning to sea level (1.9 $U \cdot L^{-1}$). All blood parameters, except for Ret, increased (range: +8.1 to +18.2 %) after 35 days of altitude exposure compared to baseline. Compared to pre-altitude, resting BF increased during the first week and remained slightly elevated until the last week of the camp (5.1 ± 0.4 vs. 9.1 ± 1.6 and 6.6 ± 0.8 Breaths $\cdot$ min^{-1}). **Conclusions:** five-weeks of altitude training at 3860 m triggered polycythemia and elevations in resting BF, as indications of an effective hypoxic acclimation, in a professional wheelchair marathoner.

Key-words: Altitude training, marathon, Paralympic, erythropoiesis, ventilatory drive.

Introduction

Larger increases in red cell volume (RCV) occur at a terrestrial altitude of 4000 m after a stay of 4 weeks compared to shorter exposure. [37] Expected RCV increases vary between 5 and 9 %, [2] while Hb_{mass} gains are of ~1.1 % · 100 h^{-1} of hypoxic exposure. Moreover, plasma EPO levels generally expand after only 2 h upon arrival to altitude and up to 3-4 days, [16] with values returning to pre-altitude levels 1-4 weeks after removal of the hypoxic stress.[253]

Training at altitude significantly exacerbates relative exercise intensities when completing a given workout, yet these adjustments may be of a smaller magnitude in wheelchair athletes. We have recently reported, in a professional wheelchair marathoner, a ~3 % decrement in 2000 m repetitions at second ventilatory threshold (VT2) at 4090 m compared to sea level. [311] However, a 20 % reduction in one mile (1619 m) time trial was observed in able-bodied athletes training at similar altitude. [211] After returning to sea level, the wheelchair athlete produced a 13.6 % greater peak power output during an incremental test, improving his 3000 m performance by 0.9 %. While this may relate to a more favorable blood profile after chronic hypoxic exposure, this later study didn't assess blood or cardio-respiratory parameters during the first week at altitude. Furthermore, it is now possible to reliably assess breathing frequency (BF) through the use of smart T-shirt. [3] This approach may be useful to determine if athletes cope well with the stress of severe, chronic altitude exposure.

The aim of this study was to analyze changes in red blood cell parameters and resting BF as markers of altitude acclimation in response to a 5-week live high-train high (LHTH) altitude training camp (3860-4090 m) in a professional wheelchair marathoner.

Methods

Athlete description

One professional male wheelchair marathoner (age, 36 yrs; body-height, 176 cm; body-mass, 52.6 kg; VO_{2max}, 52 ml · kg^{-1} · min^{-1}), class T52 (Tetraplegic category), holding thirteen world records, took part in the study. This training camp was part of his marathon base preparation for the Boston and London marathons, in which he finished first (with course record) and second, respectively. He provided written informed consent. Research conformed the Declaration of Helsinki, being approved by the Ethics Research Committee of the Miguel Hernandez University from Elche, Spain.

Design

This study is part of a multidimensional case report, [311] in which repeated observations were made on a single athlete during: 1) A 6-week leading phase at sea level (16 m altitude), including 74 training sessions and the completion of 1280 km; 2) 1 week as pre-altitude (W_{-1}); 3) A 5-week altitude camp in the Peruvian Andes (3860-4090 m above sea level) (W_{1-5}); 4) 1 week as post-altitude (W_{+1}). The details of training have been published elsewhere. [311]

The athlete ensured iron deposits by the daily intake of 105 mg of ferrous sulphate (Ferogradumet®, Ross, Abott Científica) after taking breakfast.

Blood Parameters Assessment

Blood tests were conducted under fasting conditions after a day of rest. Blood was withdrawn: i) first day of season or 7 weeks before the altitude camp ($T_{-7\ weeks}$); ii) 7 days before the altitude camp ($T_{-7\ days}$); iii) at the altitude camp on days eight, fifteen, twenty-one, twenty-eight and thirty-five ($T_{8,15,21,28,35\ days}$);

iv) 6 days after returning from altitude ($T_{+6\ days}$). Plasma EPO was assessed: i) 7 days before the altitude camp ($T_{-7\ days}$); ii) 35 h after altitude arrival (T_{35h}); iii) on the 21st day at the altitude camp ($T_{21\ days}$); iv) 16 days after returning from altitude ($T_{+16\ days}$). Hb and erythrocytes were measured with a Coulter T 840 Counter (Coulter Electronics, Krefeld, Germany). Hct was determined with microcentrifugation.[312] Ret were measured with flow cytometry (Epics XL, Beckmann). A 7 ml blood sample was centrifuged (3000 rpm, 10min, 4 degrees Celsius) and measured (Erythropoietin ELISA, IBL, Hamburg, Germany) for serum EPO. Ferritin was measured photometrically (EIAgen Ferritin Kit, Adaltis, Freiburg, Germany).

Breathing Frequency Assessment

An Hexoskin wearable body metrics shirt (Hexoskin, Carré Technologies Inc. San Francisco, CA), which was found valid and reliable, [218] was used for assessing resting BF. Measurements were obtained upon awaking in supine position during 5 min, with the average value for the last 60 s retained for final analysis.

Statistical Analysis

Blood parameters and ferritin data are presented as raw values (Table 5), while BF data are presented as mean ± SD (Table 10). A ΔEPO was calculated with reference to $T_{-7\ days}$ that was assigned the value 100 %. Δerythrocytes, ΔRet, ΔHb, ΔHct and Δferritin were calculated with reference to $T_{-7\ weeks}$ that was assigned the value 100 %. Repeated-measures ANOVA (time) were carried out. Effect size (d) associated with change in BF were calculated using Cohen's d and were interpreted as small ($d \leq .2$), moderate ($d \sim .5$), and large ($d \geq .8$). [240] 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.

Results

Blood Parameters

From $T_{-7\ days}$ to $T_{35\ days}$ erythrocytes, Hb and Hct increased by 17.9 %, 21.2 % and 23.8 % respectively. Moreover, Ret decreased by 38.3 % from $T_{-7\ days}$ to $T_{35\ days}$, and by 42.7 % from $T_{35\ days}$ to $T_{+6\ days}$. At $T_{+6\ days}$ erythrocytes, Hb and Hct were enhanced by 8 %, 8.8 % and 12.1 % respectively, compared to $T_{-7\ days}$. However, erythrocytes at $T_{+6\ days}$ were 1 % lower compared to $T_{-7\ weeks}$. Compared to $T_{-7\ days}$, EPO increased by 259 % at T_{+35h}, while lower values were observed at $T_{21\ days}$ (-29 %) and $T_{+16\ days}$ (-84 %) (Table 5).

Ferritin

Compared to $T_{-7\ days}$, ferritin values were 56 % lower at $T_{8\ days}$ (247 vs. 110 ng · mL^{-1}) but 36.4 % higher (150 ng · mL^{-1}) at $T_{35\ days}$ and remained 12.2 % lower at $T_{+6\ days}$.

Breathing frequency

Compared to sea level, resting BF was elevated during the 5-week altitude training camp (pooled values: 7.7 ± 1.8 vs. 5.3 ± 0.6 Breaths · min^{-1}). (Table 10).

Table 10. Resting breathing frequency (BF) pre-altitude, during, and post-altitude and the magnitude of change.

Table 2. Breathing Frequency (BF) pre-altitude, during, and post-altitude and the magnitude of change.

	W_{-1}	W_1	W_2	W_3	W_4	W_5	W_{+1}
BF (Breaths · min^{-1})	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&#$
Cohen´s d		3.4	0.1	-1.4	-0.7	0.2	-1.4

W_{-1}: Pre-altitude week. $W_{1,2,3,4,5}$: Weeks at altitude **Post**: Post-altitude week. **BF**: Breathing frequency upon wakening in resting conditions.

* Differences from W_{-1} (p<.001); & Differences from W_1 (p<.001); # Differences from W_2 (p<.001); $ Differences from W_3 (p<.001); ¤ Differences from W_4 (p<.001); ¥Differences from W_5 (p<.001). Cohen´s d:difference in mean scores over time divided by pooled SDin reference to previous time point.

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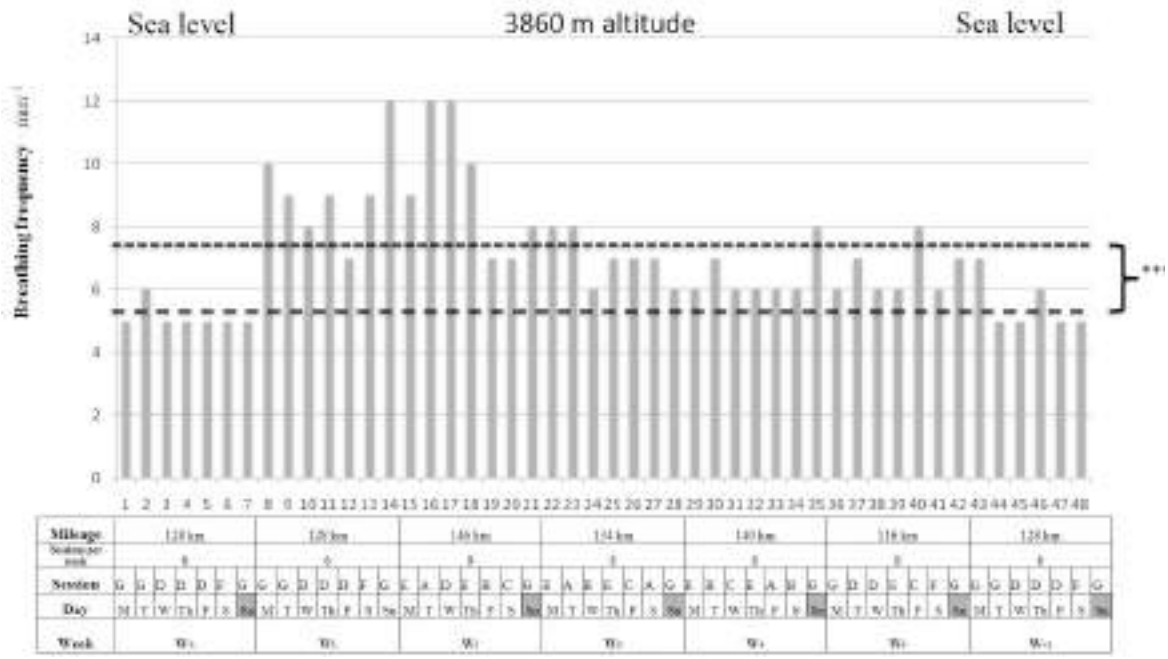

Figure 9. Breathing frequency under normoxic and hypoxic conditions

Session A: performed on a plateau at 4090 m. 8 km + technique drills + 5 x 80 m strides + 20 x 400 m (VT2), recovery repetitions 75 s + 2 km.

Session B: 2 hours at first ventilatory threshold (VT1).

Session C: performed on a plateau at 4090 m. 8 km + technique drills + 5 x 80 m strides + 6 x 2000 m (VT2), recovery repetitions 120 s + 2 km.

Session D: 20 km (< VT1) in the morning + 16 km (< VT1) in the afternoon.

Session E: 16 km (< VT1) in the morning + gym session in the afternoon (4 sets x 8 repetitions, recovery sets 150 s at 80 % RM). Exercises for resistance session: press bench, close grip, dumbbell press, seated military press and seated cable row).

Session F: 20 km (< VT1) in the morning + resting afternoon.

Session G: Rest Day.

Dash line: Represents mean breathing frequency (5.3 ± 0.6 Breaths · min^{-1}) in normoxic conditions.

Square dot line: Represents mean breathing frequency (7.7 ± 1.8 Breaths · min^{-1}) at 3860 m altitude.

Differences from mean breathing frequency under normoxic conditions: *** $p<0.001$

Discussion

Our novel findings are: 1) Living at 3860 m altitude and training at 3860-4090 m altitude for 5 weeks in a professional wheelchair marathoner triggered polyglobulia; 2) peak erythrocytes, Hb and Hct values were observed towards the end of the camp; 3) resting BF increased at altitude compared to pre-altitude, and remained elevated until camp completion.

We observed that 6-week of training at sea level increased Ret (+25.8 %) as a result of endurance training. [249] In contrast, a study of elite biathletes conducted at 2050 m altitude demonstrated no differences between EPO measured 16 days post- compared to pre-altitude.[31] In our study, the EPO suppression observed at $T_{21\ days}$ (-29 %) and $T_{+16\ days}$ (-84 %), might explain the 8.5 % decrease in erythrocyte values from $T_{35\ days}$ to $T_{+6\ days}$. Likewise, EPO suppression might explain the 38.3 % Ret decrease at altitude. Furthermore, the slight decrease in erythrocytes, Hb and Hct observed at W_3 compared to W_2 might be explained by the four specific sessions performed during the previous week (W_2), [311] showing probably incomplete recovery time between sessions. Observation of greater Hb and erythrocyte levels at W_4 and W_5 compared to $W_{1,2,3}$, is in line with previous reports of maximal increase in RBC occurring by the fourth week of altitude exposure. [37] Furthermore, greater Hb, erythrocytes and Hct values observed one week after returning to sea level confirm findings from previous LHTH studies conducted at lower altitude. [31] This suggests that LHTH at high altitude would allow positive red blood cell adaptations in elite wheelchair athletes.

Interestingly, the decrement in ferritin observed at altitude in our study, exceeded that of a previous study (-56 vs. -15 %) with elite biathletes. [31] However, the differences in altitude elevation (3860 vs. 2050 m) could partly

explain this observation. Therefore, amounts greater than 105 mg per day of ferrous sulphate should be prescribed to athletes residing and training at ~ 4000 m.

Noteworthy, a decrease in resting BF at W_3 occurred concomitantly with 12-14 % increases in erythrocytes and Hb in reference to W_{-1}. This is an important observation, considering the difficulty for hematological testing when residing/training at ≥3500 m altitude venues. During altitude acclimatization, BF was enhanced, which was accompanied by an increase in oxygen desaturation compared to W_{-1} (88.3 ± 2.5 vs. 98.6 ± 0.1 %) reported in other studies. [311] Therefore, resting BF oscillations at altitude might be considered by those athletes training specially in remote places, in which blood tests are not possible.

Practical applications

i) A LHTH camp at high altitude (~ 4000 m) improves hematological variables in a well-trained wheelchair athlete.

ii) Resting BF, as measured with a smart T-shirt, is sensitive to exposure to chronic hypoxia.

Conclusions

Blood markers were significantly enhanced in response to 5 weeks of LHTH at 3860-4090 m terrestrial altitude in an elite wheelchair marathoner. Interestingly, EPO suppression observed by mid-camp did not affect blood markers peak towards the end of exposure. Finally, evaluating resting BF adjustments could be considered as a proxy to establish the ability of athletes to cope with the stress of hypoxia when living/training at terrestrial altitude in remote mountainous areas, in which conducting blood evaluations might be challenging.

Acknowledgments

The authors wish to thank the subject who voluntarily participated in this case study, assisting with all data collection.

9 PUBLISHED MANUSCRIPT III

sports

MDPI

Case Report

Influence of Training Load on Mood Disturbance at Sea Level and 3900 m Altitude: A Case Study of a Wheelchair Athlete

Santiago Sanz-Quinto [1], Gabriel Brizuela [2], Raúl López-Grueso [1], Ian Rice [3] and Manuel Moya-Ramón [1,4,*]

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Abstract: The purpose of this case study was to investigate the influence of a training load (TL), oxygen saturation (SO_2) and blood pressure (BP) on mood states in a wheelchair marathoner during (7 weeks at sea level (SL), 5 weeks at 3860 m altitude, 1 week returning to SL). TL was obtained with Foster's equation while mood states were obtained with the Profile of Mood States Questionnaire (POMS). Furthermore, SO_2 and BP were assessed upon wakening. SO_2 (%) decreased at altitude, compared to SL (88.31 ± 2.46 vs. 98.52 ± 0.11) and increased until the last week at altitude (92.64 ± 1.12). Systolic pressure (SP) increased at altitude compared to pre-altitude (126.0 ± 5.1 vs. 107.6 ± 4.4 mmhg), and was not different from the last week at altitude. Controlling for SO_2 and SP, differences were also observed in fatigue (97.66 ± 18.92 vs. 17.39 ± 13.71) and vigor (73.23 ± 8.62 vs. 26.48 ± 11.89) as a function of altitude. Upon return to SL, fatigue, vigor, SO_2 and SP returned to pre values. This case study demonstrated the POMS was sensitive to worsening patterns in fatigue and vigor at altitude through a practical survey approach combined with daily physiological assessment.

Keywords: hypoxic environment; POMS; athletics; paralympic; baroreflex sensitivity

1. Introduction

The Profile of Mood States questionnaire (POMS) [1] reflects the individual mood in six primary dimensions (Depression-Dejection, Tension-Anxiety, Anger-Hostility, Vigor-Activity, Fatigue-Inertia, Confusion-Bewilderment) and is widely used in sports to evaluate the psychological state of athletes. High values on the vigor-activity scale and low values in the remaining scales are desirable for athletic performance and resemble an iceberg formation when plotted. Exercise has been suggested to alter mood, depending on the type, volume, duration and intensity [2]. An optimal balance between training and rest may prevent overtraining symptoms, which have been characterized by fatigue, performance decrements, mood changes, irritability, and loss of motivation [3]. Moreover, during periods of overtraining, athletes generally report undesirable changes in Total Mood Disturbance (TMD), which represents the sum of the five negative scales of POMS, subtracting vigor score and adding a constant of 100 [4]. Therefore, flattened or even inverted iceberg profiles on the POMS subscales have been observed in non-functional, overreaching, or over trained athletes [5], as high

The study was previously published as:
Santiago Sanz-Quinto, Gabriel Brizuela, Raúl López-Grueso, Ian Rice, Manuel Moya-Ramón. Influence of training load on mood disturbance at sea level and 3900 m altitude: A case study of a wheelchair athlete. *Sports (Basel).* 2018;6(4). DOI: 10.3390/sports6040122.

The version displayed in this doctoral thesis corresponds to the major review submitted to SPORTS journal on October 8Th 2018. However, on October 22Nd 2018 Sports journal published the version submitted on September 25Th 2018.

Abstract

The purpose of this case study was to investigate the influence of a training load (TL), oxygen saturation (SO_2) and blood pressure (BP) on mood states in a wheelchair marathoner during 7 weeks at sea level (SL), 5 weeks at 3860 m altitude, 1 week returning to SL. TL was obtained with Foster´s equation while mood states was obtained with the Profile of Mood States Questionnaire (POMS). Furthermore, SO_2 and BP were assessed upon wakening. SO_2 (%) decreased at altitude, compared to SL (88.31 ± 2.46 vs. 98.52 ± 0.11) and increased until the last week at altitude (92.64 ± 1.12). Systolic pressure (SP) increased at altitude compared to pre-altitude (126.0 ± 5.1 vs. 107.6 ± 4.4 mmhg), and it was not different from the last week at altitude. Controlling for SO_2 and SP, differences were also observed in fatigue (97.66 ± 18.92 vs. 17.39 ± 13.71) and vigor (73.23 ± 8.62 vs. 26.48 ± 11.89) as a function of altitude. After returnig to SL, fatigue, vigor, SO_2 and SP returned to pre values. This case study demonstrated the POMS was sensitive to worsening patterns in fatigue and vigor at altitude through a practical survey approach combined with daily physiological assessment.

Keywords: Hypoxic environment; POMS; Athletics; Paralympic; Baroreflex sensitivity

Introduction

The Profile of Mood States questionnaire (POMS) [100] reflects the individual mood on six primary dimensions (Depression-Dejection, Tension-Anxiety, Anger-Hostility, Vigor-Activity, Fatigue-Inertia, Confusion-Bewilderment) and is widely used in sports to evaluate the psychological state of athletes. High values on the vigor-activity scale and low values in the remaining scales are desirable for athletic performance and resemble an iceberg formation when plotted. Exercise has been suggested to alter mood, depending on the type, volume, duration and intensity. [105] An optimal balance between training and rest may prevent overtraining symptoms, which have been characterized by fatigue, performance decrements, mood changes, irritability, and loss of motivation. [99] For example, a group of 10 elite judoists experienced increased anger, tension, and fatigue according to training load (TL), supporting the idea of a negative psychological state reaction to demanding training in athletes. [266] Moreover, during periods of overtraining athletes generally report undesirable changes in Total Mood Disturbance (TMD), which represents the sum of the five negative scales of POMS, subtracting vigor score and adding a constant of 100. [106] Therefore, a flattened or even inverted iceberg profile on the POMS subscales have been observed in non-functional, overreaching, or overtrained athletes, [101] as high values on the vigor-activity scale and low values in the remaining scales are desirable for athletics performance. Furthermore, when TL is reduced during taper periods, athletes have reported improvements in mood as reflected by a return of POMS scores to baseline within 10 days of lowered TL. [271] Moreover, it has been suggested that a 50 % or greater increase in an individual's basal off-season

TMD score may be reflective of an overreaching state. This was demonstrated in world-class canoeists preparing for the Olympic Games. [108]

High-altitude exposure alters many mood factors including fatigue, depression, confusion, anger, tension and vigor. [268] Numerous researchers have reported an increase in negative mood states at altitudes above 3050 m, [269] 3700 and, 4400m compared to normoxic conditions at 400 m. [114] For example, in a recent publication in which the POMS was administered to a 9 people team during a 6000 m ascent of Mount Everest, the authors reported oscillations in fatigue and vigor during the ascent, with no changes in the depression scale. [113] More specifically, upon psychological evaluation of the athletes using the POMS, mood changes are most severe during the first or second day at altitude and then gradually recede over the next 2-4 days. [111] However, evidence supports that longer pre-altitude acclimatization before ascending to higher elevations is not protective for negative mood states when an individual reaches high altitudes. [114]

To the best of our knowledge, the use of the POMS to assess elite marathoners is absent from the literature, despite the fact that marathon running has been shown to affect mood states more negatively than low to moderate intensity exercise. [313] Therefore, it may be reasonable to surmise that similar mood disturbance may be precipitated by a combination of intense marathon training performed under normoxic and hypoxic environments. However, to date, there is a lack of literature pertaining to mood response of wheelchair athletes in training scenarios at SL or in hypoxic conditions, except one study which found elite athletes with and without disabilities possessed similar iceberg profiles and thus superior emotional health compared to general population implying “elite” rather than “ability” was most influential to mood status. [314] Moreover, it must be considered that only one study has assessed the physiological-psychological

response of a Paralympic athlete under altitude conditions. [311] In fact, recently the same study reported a disturbance in oxygen saturation (SO_2) and brachial blood pressure (BP) upon arrival to altitude. However, to our knowledge there are no studies that have analyzed the influence of these physiological variables on mood states.

Despite the aforementioned finding, the extent to which wheelchair athletes experience mood responses similarly to able-bodied athletes is largely unknown. Therefore, the purpose of this study was to investigate the psychological response of an elite-wheelchair marathoner to a TL under both, normoxic and hypoxic conditions. Furthermore, we examined the influence of SO_2, systolic and diastolic blood pressure (SP and DP) on mood states, when these physiological variables were added as covariates. To achieve this goal our study was divided into 4 aims:

Aim 1 – To examine the psychological response of an elite-wheelchair marathoner during a TL at sea level (SL) with the (POMS) mood state assessment tool, considered as baseline.

Aim 2 – To examine how a TL conducted at altitude (3860 m) impacts on the psychological response in the same athlete.

Aim 3 – To examine the influence of previously analyzed, [312] salient physiological variables, as SO_2, SP and DP have on mood states.

Aim4 –To determine if any changes in mood states occurring at altitude would return back to baseline when training resumed at SL under a reduced TL.

Material and Methods

Participant

The athlete was a 36 year-old, professional wheelchair marathoner, International Paralympic Committee (IPC) class T52 (wheelchair athletes

with arm involvement), multiple times world champion, Paralympic Games silver medalist, thirteen world records; height = 1.76 m; Body Mass = 52.6 ± 0.4 kg; VO_{2max} = 52 ml · kg^{-1} · min^{-1}; VO_2 at second ventilatory threshold (VT2) = 48 ml · kg^{-1} · min^{-1} Training 8000 km per year. The athlete was familiarized with altitude training camps set at 1600-2900 m in the last thirteen years with next models: live high-train high (LHTH), live high-train low (LHTL) and high-high-low (LHTHTL). [2] The following altitude camp was performed as a preparation for The Boston and London marathons, in which he finished first with course record and second respectively in his division. The participant provided written informed consent for participation and the study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Research Committee of the University (project identification code # DPS.MMR.02.15).

Procedures

Training features at sea level and altitude

Our study occurred between SL at 16 m altitude and the Peruvian Altiplano (Puno, 3860 m altitude). The study period occurring from November 23, 2015 to February 21, 2016 (13 weeks; 91 days), was composed of the athlete´s introductory cycle I (I1) (weeks 1 to 4) at SL, in which training contents included arm ergometer workouts, over ground Nordic ski sessions and workouts below the first ventilatory threshold (<VT1) with the racing chair. Fundamental cycle I (F1) (weeks 5 and 6) occurred at SL in which training included arm ergometer workouts, over ground Nordic ski, long moderate workouts at approximately VT1, two interval sessions at approximately VT2, and strength sessions in the gym. Introductory cycle 2 (I2) (weeks 7 and 8) occurred at SL week 7 and shifted to 3860 m altitude week 8, in which the athlete performed the same 16 to 20 km workouts <VT1 during the pre-altitude and acclimatization week. Fundamental cycle II (F2)

(weeks 9 to 12) at 3860 m altitude, involved only intense sessions if the athlete reached a reference value of his Heart Rate Variability (HRV), considered as optimal to perform intense sessions, explained in detail previously. [311] The Introductory cycle 3 (I3) (week 13) occurred at SL, as post-altitude, and involved training identical to I2 (See Figure 10).

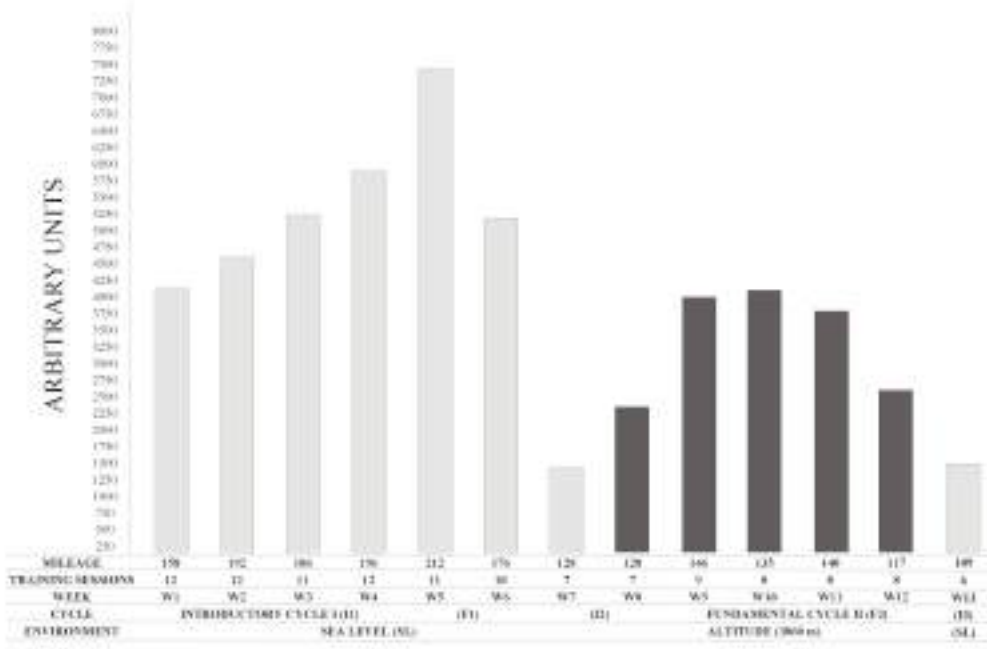

Figure 10. Weekly training load.

Mood states assessment and training load calculation

Mood states were assessed using the Spanish short form version of the Profile of Mood States self-report questionnaire (POMS). This version consists of 58 adjectives; each rated on a five-point scale. [219] From week 1, every Sunday (rest day) and after breakfast, our athlete responded to the question of how he had felt during the previous week including that day. First week results constituted baseline. Week 7 data was not included for analysis because it

coincided with overseas travel, which reportedly caused physiological disturbances in this athlete. [315]

Physical TL was defined with Foster´s equation [220] as the product of the rating of perceived effort (RPE), using Borg scale 1-10 (in which 1 means nothing at all and 10 extremely strong) [120] multiplied by the active time of session duration. RPE was recorded thirty minutes after morning and afternoon sessions and it was calculated based on the morning sessions (one-day training) or as the average of morning and afternoon (two session training day). Daily TL was calculated by adding up the Fosters values for morning and afternoon sessions. Weekly TL was calculated as the addition of daily values from Monday to Saturday (See figure 10).

Oxygen saturation and brachial blood pressure assessment

The SO_2 was measured with a finger pulse oximeter (Colson 650 2100, Frouard, France) in a seated position after waking.

Brachial blood pressure (BP) was measured in a seated position, with the validated (Omron HEM-705CP) oscillometric sphygmomanometer. Measurements were made in triplicate and averaged. Both systolic (SP) and diastolic (DP) blood pressure were recorded as well.

The assessment, results and discussion of both, SO_2 and BP, have been previously reported. [311]

Statistical analysis

Kolmogorov-Smirnov was used to assess data normality. A repeated measures ANOVA was performed for POMS dimensions with TIME at two levels (SL and altitude). Oxygen saturation (SO_2) systolic blood pressure (SP) and diastolic blood pressure (DP) were added as covariates. A post hoc LSD multiple range test determined differences between factor levels. Pearson's correlation coefficients were calculated for tension, fatigue, vigor,

anger, depression, confusion, TMD, weekly Foster, SO_2, SP and DP. Effect size (d) associated with change in every POMS dimension was calculated using Cohen's d (difference in mean scores over time divided by pooled SD) and it was interpreted as trivial (≤ 0.19), small (0.20-0.49), medium (0.50-0.79), and large (≥ 0.08). [240] Statistical significance was set at alpha = 0.05. Statistical analyses were performed using SPSS version 22.0 (SPSS, Inc., Chicago, IL, USA) software.

Results

Results can be seen in table 11 and 12.

Table 11. Weekly POMS dimensions values and rate of perceived exertion.

	TENSION	FATIGUE	CONFUSION	DEPRESSION	ANGER	VIGOR	TMD	BORG
W1	55	35	31	39	39	64	98	4
W2	57	37	31	39	42	57	106	4
W3	37	47	31	39	39	62	94	5
W4	55	64	33	41	51	51	134	5
W5	54	76	33	39	56	46	145	6
W6	29	61	31	40	38	49	104	5
W7	72	64	60	44	76	48	182	4
W8	29	35	31	39	39	64	81	4
W9	28	40	31	39	39	64	83	5
W10	28	42	33	43	46	48	103	6
W11	35	62	33	41	48	42	123	5
W12	34	64	31	39	44	49	113	5
W13	40	47	43	42	46	49	117	4

TMD = total mood disturbance; W1 to W7 and W13, weeks at sea level; W8 to W12, weeks at altitude (3860 m). W7 data was not included for analysis because it coincided with overseas travel; BORG, is reported as the integer value from the weekly average of morning and afternoon sessions.

Table 12. Weekly oxygen saturation, systolic and diastolic blood pressure.

	SO_2 (%)	**SP (mmHg)**	**DP (mmHg)**
W1	98.33 ± 0.25†‡§¶**	113.8 ± 4.3†‡§¶**	72.6 ± 3.1*†‡§¶
W2	98.77 ± 0.14†‡§¶**	117.9 ± 3.6†‡§¶**	77.4 ± 3.9*†‡§¶
W3	98.11 ± 0.42†‡§¶**	115.4 ± 6.1†‡§¶**	75.1 ± 4.5*†‡§¶
W4	98.56 ± 0.24†‡§¶**	112.7 ± 9.1†‡§¶**	71.4 ± 3.9*†‡§¶
W5	98.74 ± 0.17†‡§¶**	112.3 ± 6.8†‡§¶**	70.9 ± 5.8*†‡§¶
W6	98.52 ± 0.11†‡§¶**	107.6 ± 4.4†‡§¶**	68.6 ± 3.8*†‡§¶
W7	98.64 ± 0.14†‡§¶**	111.0 ± 3.3	68.4 ± 3.3
W8	88.31 ± 2.46*	126.0 ± 5.1	80.4 ± 5.2*
W9	91.19 ± 0.76†	127.1 ± 4.8*	81.1 ± 3.9*
W10	91.92 ± 0.82†	132.4 ± 3.4*	83.4 ± 4.1*
W11	92.35 ± 1.14†‡	125.7 ± 6.9*	80.0 ± 2.1*
W12	92.64 ± 1.12†	124.9 ± 3.5*§	77.7 ± 2.1*§
W13	98.08 ± 0.26†‡§¶**	111.3 ± 7.6†‡§¶**	73.7 ± 4.7*†‡§¶

SO_2 = oxygen saturation; SP = systolic blood pressure; DP = diastolic blood pressure. * Differences from W7 ($p < .01$); † Differences from W8 ($p < .01$); ‡ Differences from W9 ($p < .01$); § Differences from W10 ($p < .01$); ¶ Differences from W11 ($p < .01$); ** Differences from W12 ($p < .01$).

POMS

We observed significant differences between SL and altitude in two dimensions of the POMS (fatigue and vigor), while considering SO_2 and SP as covariates. Fatigue was significantly higher at altitude compared to SL (97.66 ± 18.92 vs. 17.39 ± 13.71, p = 0.0362, d = 6). Vigor was also significantly lower at altitude (73.23 ± 8.62 vs. 26.48 ± 11.89, p = 0.0484, d = 6.1). No significant differences were observed between SL and altitude in any POMS dimension when DP was considered as a covariate. We observed the following correlations between vigor and Borg (r = - 0.66; p = 0.0264); vigor and anger (r = - 0.71; p = 0.0102); confusion and Borg (r = 0.74; p = 0.0100); anger and Borg (r = 0.63; p = 0.0366); fatigue and anger (r = 0.70; p = 0.0109); confusion and depression (r = 0.61; p = 0.0367).

Oxygen saturation.

The SO_2 (%) improved with altitude exposure from acclimatization until the fifth week of exposure (88.31 ± 2.46 vs. 92.64 ± 1.12, p = 0.001, d = 2.7), although it remained stable at SL (98.08 ± 0.26 to 98.77 ± 0.14). We observed the following correlations between SO_2 and SP (r = - 0.90; p = 0.0001); SO_2 and DP (r = - 0.84; p = 0.0004); SP and DP (r = 0.96; p = 0.0006).

Brachial blood pressure.

SP remained significantly higher (p = 0.001) at altitude compared to SL. Values were maximal during the third week of exposure (132.4 ± 3.4 mmHg) and reached their lowest magnitude by the last week at altitude (124.9 ± 3.5 mmHg). After returning to SL SP become significantly lower compared to last altitude week (113.1 ± 3.1 mmHg, p = 0.001, d = 2). A deeper report on SO_2 and BP during the experiment can be read in Sanz-Quinto et al. [311]

Oscillations in POMS dimensions can be found in Figure 3.

Total mood disturbance.

We observed the greatest values in TMD in week 5 (145 A.U.) and week 4 (134 A.U.), in which weekly Foster reached its greatest level (7406.86 A.U. in week 5 and 5916.1 A.U in week 4). Indeed, a strong correlation was observed between TMD and weekly Foster (r = 0.66; p = 0.0258). In week 5, when SO_2 remained high at SL, value for fatigue reached the greatest level (76 A.U.), the same as anger (56 A.U.), which was not influenced by SO_2, SP and DP when they were considered as covariates. Furthermore, we found a strong correlation between fatigue and anger (r = 0.70; p = 0.0109). Both depression and confusion remained similarly stable during the whole experiment. However, in the last week confusion increased 38.7 % and the

magnitude of change compared to the last altitude week was large (d = 3.7). Moreover, we found a strong correlation between depression and confusion (r = 0.60; p = 0.0367), and almost a perfect correlation between TMD and anger (r = 0.91; p = 0.0001). Finally, tension was greater pre-altitude and a strong correlation was found between tension and weekly mileage (r = 0.67; p = 0.0244).

Discussion

The purpose of this study was to investigate the psychological response of an elite-wheelchair marathoner to a TL under both, normoxic and hypoxic conditions. In addition, we examined the influence of SO_2, SP and DP on the psychological response, through their addition as covariates. Finally, we examined if mood changes occurring from SL to altitude would return to baseline levels when training resumed at SL under reduced TL.

Consistent with previous studies performed on able-bodied athletes at altitudes ranging from 3080 m to 6000m, [111,113,114] we found increased fatigue and decreased vigor with altitude. In addition, we observed increased TL at SL had a negative effect on fatigue and TMD, which peaked in week 5 when TL was maximal, the same as shown in a group of elite judoists. [266] In contrast with our findings, a recent study did not find differences in mood states between cyclists diagnosed with acute fatigue and cyclists diagnosed with functional overreaching. [276] However, this study was not performed under hypoxic conditions. [111,113,114,268,269] Furthermore, our findings resemble those of elite swimmers who showed decreased vigor after one day of a 2/3 volume increase and elevated fatigue and overall mood scores after two days of increased TL. [270] Interestingly, TMD in the current study peaked in week 5 although training volumes in weeks 4 and 5 were similar (196.4 vs. 211.6 km), which may suggest a Foster´s threshold was reached and might have

affected TMD significantly. In fact, a TMD 48 % greater than baseline may be reflective of overreaching symptoms. [108] It seems that altitude had a lower impact in TMD than greater volume of training performed at SL; however, we must consider training volume at altitude was 1/3 lower compared to SL. Moreover, an inverted iceberg profile occurred in weeks 5 and 12, which again may signify overreaching symptoms. [101] Similarly, we found confusion and TMD were negatively affected upon return to SL where levels exceeded those observed during pre-altitude. Despite a strong positive correlation between tension and mileage, high tension scores occurred in the first two weeks which may reflect stress imposed by equipment preparation for the season. Furthermore, we found that greater Borg had a strong correlation with vigor and anger. In fact, there may be a connection between TL and TMD, as we found a strong correlation between TMD and weekly Foster, which supports previous findings in which increased anger, tension and fatigue were associated with overloading. [266] Therefore, under stressful conditions like altitude, one should consider lowering TL to avoid an increase in TMD. Finally, we observed fatigue returned to baseline levels upon return to SL with a reduction in TL of 42.7 %. Similar results were reported from a master track athlete, whose POMS returned to baseline within 10 days after TL was lowered 41 %. [271]

A limitation of the current study was evident in our inability to find current literature with similar experimental designs among elite marathon populations. Indeed, only one study with middle distance collegiate athletes was conducted at a similar altitude (4000 m). [211] However, the study was carried out in 1967 when mood state was not assessed. Another possible limitation might be the influence of loneliness on mood states at weeks four and five at altitude; however, the athlete feedback on social relations with the hotel´s workers and area coaches was quite positive. Finally, this study was

also limited with regard to the influence of poor wheelchair accessibility in living conditions on tension and anger dimensions. However, a positive aspect of the training environment included the existence of perfect tarmac road conditions where the athlete had no incidences, other than one flat tire, which is uncommon for racers as they commonly experience frequent flats.

Conclusions

In conclusion, our study shows that despite the implementation of only one questionnaire per week, compared to daily physiological variables assessment, [311] POMS was sensitive to worsening patterns in fatigue and vigor at altitude, which might be influenced by a disturbance in SO_2 and SP. To the best of our knowledge, this is the first study analyzing the influence of several physiological variables on mood states at altitude. Furthermore, effects on fatigue and TMD may be more pronounced at SL once TL reached its peak. In line with our findings and considering that marathoners train in excess of 200 km per week, we might be cautious when overloading training periods at SL. Fortunately, the POMS is non-invasive, easy to assess, inexpensive, and has excellent internal consistency and reliability [(Cronbach´s α) range from .84 to .95]. [100] Furthermore, while session-RPE showed intraindividual variability, POMS reflected a 45 % increase in fatigue, and a 24 % decrease in vigor, comparing the first day with the last in a 5 days training camp with young triathletes. [316] Therefore, we encourage POMS use for coaches and athletes interested in monitoring oscillations in psychological response to TLs during training at varying altitudes.

Considering that we observed decreased vigor and increased anger, fatigue and TMD when TL was at its peak, we suggest coaches decrease TL to 40 %, once they observe a significant decrease in vigor, or significant greater TMD,

fatigue and anger, as fatigue has shown to return to baseline values within 10 days [107] and in our study within 7 days, once lowering TL ~ 40 %.

Quite often altitude training camps must be completed by athletes in remote locations, without direct assistance from coaches, physicians, doctors or psychologists. This study shows that a wheelchair-athlete could self-monitor TL with simple and not-expensive devices, along with use of a weekly mood profile assessment (POMS), to better convey his (physiological-psychological) state to technical staff during training program under greater stressful conditions, such as challenging environment. Our research may help to inform the design and power of future studies performed under similar conditions. Future studies should continue to examine greater numbers of elite wheelchair marathon racers with more diverse disability characteristics (paraplegics, tetraplegics, spina bifida, etc.) to replicate and validate our findings.

9.1 Manuscript III post-published rectifications.

The following issues were observed while reading this published article and should be considered as rectifications.

- We found SP significantly greater in W12,11,10,9,8 compared to W7 ($p < 0.0001$)

10 PUBLISHED MANUSCRIPT IV

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CASE REPORT Open Access

Nutritional strategies in an elite wheelchair marathoner at 3900 m altitude: a case report

Santiago Sanz-Quinto[1], Manuel Moya-Ramón[1,2], Gabriel Brizuela[3], Ian Rice[4], Tomás Urbán[1] and Raúl López-Grueso[1*]

Abstract

Background: Altitude training is a common practice among middle-distance and marathon runners. During acclimatization, sympathetic drive may increase resting metabolic rate (RMR), therefore implementation of targeted nutritional interventions based on training demands and environmental conditions becomes paramount. This single case study represents the first nutritional intervention performed under hypobaric hypoxic conditions (3900 m) in Paralympic sport. These results may elucidate the unique nutritional requirements of upper body endurance athletes training at altitude.

Case presentation: This case study examined the effects of a nutritional intervention on the body mass of a 36-year-old professional wheelchair athlete (silver medalist at the Paralympic Games and 106 victories in assorted road events) during a five-week altitude training camp, divided into pre-altitude at sea level (B_N), acclimatization to altitude (Puno, 3860 m) (B_A), specific training ($W_{1,2,3,4}$) and return to sea level (Post) phases. Energy intake (kcal) and body mass (kg) were recorded daily. Results demonstrated significant decrease in body mass between B_N and B_A (52.6 ± 0.4 vs. 50.7 ± 0.5 kg; $P < 0.001$) which returned to pre-altitude values, upon returning to sea level at Post (52.1 ± 0.5 kg). A greater daily intake was observed during B_A (2899 ± 670 kcal) and $W_{1,2,3}$ (3037 ± 490; 3116 ± 170; 3101 ± 385 kcal) compared to B_N (2397 ± 242 kcal, $P < 0.01$) and Post (2411 ± 137 kcal, $P < 0.01$). No differences were reported between W_4 (2786 ± 375 kcal), B_N and Post. The amount of carbohydrates ingested ($g \cdot kg^{-1}$) was greater in $W_{1,2,3}$ (9.6 ± 2.1; 9.9 ± 1.2; 9.6 ± 1.2) than in B_N (7.1 ± 1.2) and Post (6.3 ± 0.8, $P < 0.001$). Effect sizes (Cohen's d) for all variables relative to B_N (all time points) exceed a large effect ($d > 0.80$).

Conclusions: These results suggest an elite wheelchair marathoner training at 3860 m required increased nutrient requirements as well as the systematic control needed to re-adapt a nutritional program. Moreover, our findings highlight training and nutritional prescription optimization of elite wheelchair athletes, under challenging environmental conditions.

Keywords: Hypoxia, Nutritional intervention, Paralympic, Energy intake, Body mass

Background

In recent years, there has been emerging interest in the optimization of nutritional strategies to help athletes reach their fitness goals during hypoxic training conditions [1]. However, nutritional guidelines for athletes training at 4000 m altitude remain unclear as most nutritional and exercise metabolism studies have been completed at lower altitudes [1, 2], and the data reflects athletes participating in activities less than marathon distances [3–9]. For example, in distance running only one study has examined well-trained runners at an altitude of 4000 m [10] and, recently a case study reported physiological data on an elite wheelchair marathoner training at 3900 m altitude [11]. Loss of body fat and fat free mass have been reported during high altitude sojourns in people eating ad libitum [12–15], suggesting

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Conclusions: These results suggest an elite wheelchair marathoner training at 3860 m required increased nutrient requirements as well as the systematic control needed readapt to a nutritional program. Moreover, our findings highlight training and nutritional prescription optimization of elite wheelchair athletes, under challenging environmental conditions.

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Background

In recent years, there has been emerging interest in the optimization of nutritional strategies to help athletes reach their fitness goals during hypoxic training conditions. [221] However, nutritional guidelines for athletes training at 4000 m terrestrial altitude remain unclear as most nutritional and exercise metabolism studies have been completed at lower altitudes, [63,221] and the data reflects athletes participating in activities less than marathon distances. [58,59] For example, in distance running only one study has examined well-trained runners at an altitude of 4000 m [211] and, recently a case study reported physiological data on an elite wheelchair marathoner training at 3900 m altitude. [311] Loss of body fat and fat free mass have been reported during high altitude sojourns in people eating *ad libitum*, [52,53,54,317] suggesting that strict altitude imposed dietary controls can attenuate daily energy deficits and partially mitigate weight loss. [50] Loss of fat free mass at high altitude increases the risk of illness and injury in extreme environments. [55,56,57,318]

During acclimatization there is a reduction of intra and extracellular water combined with a decrease in plasma volume, [48,49] which can result in body mass loss of up to 2 kg. [52] Furthermore, during the acute phase exposure, total exogenous glucose oxidation appears to be lower than at sea level, remaining lower 21 days after being exposed to an altitude of 4300 m, suggesting oxidation rates under hypoxic conditions do not cover the energy demands of athletes at altitude. [58] Alternatively, other studies suggest individuals have an increased dependence on glucose as a fuel source at high altitude, especially during exercise. [59,60,61]

Increased resting metabolic rate (RMR) has also been observed at altitude, which could be due to increased sympathetic drive and a subsequent rise in adrenaline levels. [65] Recent research found that RMR in elite middle-distance runners increased by ≈ 19 % at a moderate altitude (2100 m) compared to SL conditions [63] and 10 % at high altitude (3800 m). [64] In contrast, a small decrease in RMR was reported in a group of Olympic rowers training at 1800 m. [67] Moreover, RMR is more pronounced over the first 2-3 days after arrival [16, 24]. [50,66] However, elevated RMR (≥ 17 %) can persist for up to 21 days after initial high altitude exposure. [56] Ultimately, energy expenditure, which is elevated at altitude, may be equivalent to high intensity exercise conducted at sea level. [68]

Due to the aforementioned factors, one of the primary nutritional goals for managing a successful altitude training camp involves matching the energy intake to the daily expenditure in order to minimize body mass loss. [69] In fact, it was reported that, a total of 7.6 g · kg^{-1} body mass of carbohydrates (CHO) per day did not cover the energetic demands of cyclists living and training at 4300 m. [70] Importantly, up to 70 % of the chronic altitude exposure-related weight loss is said to be due to reductions in muscle mass itself. [71] To consider, D´Hulst & Deldique [73] recently suggested that based on the hypoxic

dose theory, [72] an exposure of 5000 km · h^{-1} is the cutoff point above which muscle loss starts to occur. However, at altitude the stimulation of protein synthesis after exercise might be blunted by hypoxia, as it was shown that the increase in muscle protein synthesis following walking at 4559 m [74] was much lower than a comparable study with exercise performed at sea level. [75] Interestingly, in a separate study, body mass was maintained in ski mountaineers following an isocaloric diet of 4000 kcal · d^{-1}, supplemented with 1.5 g or 2.5 g · kg^{-1} body mass casein protein per day during seven days at 2500-3800 m. [232] Moreover Bigard and colleagues examined the effects of branch chain amino acids (BCAA) (7.8 g leucine, 3.4 g isoleucine, 11.2 g valine; 1.44 g protein · kg^{-1} · d^{-1}) compared to carbohydrate supplementation on body composition following six days of ski mountaineering at 2500-3800 m. Body composition and muscular performance were unaffected by BCAA. However, significant weight loss only occurred in the carbohydrate-supplemented group (-1.55 vs. -0.8 kg). [232]

The purpose of this study was to examine the effects of a nutritional intervention on the body mass of an elite wheelchair marathoner during a five-week training camp performed between sea level and 3900 m altitude. The intervention was designed to anticipate increases in RMR due to the combined effects of both environmentally induced hypoxia and the demands of marathon training.

Case presentation

The study athlete was a 36-year-old, elite wheelchair marathoner, functional class T52 (upper limb involvement category). Some of his achievements include winning a silver medal at the Paralympic Games and 107 victories in different road events, including a win at the 2016 Boston Marathon, ten weeks after returning to sea level from Los Andes (Peruvian Altiplano). Our

participant's height = 1.76 m; body mass = 52.6 ± 0.4 kg; power output at second ventilatory threshold = 62 W; training 8000 km per year; former world record-holder in the T52 division in 800 m (1min:56s); 1500 m (3min:36s); world record-holder in 5000 m (12min:37s); half marathon (50min:28s) and fourth best ever time in marathon (1h:42min:05s). Additionally, he has more than ten years of altitude training experience, with training camps performed in Boulder, CO (1655 m), Navacerrada, Spain (1858 m), Flagstaff, AZ (2106 m), Sierra Nevada, Spain (2320 m), Keystone, CO (2796 m) and Breckenridge, CO (2926 m), performing both altitude models: Live High-Train High (LHTH) and Live High-Train Low (LHTL) and has been exposed to more than 8000 hours of normobaric-hypoxia. For the last five seasons prior to the current study, the athlete trained at moderate altitudes (1655 up to 2926 m) for: 78, 82, 101, 79 and 62 days.

The athlete requested advice for the development of an individualized nutritional program based on training loads to prepare for his upcoming season. Therefore, after consultation with laboratory members a nutrition program was designed, according to his training load (Table 13).

Table 13. Main meals designed for each type of session under altitude conditions

Table 1 Main meals designed for each type of session under altitude conditions

Session	Breakfast	Lunch	Dinner	Energy intake (kcal)	Carbohydrate Protein Fat (g)
A	62 g cereals, 204 g soy milk, 26 g white bread, 18 g jam, 3 g black tea in ~ 200 ml water, 12 g sugar	180 g (dry) spaghetti, ~ 150 g alpaca, 8 g olive oil	180 g (dry) steamed rice, 180 g emperor fish, 10 g olive oil	2395	383 111 49
B	62 g cereals, 204 g soy milk, 3 g black tea in ~ 200 ml water, 12 g sugar	180 g (dry) spaghetti, ~ 150 g alpaca, 8 g olive oil	180 g (dry) steamed rice, 180 g emperor fish, 10 g olive oil, 8 g parmesan cheese	2357	353 116 52
C	80 g cereals, 200 g soy milk, 3 g black tea in ~ 200 ml water, 12 g sugar	180 g (dry) spaghetti, 140 g beef, 10 g olive oil, 14 g parmesan cheese	180 g (dry) spaghetti, 125 g tuna, 12 g olive oil, 10 g parmesan cheese, 180 g fat free yoghurt	2424	365 119 42
D	80 g cereals, 200 g soy milk, 130 g coffee, 25 g fat free milk, 14 g sugar	180 g (dry) spaghetti, ~ 150 g alpaca, 8 g olive oil, 8 g parmesan cheese	140 g (dry) rice, 120 g omelette, 170 g tuna canned	2639	348 135 75
E	80 g cereals, 204 g soy milk, 3 g black tea in ~ 200 ml water, 12 g sugar	160 g (dry) rice, ~ 160 g chicken breast, 14 g olive oil, 1 kiwi	180 g (dry) spaghetti, 135 g tuna, 20 g parmesan cheese, 6 g olive oil	2351	374 114 36
F	80 g cereals, 204 g soy milk, 3 g black tea in ~ 200 ml water, 10 g sugar	180 g (dry) spaghetti, ~ 160 g chicken breast, 14 g olive oil, 8 g parmesan cheese, 180 g fat free yoghurt	170 g alpaca, 275 g sweet potato	2091	282 145 43
G	50 g cereals, 200 g soy milk, 17 g coffee, 25 g fat free milk, 12 g sugar	180 g fat free yoghurt 140 g (dry) rice, 120 g omelette, 12 g olive oil	500 g Margherita (pizza)	2618	343 115 89

Session A: 20 × 400 m ~ VT2; Session B: 2 h ~ VT1; Session C: 6 × 2000 m ~ VT2; Session D: 20 km < VT1 in the morning + 16 km < VT1 in the afternoon; Session E: 16 km < VT1 in the morning + Gym session in the afternoon; Session F: 20 km < VT1 in the morning; Session G: Day off

The research participant provided written consent prior to participation in the current study and read the manuscript before submission. Research was approved by the Ethics Research Committee of Miguel Hernandez University.

Training protocol

Both pre-altitude (B_N), at 16 m and acclimatization (B_H) at 3900 m incorporated identical training loads (128 km of mileage each). However, the first two days of B_H incorporated no training to minimize the effects of jet-lag, and acute mountain symptoms (AMS), like headache. [319] Two daily training sessions were performed from Wednesday to Friday under the first ventilatory threshold (<VT1). The morning session involved 20 km of distance training and the afternoon session 16 km. A 20 km workout was performed on Saturday <VT1. Sunday was a rest day. Specific training weeks

"W_1, W_2, W_3 & W_4" were based on a day-to-day basis periodization, according to level of heart rate variability (HRV). [142] When the HRV reached a reference value (RV), the subject completed a specific session in the morning, followed by an evening off. If the RV was not reached, two workouts <VT1 were performed: 20 km in the morning and 16 km in the afternoon. On three days the training was fixed; On Mondays and Thursdays the AM sessions were 16 km <VT1, while the PM sessions involved resistance training and Sundays were off. The specific sessions were known as: A (20 x 400 m at ~ second ventilatory threshold (VT2) in a plateau at 4090 m altitude; recovery repetitions: 75 s); B (30 km ~ VT1) and C (6 x 2000 m ~ VT2 in a plateau at 4090 m altitude; recovery repetitions: 120 s).

As a way to induce muscle hypertrophy, resistance sessions were performed at 80 % of 1 RM [320] with 4 sets of 8 repetitions with 150 s recovery, aimed at avoiding loss of muscle mass induced by chronic hypoxia. RM test was not performed under altitude conditions due to the high risk of injury, so it was done four days before flying to Peru. More details on the experimental design have been previously reported. [311]

Daily recording

Throughout the experiment, basal body mass was recorded in fasting conditions, naked, after waking up, with a digital scale (Tanita BC-601®, TANITA Corporation, Tokyo, Japan). Utilizing a food recording system previously reported, [221] a nutritional diary was maintained by the subject to record daily intake, which included main meals (breakfast, lunch and dinner), two small snacks and all training activities that occurred post-intake (Figures 11 and 12).

Total energy (kcal), carbohydrates, proteins and fats ($g \cdot kg^{-1}$ body mass) were estimated according to a nutritional composition database supported by

the Spanish Ministry of Science and Innovation. [221]

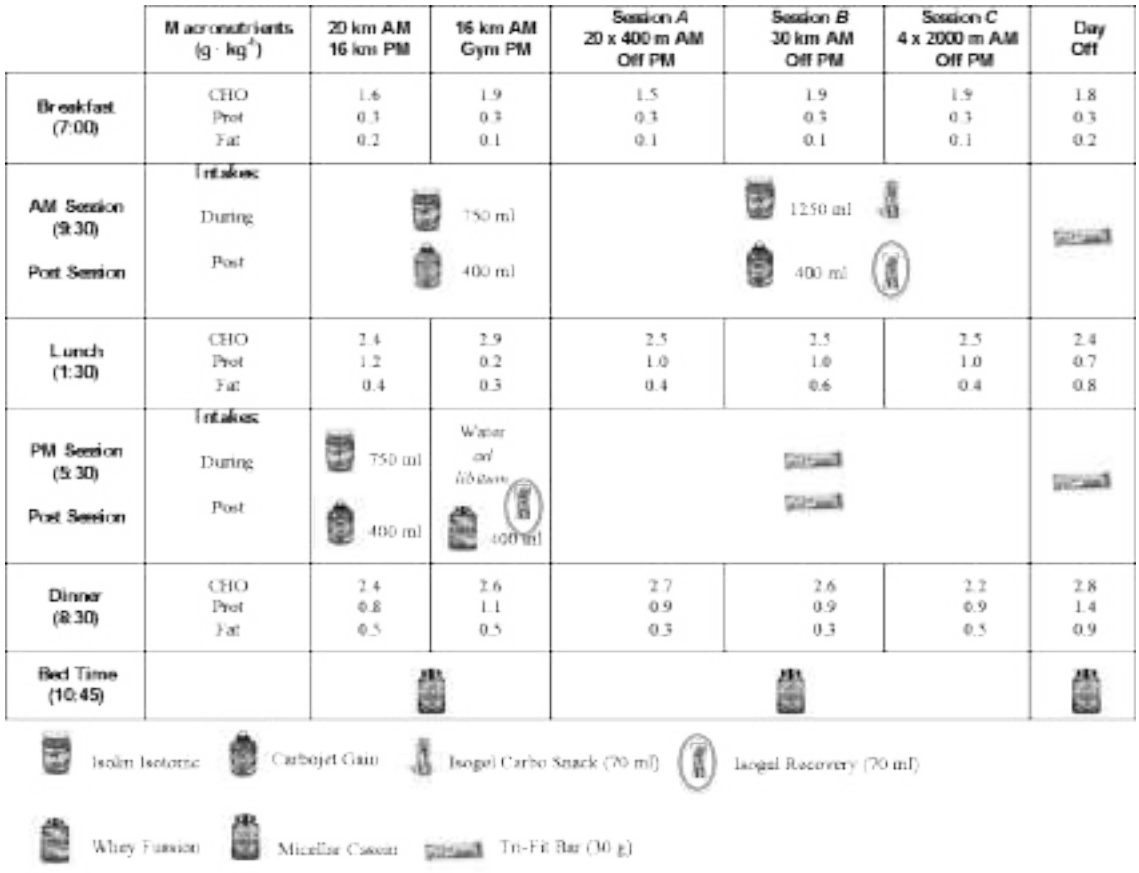

Figure 11. Timing of Daily Food and Fluid Intake during Altitude, based on different training routines.

Nutritional program

The athlete was instructed by a nutritionist to prepare all meals which included weighing both ingredients prior to cooking and left overs prior to disposal. On days when the athlete ate at restaurants, which occurred on four occasions, he was instructed to send pictures of these meals to the research team. [224] A personal chef was contacted to buy and cook all foods/ingredients

for the athlete on a daily basis according to the athlete´s instructions while the weighing and cooking process occurred under the athlete's supervision. Additionally, the athlete was instructed to prepare all training drinks and post-training recovery solutions. To prevent contamination, the athlete did not eat raw foods or unpeeled fruits or vegetables and no water from the tap was consumed. [76] At sea level the athlete cooked all meals at home.

Daily energy intake was increased ~20 % from pre-altitude (B_N), to arrival at altitude (B_H) to avoid body mass loss from increased RMR which is common while living and training at higher altitudes. [63,64] Moreover, main meals were designed according to the type of training session performed, [63] as we have recently reported that during specific training weeks ($W_{1,2,3,4}$) the number of A, B, C, sessions differed between specific training weeks, according to a training program based on HRV, [311] which explains why the greatest amount of CHO was ingested at W_2 (9.9 ± 1.2 g · kg^{-1} body mass), and why during B_H and W_4 the total amount of CHO tended to be lower than in $W_{1,2,3}$ (Table 14). Moreover, main meals were accompanied by two rich-carbohydrate snacks, based on reports that the inclusion of several rich carbohydrate snacks, more optimally covers increased energy requirements than three standalone main meals. [76] Furthermore, regarding proteins, a minimum intake of 2.4 g · kg^{-1} body mass was targeted in the current nutritional design to avoid loss of lean mass. [225] To avoid gastrointestinal issues (GI) and fullness, [226] a low protein/fat intake was provided for breakfast and PM sessions; however, the percentage of lipids at lunch was lower than at dinner. Protein intake at lunch and dinner were ≈ 1 g · kg^{-1}, given that specific and, more demanding sessions (A, B, C) were performed in the morning, and muscle tissue repair is a main meal target. The ingestion of lipids was set at a minimum of 1 g · kg^{-1} body mass throughout the sea level and altitude camps, as fat cells increase their sensitivity to hormonal stimulation after training,

resulting in a greater mobilization of fatty acids. [227] Moreover, an Iso-Lyn Isotonic (AMIX) sports drink was used for workouts < VT1 shorter than 65 min (20 and 16 km). The athlete was instructed to drink a solution with 750 ml of water and 56.4 g of CHO, while a solution of 1250 ml with 80 g of CHO was recommended for specific sessions. The CHO rate was 0.5 to 1 g · kg^{-1} body mass per hour. [228] Despite these recommendations, the athlete and team elected to preserve his natural drinking habits that involved consuming drinks every 10 min. This decision was made because fluid consumption for a wheelchair racer can be precarious during propulsion, as they must come out of their natural prone/kneeling body position to drink. This action can force loss of vision, which increases the risk of collision or crashing. Because our participant never experienced GI in his career with the use of carb gels, [321] he drank a 42 g CHO (Glucose + Fructose) Iso-Gel carbo snack (AMIX) during specific sessions workouts. [229] Gels were consumed in the A session after fourteen 400 m rep, in the B session 90 min after starting, and in the C session after four 2000 m rep. Both types of carbs used in the solution and gels were multiple transportable carbohydrates, as directed by Jeukendrup. [230] During the gym sessions water was consumed *ad libitum*, and immediately after gym sessions the athlete co-ingested a rich leucine whey protein (23.6 g) (Whey Fussion, AMIX) dissolved in 400 ml of water and a carbohydrate gel (Iso-Gel Recovery, AMIX) (37.6 g maltodextrin + fructose + Vitargo ®) as directed to speed glycogen synthesis up to 25 %. [230] For refueling purposes carbohydrate guidelines, [228] suggest aiming for post-exercise rapid recovery of muscle glycogen deposits, with 1 g · kg^{-1} body mass of CHO, repeated every 2-3 hours. After specific sessions, a carbohydrate shake was taken with a carbohydrate gel, providing 1.4 g · kg^{-1} body mass. In the hour immediately after the 16 km and 20 km < VT1, the subject drank a carbohydrate solution (Carbojet Gain, AMIX) (34 g CHO, 7.5 g prot, 1.8 g fat) dissolved in 400 ml

of water, and after specific sessions he ingested a combination of the same drink plus Iso-Gel Recovery. To consider, 2.4 g · kg^{-1} body mass, CHO were consumed (Figure 11) at lunch which occurred approximately two hours post-exercise meal, in order to achieve 3.1 g · kg^{-1} body mass of CHO 3h post-exercise for our athlete vs. 3 g · kg^{-1} body mass as suggested by Burke and colleagues. [228]

On specific session days, rest was provided in the evenings along with a snack at 5:30 PM, to meet increased energy requirements. [76] This snack included two 30 g cereal bars (Tri-Fit Bar, AMIX) (34.9 g CHO, 3.9 g prot, and 10.1 g fat).

In a manner to avoid loss of body mass [232] and enhance muscle protein synthesis [233] the athlete consumed 2.5 g leucine, 1.5 g isoleucine, and 1.5 g valine) immediately after each session (BCAA Elite Rate, AMIX). Before bedtime, 30 g of casein protein (Micellar Casein, AMIX) (1.7 g CHO, 24 g prot, 0.6 g fat) was ingested as suggested by Snijders et al. [234]

Finally, the athlete maintained iron levels through a daily intake of 105 mg of ferrous sulphate (Ferogradumet®, Ross, Abbott Científica), as ferrous sulphate intake has been related to the production of Hb and RBC. [235,236] To comply with World Anti-Doping Agency (WADA) regulations, none of the aforementioned supplements contained any prohibited substance.

For a description of the macronutrients intake during main meals in each session see Figure 11.

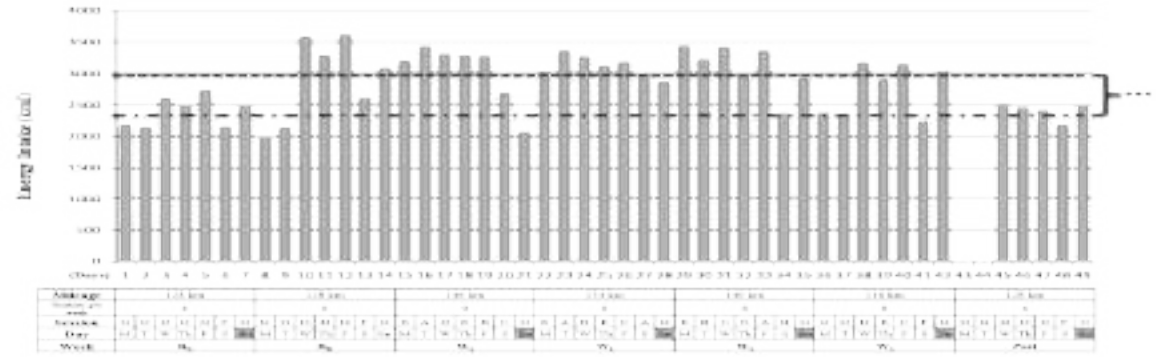

Figure 12. Training program and energy intake during B_N, B_H, $W_{1,2,3,4}$ and Post.

B_N, baseline in normoxia; B_H, baseline in hypoxia; $W_{1,2,3,4}$, specific training weeks in hypoxia; Post, returning sea level week.

Session A: performed on a plateau at 4090 m; 8 km + technique drills + 5 x 80 m accelerations + 20 x 400 m ~ VT2 + 2 km. Recovery repetitions 75 s.

Session B: 2 hours ~ VT1.

Session C: performed on a plateau at 4090 m; 8 km + technique drills + 5 x 80 m accelerations + 6 x 2000 m ~ VT2 + 2 km. Recovery repetitions 120 s.

Session D: 20 km <VT1 in the morning + 16 km <VT1 in the afternoon.

Session E: 16 km <VT1 in the morning + gym session in the afternoon (4 sets x 8 repetitions recovery sets 150 s at 80 % RM). Exercises for resistance session: press bench, close grip, dumbbell press, seated military press and seated cable row).

Session F: 20 km <VT1 in the morning + resting afternoon.

Session G: Day off.

Dash line: Represents mean energy intake (2423 kcal) in normoxic conditions at sea level.

Round dot line: Represents mean energy intake (3017 kcal) in hypoxic conditions at 3900 m altitude.

Differences from mean energy intake under hypoxic conditions: *** $P < 0.001$

Statistical analysis

All data are presented as mean ± SD. A repeated-measures ANOVA was carried out for all the variables including the factor TIME with levels B_N, B_H, W_1, W_2, W_3, W_4 and Post. A post hoc least significance difference (LSD)

multiple-range test was performed to determine differences between the factor levels. Effect size (d) associated with change in body mass was calculated using Cohen's d (difference in mean scores over time divided by pooled SD) with its 95 % confidence limits (CL) [241] and they were interpreted as trivial (≤ 0.19), small (0.20-0.49), medium (0.50-0.79), and large (≥ 0.80). [240] 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.

Results

Our nutritional intervention results can be found in Table 14.

Body mass

A significant decrease in body mass was observed from B_N to B_H [$P < 0.001$; $d = 4.16$, 95 % CL (2.02 ; 5.71)] but it returned to near baseline levels during Post. There was no significant effect for time during the $W_{1,2,3}$ period; however, we observed a significant increase in body mass from W_1 to W_4 [$P < 0.001$; $d = 2.35$, 95 % CL (0.86 ; 3.51)].

Energy intake

Results show a greater amount of kcal in B_H [$P < 0.01$; $d = 0.96$, 95 % CL (-0.25 ; 2.04)] and W_1 [$P < .01$; $d = 1.61$, 95 % CL (0.27 ; 2.73)], W_2 ($P < 0.01$; $d = 3.49$, 95 % CL (1.59 ; 4.91)], W_3 [$P < 0.01$; $d = 2.15$, 95 % CL (-0.66 ; 3.33)] than in B_N. The same differences were observed within B_H [$P < 0.01$; $d = 0.97$, 95 % CL (-0.24 ; 2.05)], W_1 [$P < 0.01$; $d = 1.68$, 95 % CL (0.31 ; 2.80)], W_2 [$P < 0.01$; $d = 4.52$, 95 % CL (2.26 ; 6.16)], W_3 [$P < 0.01$; $d = 2.31$, 95 % CL (0.78 ; 3.51)] and Post. No differences were reported between W_4, B_N and Post.

Carbohydrates

The amount of CHO ingested ($g \cdot kg^{-1}$ body mass) was greater in W_1 [$P <$

0.001; d = 1.43, 95 % CL (0.12 ; 2.53)], W_2 [P < 0.001; d = 2.33, 95 % CL (0.80 ; 3.54)], W_3 [P < 0.001; d = 2.08, 95 % CL (0.62 ; 3.26)] than in B_N. Differences were observed within W_1 [P < 0.01; d = 2.01, 95 % CL (0.56 ; 3.17)], W_2 [P < 0.01; d = 3.47, 95 % CL (1.58 ; 4.88)], W_3 [P < 0.01; d = 3.18, 95 % CL (1.38 ; 4.53)] and Post.

Proteins

Protein intake (g · kg^{-1} body mass) was greater in B_H (P < 0.001; d = 2.54, 95 % CL (0.95 ; 3.79)] and W_1 (P < 0.001; d = 2.03, 95 % CL (0.58 ; 3.20)], W_2 (P < 0.001; d = 2.16, 95 % CL (0.67 ; 3.34)], W_3 (P < 0.001; d = 2.03, 95 % CL (0.58 ; 3.20)], W_4 (P < 0.001; d = 2.31, 95 % CL (0.78 ; 3.52)] than in B_N. The same differences were found within B_H (P < 0.01; d = 2.38, 95 % CL (0.83 ; 3.59)], W_1 (P < 0.01; d = 1.90, 95 % CL (0.48 ; 3.05)], W_2 (P < 0.01; d = 1.96, 95 % CL (0.52 ; 3.11)], W_3 (P < 0.01; d = 1.90, 95 % CL (0.48 ; 3.05)], W_4 (P < 0.01; d = 2.00, 95 % CL (0.56 ; 3.16)] and Post.

Lipids

No differences were found in lipids intake (g · kg^{-1} body mass) within any period.

Table 14. Body mass and nutritional parameters during sea level and altitude.

Phase	Body Mass (kg)	Daily Intake (kcal)	CHO (g · kg^{-1})	Prot (g · kg^{-1})	Fat (g · kg^{-1})
B_N	52.6 ± 0.4 (52.25; 53.04)	2397 ± 242 (2173; 2621)	7.1 ± 1.2[W] (5.97; 8.19)	1.9 ± 0.2[W] (1.74; 2.11)	1.0 ± 0.2 (0.82; 1.14)
B_H	50.7 ± 0.5[B_N] (50.23; 51.17)	2899 ± 670[B_N] (2280; 3518)	8.1 ± 2.2[B_N] (6.04; 10.11)	2.9 ± 0.5 (2.46; 3.38)	1.4 ± 0.5 (0.92; 1.99)
W_1	50.6 ± 0.7[B_N] (50.39; 50.78)	3037 ± 490[B_N] (2584; 3490)	9.6 ± 2.1 (7.68; 11.55)	2.7 ± 0.5 (2.18; 3.17)	1.2 ± 0.4 (0.81; 1.49)
W_2	50.8 ± 0.4[B_N] (50.45; 51.09)	3116 ± 170[B_N] (2959; 3273)	9.9 ± 1.2 (8.79; 11.09)	2.6 ± 0.4[B_N] (2.17; 2.99)	1.1 ± 0.5 (0.65; 1.60)
W_3	50.9 ± 0.3[B_N] (50.68; 51.15)	3101 ± 385[B_N] (2744; 3457)	9.6 ± 1.2 (8.53; 10.73)	2.7 ± 0.5[B_N] (2.25; 3.22)	1.2 ± 0.5 (0.75; 1.64)
W_4	51.2 ± 0.3[B_N,B_H] (50.93; 51.47)	2786 ± 375 (2439; 3133)	8.6 ± 1.3 (7.39; 9.73)	2.5 ± 0.3[B_N] (2.21; 2.77)	1.1 ± 0.5 (0.57; 1.56)
Post	52.1 ± 0.5[B_N,W_1,W_2,W_3] (51.54; 52.66)	2411 ± 137[W_1,W_2,W_3,W_4] (2241; 2580)	6.3 ± 0.8[B_N,W_1,W_2,W_3] (5.41; 7.27)	1.9 ± 0.3[B_N,W_1,W_2,W_3] (1.55; 2.31)	1.3 ± 0.3 (0.94; 1.69)

B_N, Baseline in normoxia at 16 m altitude; B_H, Baseline in hypoxia at 3860 m altitude; $W_{1,}$ First week of specific training; $W_{2,}$ Second week of specific training; $W_{3,}$ Third week of specific training; $W_{4,}$ Fourth week of specific training; Post, values after altitude training camp at 16 m altitude; Body Mass: wake up body mass, kg; Daily intake, is the amount of daily kilocalories; CHO, is the amount of daily carbohydrates related to the AM body mass; Prot, is the amount of daily proteins related to the AM body mass; Fat, is the amount of daily fats related to the AM body mass; Mean ± SD (95% CL).

Differences from B_N: [a] $P < 0.01$; * $P < 0.001$;

Differences from $B_{H:}$ [b] $P < 0.01$; [h] $P < 0.001$;

Differences from $W_{1:}$ [c] $P < 0.01$; [i] $P < 0.001$;

Differences from $W_{2:}$ [d] $P < 0.01$; [j] $P < 0.001$;

Differences from $W_{3:}$ [e] $P < 0.01$; [k] $P < 0.001$;

Differences from $W_{4:}$ [f] $P < 0.01$; [l] $P < 0.001$.

Discussion

The aim of this case study was to assess the effectiveness of an evidence based individualized nutrition program applied to an elite wheelchair marathoner during a five-week altitude training camp, carried out in the Peruvian Altiplano (Puno, Peru) at 3900 m. The program was designed based on existing literature for its ability to sustain the athlete's body mass and meet the energetic demands of intense training, while promoting substrate availability, nutrient recovery, and muscle tissue repair. Interestingly, the designed nutritional intervention helped to: 1) maintain the athlete´s body mass throughout the altitude camp, 2) minimize performance deficits during intense training at altitude compared to sea level (~20 to ~24 % in 1609 m and 3218 m repetitions respectively), [211] as evidenced by recently reported data demonstrating a ~3 % reduction in repetitions (2000 m), [311] 3) facilitate intra-sessions recovery through faster glycogen restoration, helping the athlete to perform during physiological demanding sessions (~ VT2) when completed consecutively, or until two sessions of ~2 hours at ~ VT1 at W_2,

[311] and 4) maintain quality training sessions at altitude as evidenced by : a) improved power output, 11-days post-altitude compared to 4-days pre-altitude (44 W vs. 50 W), b) time reductions during 3000 m races 12-days post-altitude compared to 3-days pre-altitude (472 s vs. 456 s). [311]

At 4300 m there can be an increase in respiratory water loss, due to greater ventilation and an increase in urinary water loss that can increase up to 500 ml per day. [56] This could explain the nearly 2 kg weight loss observed from baseline (B_N) to acclimatization phase (B_H) and the return to pre-altitude levels in post (Table 14). It should be noted that there was an increment of energy intake of ≈ 500 kcal · d^{-1} in hypoxic conditions compared to normoxic conditions (P=0.001) and the same training was done in B_N and B_H (Figure 12). Of note, all effect sizes associated with statistically significant changes in body mass far exceeded Cohen's convention for a large effect.

Increased RMR has been reported in athletes who live and train at altitude. [63] For this reason, to maintain body mass in the current study, there was a significant increase in the amount of carbohydrates per kilogram of body mass and proteins per kilogram of body mass provided at altitude compared to sea level. We suspect that the slight increase in body mass observed in W_4 was induced by the different number of specific sessions performed from W_1 to W_4; 2 in W_1, 3 in W_2, 2 in W_3 and 1 in W_4. [311] To increase energy supply, as a result of a greater energy demand and to avoid GI, six meals (breakfast, post-training AM, lunch, snack or post-training PM, dinner and bedtime) were projected in an elapsed time within three hours from each other (Figure 11), as it has been recommended to include several rich carbohydrate snacks, rather than three main meals. [76] We did not find differences in energy intake between acclimatization (B_H) and specific training weeks (W_1 to W_4); however, this could be due to the fact that when the athlete performed a specific session in the morning, a rest afternoon was followed, in spite of two

sessions performed daily during acclimatization with 36 km volume (Figure 12). Furthermore, we did not consider a slightly lower exogenous glucose oxidation rate during acclimatization and chronic altitude, [272] as it has been reported that such observations should be contrasted with fully fed individuals, although evidence exists to the contrary. [59, 61] Three hours before training sessions, a rich CHO meal was consumed, as it has been shown to increase glycogen availability. [228] We recommended that the athlete changed from cereals to a lower fiber food like white bread to avoid GI distress; however, because of disability imposed manual dexterity deficits which prevent cutting bread slices and spreading fruit jam, he decided to use cereals. The research team also had to consider that the athlete ate breakfast by seven in the morning, which was nearly two and a half hours before training sessions. However, the athlete commonly practiced training in a fasted state like this during training sessions at home, to minimize GI. Despite the athlete's comfortability with this practice, it was discarded in Puno because temperatures were extremely cold at 7am (~ 0º C) and he trained barefoot.

To avoid a loss of muscle mass, high-protein foods were spread out across all meals (Figure 11), while whey and casein protein training products were consumed to ensure minimum requirements of 2.4 $g \cdot kg^{-1}$ body mass were reached. [225] However, we have to consider that the hypoxic dose [72] of this training camp was 3300 $km \cdot h^{-1}$, not reaching the cut off point, in which muscle loss begins. [73] Due to personal preferences, protein delivery by meat was introduced at lunch, while fish was eaten at dinner. No eggs were eaten while training but the athlete ate an omelet for lunch during rest days (Table 13).

Limitations

The main limitations of this study are evident in the absence of outcomes like upper body skinfolds, and upper arm circumference measurements, which could help us to know if body fat percentage and loss of muscle mass occurred in our athlete which was reported previously in subjects eating *ad libitum* under hypoxic conditions [12-15]. [52,53,54,317] Moreover, RMR was not assessed, as recently reported [67] in Olympic rowers training at 1800 m who did not show an increase in RMR. However, our athlete was exposed to more intense hypoxic conditions, so sympathoexcitation may have occurred [39] leading to elevated adrenaline levels and subsequent greater energetic demands. Another limitation was evident in the use of a self-reported intake diary conducted without supervision from a nutritionist, however the athlete was providing instructions for meal preparation as described previously. Importantly, similar self reported nutritional tools have been validated for estimating energy and nutrient intake. [224]The use of pictures on four occasions to record restaurant meal consumption must also be considered as a limitation. However, this methodology has been supported by exercise nutritionists as a useful strategy, particularly when research teams are not present. [221] Finally, the absence of muscular biopsies did not allow us to measure glycogen and protein muscle content.

Conclusions

The aim of the daily meal distributions (Figure 11) was to cover the energetic demands of training sessions and to ensure substrate availability, nutrients recovery, and muscle tissue repair according to literature recommendations.

This paper can help us to better understand the unique nutritional requirements of upper body endurance athletes during altitude training conditions in which nutritional strategies may differ from able-bodied athletes. Importantly, to confirm and expand on the current findings specific

to the aforementioned differences between able bodied and upper limb athletes, more research is needed on both populations. However, analogous studies are scarce in able bodied athletes and nonexistent in upper limb athletes. For example, only one study, published in 1967 examined well-trained athletes at 4000 m, [211] while others have investigated nutritional interventions or exercise metabolism at moderate altitudes only (2150m). [63,221] To date, the only other studies conducted at altitudes similar to ours involved either dissimilar sports disciplines, [70] lacked a nutritional component, [211] or utilized non elite athletes. [272] Ultimately, this study represents the first nutritional intervention conducted on an elite wheelchair marathoner under altitude conditions. Since no specific nutritional interventions have been performed on able-bodied marathon runners or wheelchair athletes at 4000 m altitude, all nutritional guidelines were reflective of the literature pertaining to able-bodied athletes training at lower altitudes.

Ultimately, our nutritional intervention targeted body mass maintenance to sufficiently anticipate increases in RMR due to the combined effects of environmentally induced hypoxia and the demands of marathon training. Moreover, the intervention helped minimize performance perturbations, facilitated overall recovery, and enhanced athlete performance post-altitude. Future related studies should be designed based on considerations from the current study, however with more specificity, and therefore utilizing deeper assessment tools like biological samples. For example, biopsies could be applied to determine the protein and glycogen synthesis-breakdown cycle of athletes during periods of intense training.

List of abbreviations

AMS Acute mountain symptoms, BCAA Branch chain amino acids, B_H Altitude acclimatization, B_N Pre-altitude, CHO Carbohydrates, CL Confidence limits, GI Gastrointestinal issues, HRV Heart rate variability, LHTH Live-High-Train-High, LHTL Live-High-Train-Low, RMR Resting metabolic rate, RV Reference value, SD Standard Deviation, VT1 First ventilatory threshold, VT2 Second ventilatory threshold, W_1,W_2, W_3, W_4 Specific training weeks at altitude

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Availability of data and materials

Please contact authors for data requests

Authors´ contributions

RLG, MMR and GBC conceived the study. RLG, MMR, GBC and SSQ participated in the design of the study. Data were collected by SSQ and analyzed by SSQ, IR, RLG, MMR, TU and GBC. Data interpretation and manuscript preparation were undertaken by SSQ, IR, RLG, MMR, TU and GBC. All authors approved the final manuscript.

Ethics approval and consent to participate

This investigation had prior ethical approval by the Ethics Research Committee of the Miguel Hernandez University.

Consent for publication

The athlete provided consent for publication, after reading the last version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

11 MANUSCRIPT V (UNDER REVIEW)

Hydratus status assessment in an elite wheelchair marathoner training at 3900 m altitude: a case study

Abstract

BACKGROUND: Athletes must prevent dehydration and overdrinking to maintain performance, reduce cardiovascular and thermoregulatory strain, and avoid hyponatremia. No studies have reported hydration strategies among endurance disciplines at higher altitudes (~4000 m).

METHODS: A professional wheelchair-athlete participated in this study. Total daily fluid intake, urine excretion, fluid intake during morning and afternoon training sessions, and urine specific gravity upon wakening and before bed time (SG AM, SG PM) were assessed before (W_{-1}), during (W_{1-5}), and after (W_{+1}) a 5-week training camp at 3860-4090 m altitude. Body mass and total sodium daily intake (Na^{+}) were recorded daily.

RESULTS: an increase in total fluid intake from W_{-1} (4280.8 ± 723.0 mL) to W_1 (5552.2 ± 1302.6 mL) was observed (d = -1.21), which diminished during W_{+1} (3763.6 ± 1321.9 mL) (d = 0.49). There was also a significant reduction in fluid intake from W_2 (4628.8 ± 839.6 mL) to W_{+1} (3763.6 ± 1321.9 mL) (d = 0.78). Urine volume was greater during W_1 (4448.0 ± 444.3 mL) compared to W_{-1} and $W_{2,3,4,5}$ ($d \geq 2.85$). Diuresis was lower after returning from altitude (2526.0 ± 517.3 mL) compared to all other periods ($d \geq 1.24$). SG AM was lower at W_1 (1.010 ± 0.006) compared to W_{-1} (1.020 ± 0.002; d = 2.23), W_2 (1.019 ± 0.002; d = -2.01), and W_{+1} (1.023 ± 0.006; d = -2.17).

CONCLUSIONS: Increased fluid requirements and diuresis were observed in an endurance wheelchair athlete training and living at 3860-4090 m terrestrial altitude.

Key words: hypoxia, hydric balance, fluid replacement, marathon

Introduction

At altitude, body mass reduction typically occurs from water loss followed by malnutrition which drives fat and muscle loss.[322] During altitude acclimatization, there is a reduction in intra and extra cellular water, along with decreased plasma volume [48,49,317] which results in a body mass loss of up to 2 kg.[52] At altitudes reaching 4000 m, respiratory water loss may increase due to greater ventilation,[50] along with urinary water loss appraching 500 mL per day.[51] Therefore, rapid fluid rehydration, to compensate for sweat losses, ventilation and diuresis may be warranted during altitude training,[76] in which consumption of 100 mL of fluid every ten minutes during and after training can mitigate the effects of dehydration.[77, 78] In addition, increased sodium (Na^+) excretion is related to acute mountain sickness symptoms (AMS),[49] therfore 0.5 – 0.7 g · L^{-1} of Na^+ replacement during training,[82,83] in a daily range of 1500 to 2300 mg · d^{-1} has been recommended.[84] Sodium enriched drinks provided during exercise and recovery have been previously recommended for several reasons (i.e., stimulate thirst, increased voluntary fluid intake, enhanced glucose and water intestinal absorption, optimized extracellular and intracellular fluid balance), and prevention of hyponatremia (plasma sodium < 135 mmol · L^{-1}).[85,86,87,88] Moreover, a study with seven trained males performed at 5050 m altitude demonstrated the urinary Na^+ concentration was only significantly greater compared to sea level (166 ± 34 mEq · d^{-1}) in the acute exposure phase (427 ± 46 mEq · d^{-1}). Furthermore, three weeks after arrival at altitude, values did not differ significantly from sea level ones (257 ± 34 mEq · d^{-1}).[89]

The purpose of this case study was to evaluate an individualized hydration intervention, and its effects on an elite wheelchair marathon racer's body

mass, diuresis, and hydration status, during a 5-week training camp at 3860-4090 m terrestrial altitude, and after returning to sea level.

Case report

Participant

The participant was a 36 year-old, professional wheelchair athlete, Paralympic silver medalist; height = 1.76 m; Body Mass = 52.6 ± 0.4 kg; class T52 (upper limb affection), and diagnosed with Charcot Marie Tooth disease (CMT). [206] 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 Miguel Hernández University (Elche, Spain), being chairperson of this Committee Alberto Pastor Campos (project identification code #DPS.MMR.02.15; date of approval December 17Th 2015).

Procedures

A case study was performed in conjunction with a base preparatory marathon live high – train high (LHTH) training camp, in Puno (Peruvian Altiplano) at 3860-4090 m terrestrial altitude. Pre-altitude (W_{-1}), altitude acclimatization (W_1) and post-altitude (W_{+1}) involved the same training loads at intensities not exceeding the first ventilatory threshold (VT1), while specific training was performed from weeks 2-5 at altitude (W_{2-5}). The training program has been recently published. [311] From W_2 to W_5 the athlete performed a specific training session (i.e., 20 x 400 m at VT2, 6 x 2000 m at VT2, 2 hours at VT1), if a reference value of his daily morning heart rate variability was reached. Otherwise, two workouts below the first ventilatory threshold (<VT1) were completed in the morning (20 km) and in the afternoon (16 km). Sundays were resting days, while resistance sessions were performed at

a gymnasium Monday and Thursday afternoons. These sessions consisted of four exercises (bench press, close grip dumbbell press, seated military press, and seated cable row) in 4 sets of 8 repetitions 80 % RM and 150s recovery.

Ambient temperature and humidity recording

Ambient temperature and relative humidity were recorded with a portable device (Tenmars TM-183®, Taipei, Taiwan) attached to the racing wheelchair. Average temperature and humidity were recorded daily prior to training sessions.

Body mass recording

Body mass was recorded upon wakening in a fasting condition with a scale (Tanita BC-601®, TANITA Corporation, Tokyo, Japan).

Diuresis and urine specific gravity assessment

Urine was collected throughout the day (resting and training hours) with a 2000 mL recipient with 100 mL reference marks. Urine volume was weighed (Tanita kd-321®, TANITA Corporation, Tokyo, Japan), while liquid intake (Fluid) was recorded by weighing liquid consumed/leftover in bottles used for training and resting hours. Urine specific gravity (SG) was measured upon waking (AM SG) (Mission® U500, ACON Laboratories, San Diego, California) and two hours after dinner (PM SG).

Hydric balance estimation

To estimate Hydric Balance (HB) after each session, we used the formula; HB = (ingested fluid volume) - (water loss); [223] in which ingested fluid volume corresponds to the liquid intake during a session. If volume suggested (VS) for training was not consumed completely, leftover liquid (LL) was

weighed and the formula (fluid volume = VS – LL) was applied. Water loss was quantified as the difference between pre and post training body mass. In the current study, Na^+ from solid intake was estimated according to the nutritional composition from database (BEDCA, 2007) supported by Spanish Ministry of Science and Innovation.[222]

Hydration plan

The sport drink used for workout routines was Isolin Isotonic (AMIX). It was recommended that the athlete drank ~700 mL solution for workouts at <VT1 and a solution of 1250 mL plus a 70 mL carbohydrates gel for specific sessions. Recommended drinking rate was 100 mL every 10 min as previously reported.[77] Water was consumed *ad libitum* during resistance training and a minimum of 400 mL was consumed immediately after as a rehydration strategy. Overall, the daily liquid consumption target was 4 to 5 L [79] (Table 15), and SG was maintained inside normal range (≤1.20),[80] except AM SG at W_{+1}, in which liquid intake was below 4 L per day (Table 15).

Table 15. Nutritional, hydration status, and body composition parameters at sea level and altitude.

Phase	Body Mass (kg)	SG AM	SG PM	Fluid (mL)	Na^+ (mg)	Urine (mL)
W_{-1}	52.6 ± 0.4†‡§	1.020 ± 0.003	1.014 ± 0.004	4280.8 ± 723.0	2249 ± 845	3504.3 ± 652.4
W_1	50.7 ± 0.5¶	1.014 ± 0.006^{ac}	1.012 ± 0.006	5552.2 ± 1302.6^{a}	1980 ± 681	4448.0 ± 444.3$^{*\dagger\ddagger\S\|}$
W_2	50.5 ± 0.2	1.019 ± 0.002	1.008 ± 0.003*	4834.7 ± 850.7	1601 ± 244	3815.0 ± 382.9†
W_3	50.8 ± 0.4	1.018 ± 0.004	1.012 ± 0.004	4628.8 ± 839.6	1921 ± 568	3610.7 ± 476.1†
W_4	50.9 ± 0.3*	1.018 ± 0.003	1.016 ± 0.003‡	4257.1 ± 499.9^{b}	2033 ± 656	3141.4 ± 471.0‡
W_5	51.2 ± 0.3*‡	1.018 ± 0.004	1.015 ± 0.004‡	4213.1 ± 460.4^{b}	1838 ± 656	3206.4 ± 518.1‡
W_{+1}	52.1 ± 0.5*†‡§¶	1.023 ± 0.006^{b}	1.017 ± 0.006‡	3763.6 ± 1321.9^{bc}	2379 ± 750	2526.0 ± 517.3*†‡§¶

Table note: W_{-1}, pre-altitude week; B_H, $W_{1\text{-}5}$ weeks at altitude*; W_{+1}, Post-altitude week; Body Mass, is the waking up body mass; SG AM, Urine Specific Gravity after waking up; SG PM, Urine Specific Gravity before bedtime; Fluid, is the total amount of daily fluid intake; Na^+, is the total daily sodium intake; Urine, is the total daily urine excreted. Differences from W_{-1}: a $p < 0.05$; * $p < 0.001$; Differences from W_1: b $p < 0.05$; † $p < 0.001$; Differences from W_2: c $p < 0.05$; ‡ $p < 0.001$; Differences from W_3: d $p < 0.05$; § $p < 0.001$; Differences from W_4: e $p < 0.05$; || $p < 0.001$;Differences from W_5: f $p < 0.05$; ¶ $p < 0.001$.*

Statistics

All data are presented as (mean ± SD). Data were screened for normality with a Kolmogorov Smirnov test. A repeated measures ANOVA was used for all variables including factor TIME with levels W_{-1}, W_1, W_2, W_3, W_4, W_5 and W_{+1}. A post hoc LSD multiple range test was performed to examine differences between factor levels. Effect size (d) associated with change in body mass, fluid, and urine were calculated using Cohen's d (difference in mean scores over time divided by pooled SD) and they were interpreted as

trivial (d≤0.19), small (0.20-0.49), medium (0.50-0.79), and large (d≥0.80).[240] Statistical significance was set at an Alpha of 0.05 and SPSS version 22.0 (SPSS, Inc., Chicago, IL, USA) was used for all analysis.

Results

Our hydration intervention results can be found in Tables 16 & 17.

Table 16. Sessions fluid intake at sea level and altitude.

Phase	Fluid Vol.1 (mL)	Fluid Vol.2 (mL)
W_{-1}	700.0	700.0
W_1	678.8 ± 219.4[e]	707.3 ± 12.7
W_2	1089.2 ± 340.5[b]	730.0 ± 157.1
W_3	1199.0 ± 324.8[ab]	321.0 ± 29.7[abcef]
W_4	1146.7 ± 294.3[a]	620.0 ± 282.8
W_5	780.0 ± 270.7[de]	561.3 ± 134.2
W_{+1}	700.0[de]	500.0[abcd]

Table note: Fluid Vol. 1 and 2, are the fluid volumes for AM and PM respectively training sessions.

[a] Differences from $W_{-1:}$ ($p < 0.05$); [b] Differences from $W_{1:}$ ($p < 0.05$); [c] Differences from $W_{2:}$ ($p < 0.05$); [d] Differences from $W_{3:}$ ($p < 0.05$); [e] Differences from $W_{4:}$ ($p < 0.05$); [f] Differences from $W_{5:}$ ($p < 0.05$).

Results demonstrated an increase ($p < 0.05$; $d = -1.21$) in total fluid intake from W_{-1} (4280.8 ± 723.0 mL) to W_1 (5552.2 ± 1302.6 mL) which diminished ($p < 0.05$; $d = 0.49$) after returning to sea level at W_{+1} (3763.6 ± 1321.9 mL). There was also a significant reduction ($p < 0.05$; $d = 0.78$) in fluid intake from W_2 (4628.8 ± 839.6 mL) to W_{+1} (3763.6 ± 1321.9 mL). A

significant decrease ($p < 0.001$; $d = 4.19$) in body mass was observed at W_1 (50.7 ± 0.5 kg) compared to W_{-1} (52.6 ± 0.4 kg); however, it returned to near pre-altitude levels after returning from altitude at W_{+1} (52.6 ± 0.5 kg). We observed a significant increase ($p < 0.001$; $d = -1.13$) in body mass from W_2 (50.8 ± 0.4 kg) to W_5 (51.2 ± 0.3 kg). Urine volume was greater ($p < 0.001$; $d = -1.69$) during W_1 (4448.0 ± 444.3 mL) compared to W_{-1} (3504.3 ± 652.4 mL), W_2 (3815.0 ± 382.9 mL; $d = 1.53$), W_3 (3610.7 ± 476.1 mL; $d = 1.82$), W_4 (3141.4 ± 471.0 mL; $d = 2.85$) and W_5 (3206.4 ± 518.1 mL; $d = 2.57$). Diuresis at W_{+1} (2526.0 ± 517.3 mL) was lower ($p < 0.001$) than all other periods ($d \geq 1.24$). AM SG was lower ($p < 0.05$; $d = 2.23$) in W_1 (1.010 ± 0.006) compared to W_{-1} (1.020 ± 0.002), W_2 (1.019 ± 0.002; $d = -2.01$) and W_{+1} (1.023 ± 0.006; $d = -2.17$). For training session data collection see Table 17.

Table 17. Hydric balance, fluid intake, and water loss at sea level and altitude.

Date	Day	Phase	Altitude (m)	AM session (km)	PM session (km)	Duration (h-min-s)	Temp. (C)	Hum. (%)	Fluid Vol. (mL)	Fluid rate (mL · h⁻¹)	Water loss (mL)	Hydric Balance (mL)
6/01/16	5	W_{-1}	16	19.9 <VT1		1:06:30	13	56	700	631.6	500	200
6/01/16	5	W_{-1}	16		16.8 <VT1	0:59:38	16	61	700	704.3	200	900
7/01/16	4	W_{-2}	16	20.3 <VT1		1:06:17	22	52	700	633.6	200	500
7/01/16	4	W_{-2}	16		16.8 <VT1	0:52:45	23	56	700	796.2	100	600
8/01/16	5	W_{-3}	16	20.2 <VT1		1:04:22	19	53	700	652.5	300	400
8/01/16	5	W_{-3}	16		16.2 <VT1	0:50:24	23	58	700	833.3	100	600
9/01/16	6	W_{-4}	16	17.8 <VT1		0:56:53	15	63	700	738.4	150	550
9/01/16 10/01/16 10/01/16	FLIGHT ALICANTE-MADRID-LIMA-JULIACA-DRIVE TO PUNO											
13/01/16	10	W_1	3860	20.1 <VT1		1:13:40	9	44	350	275.5	0	350
13/01/16	10	W_1	3860		15.7 <VT1	1:06:55	19	48	700	627.6	200	500
14/01/16	11	W_1	3860	20.2 <VT1		1:12:49	5	41	800	659.2	100	700
14/01/16	11	W_1	3860		16.2 <VT1	0:59:17	17	51	722	731.8	300	800
15/01/16	12	W_1	3860	20.2 <VT1		1:11:10	10	40	790	666.1	100	690
15/01/16	12	W_1	3860		16.2 <VT1	0:58:58	17	44	700	712.3	200	500
16/01/16	13	W_1	3860	20.2 <VT1		1:09:46	5	49	775	666.5	100	675
18/01/16	15	W_1	3860	16.2 <VT1		1:03:06 FIRE	13	51	775	736.9	300	475
18/01/16	15	W_1	3860		Strength	1:10:00	17	47	590	505.7	300	290
19/01/16	16	W_2	4090	20 x 400 m		1:42:23	4	46	1400	820.4	100	1300
20/01/16	17	W_2	3860	20.2 <VT1		1:13:58 RIDE	11	45	775	628.7	100	675
20/01/16	17	W_2	3860		16.2 <VT1	0:55:25	17	49	700	757.9	500	200
21/01/16	18	W_2	3860	16.3 <VT1		0:54:20	10	46	785	866.9	200	585
21/01/16	18	W_2	3860		Strength	1:10:00	17	48	900	771.4	300	600
22/01/16	19	W_2	3860-4090	29.7 VT1		2:20:40	2	44	1400	597.2	100	1300
23/01/16	20	W_2	4090	6 x 2000m		2:03:54	4	45	1400	679.8	300	1100
25/01/16	22	W_3	3860	16.3 <VT1		0:54:45	11	52	785	860.3	400	385
25/01/6	22	W_3	3860		Strength	1:10:00	23	51	500	257.1	1300	-1000
26/01/16	23	W_3	4090	20 x 400 m		1:39:00	5	51	1400	848.5	200	1200
27/01/16	24	W_3	3860	30.4 VT1		1:36:54	18	53	1434	887.9	800	634
28/01/16	25	W_3	3860	16.2 <VT1		0:55:41	9	54	775	831.1	300	475
28/01/16	25	W_3	3860		Strength	1:10:00	16	44	542	293.1	0	542
29/01/16	26	W_3	4090	6 x 2000 m		2:06:08	7	51	1400	685.9	300	1100
30/01/16	27	W_4	4090	20 x 400 m		1:38:11	5	43	1400	855.5	0	1400
1/02/16	29	W_4	3860	16.3 <VT1		0:57:31	7	52	775	808.5	0	775
1/02/16	29	W_4	3860		Strength	1:10:00	16	50	820	702.9	300	520
2/02/16	30	W_4	3860-4090	29.7 VT1		2:06:20	10	48	1400	664.9	300	1100
3/02/16	31	W_5	4090	6 x 2000 m		2:01:12	9	46	1250	633.7	300	950
4/02/16	32	W_5	3860	16.3 <VT1		0:54:42	8	44	775	850.1	100	675
4/02/16	32	W_5	3860		Strength	1:10:00	14	49	420	360	0	420
5/02/16	33	W_1	4090	20 x 400 m		1:43:17	10	51	1400	813.3	600	800
6/02/16	34	W_1	3860-4090	29.7 VT1		1:59:05	8	44	1250	629.8	0	1250
9/02/16	37	W_2	3860	16.3 <VT1		0:53:52	11	53	700	779.7	100	600
9/02/16	37	W_2	3860		Strength	1:10:00	14	48	552	473.1	0	552
10/02/16	38	W_2	3860	20.3 <VT1		1:03:51	9	49	550	515.5	100	450
10/02/16	38	W_2	3860		16.5 <VT1	0:54:28	16	53	700	771.1	400	300
11/02/16	39	W_3	3860	16.3 <VT1		0:53:32	7	44	700	784.5	0	700
11/02/16	39	W_3	3860		Strength	1:10:00	16	51	700	600	200	500
12/02/16	40	W_3	4090	6 x 2000 m		2:07:13	7	49	1250	589.5	200	1050
13/02/16	41	W_4	3860	20.3 <VT1		1:07:24	14	48	700	623.1	200	500
15/02/16 16/02/16	DRIVE PUNO-JULIACA FLIGHT JULIACA-LIMA-MADRID-ALICANTE											
17/02/16	45	W_{-1}	16	20.3 <VT1		1:10:24	16	61	700	596.6	1200	-500
17/02/16	45	W_{-1}	16		16.2 <VT1	0:53:23	12	61	500	561.9	300	200
18/02/16	46	W_{-1}	16	20.3 <VT1		1:03:05	12	41	700	570.7	100	600
18/02/16	46	W_{-1}	16		16.2 <VT1	0:47:47	17	57	500	627.8	400	100
19/02/16	47	W_{-2}	16	20.3 <VT1		1:02:38	11	53	700	503.1	0	700
19/02/16	47	W_{-2}	16		16.5 <VT1	0:56:03	13	61	500	599.4	100	400

Table note: Temp., Ambient temperature; Hum., Ambient humidity: Water loss, body mass difference between pre- and post-session.

We observed positive HB in every training session, with the exception of a strength session performed at the gym, in which temperature was higher than usual. Furthermore, according to each type of session, average fluid intake per hour was: 586.8 ± 102.7 mL · h^{-1} for 20 km <VT1 sessions; 749 ± 86.5 mL · h^{-1} for 16 km <VT1 sessions; 495.4 ± 175.6 mL · h^{-1} for resistance sessions; 834.4 ± 17.9 mL · h^{-1} for 400 m interval sessions; 704.9 ± 130 mL · h^{-1} for 30 km VT1 sessions; 642.2 ± 34.7 mL · h^{-1} for 2000 m interval sessions.

A negative moderate correlation occurred between ambient temperature and HB ($r = -0.37$; $p = 0.006$), and a moderate correlation was also found between HB and fluid intake ($r = 0.53$; $p = 0.001$). Fluid intake showed a strong correlation with urine production ($r = 0.67$; $p = 0.001$). A negative moderate correlation was found between water loss and HB ($r = -0.59$; $p = 0.001$).

Discussion

This study examined the influence of a hydration assessment/intervention on body mass, diuresis, and hydration status in an elite wheelchair marathoner, during a 5-week training camp at 3860-4090 m terrestrial altitude, and after return from altitude.

At high altitude greater ventilation [50] and diuresis,[51] might explain the nearly 2 kg weight loss observed from W_{-1} to W_1, and the return to pre-altitude values after returning to sea level at W_{+1} (Table 15). In fact, from acclimatization, hydration appeared optimal, as reflected by a lower AM SG at W_1 compared to W_{-1}, and a positive HB during all training sessions, except during one gymnasium session, in which unexpectedly high temperatures were recorded. To reach the 4 L daily fluid consumption goal, researchers

utilized a strategy in which the athlete was encouraged to avoid feeling thirsty. Ultimately, this practice led to lower or equal PM SG values compared to AM SG, reflecting an optimal PM SG week by week (Table 15). Furthermore, no AMS symptoms were reported by the athlete, which could be due to an increase in diuresis at W_1, as water retention has been related to AMS. [49, 288]

Interestingly, the upper limb athlete examined in the current study exhibited 2 % body mass loss during one sea level training session (16º C ambient temperature, 61 % relative humidty), which is far less than reports of able bodied runners who displayed up to an 8 % loss.[78] Muscle mass involvement between the populations in question, and differences between exercise intensities may help explain the differences.

The aforementioned explanation may support optimal hydration levels were achieved. In fact, a lower rate of 600 mL · h^{-1} [77] would have been effective for maintaining hydration. To consider, Na^+ did not reach the minimum quantity recommended during workouts at <VT1 (0.13 g · L^{-1}) and specific sessions (0.17 g · L^{-1}).[82] However, Vrijens and Rehrer demonstrated that hyponatremia may induce lower diuresis in male endurance athletes, as they reportet that reduced urine production correlated with increased Na^+ loss ($r = -0.478$; $p = 0.0447$).[88] As this was not observed in the current study (Table 15), we believe our athlete's Na^+ requirements were met. Although our results regarding Na^+ at W_1 are contrary to those of Zaccaria et al. However, training intensity during W_1 in the current study was far less than the exhaustive exercise reported in that study.[89] Ultimately our athlete´s daily intake of Na^+ (1500 to 2300 mg · d^{-1}) met suggested quantities.[84] Finally, the decrease in diuresis at W_{+1} may have occurred because fluid consumption was reduced while flying from Peru to Spain due to the athletes mobility restrictions. Daily water intake and urine excretions are shown in Figure 13.

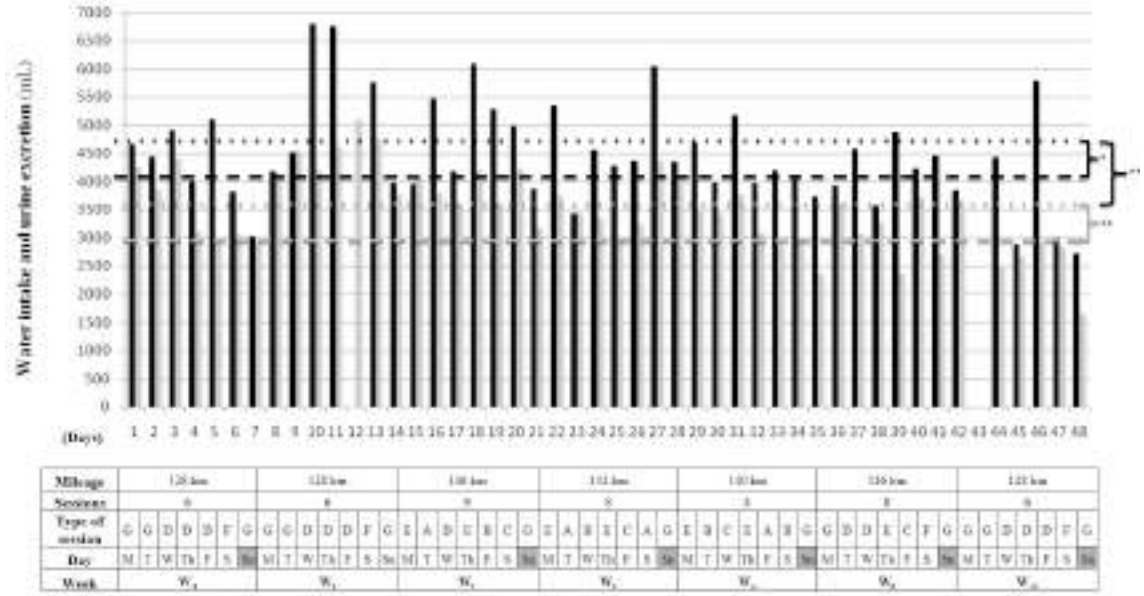

Figure 13. Training program and daily water intake plus urine excretion during W_{-1}, $W_{1,2,3,4,5}$ and W_{+1}.

Session A: 20 x 400 m VT2. Session B: 2 hours VT1. Session C:6 x 2000 m VT2 Session D: 20 km < VT1 in the morning + 16 km < VT1 in the afternoon. Session E: 16 km < VT1 in the morning + resistance session in the afternoon. Session F: 20 km < VT1 in the morning + resting afternoon. Session G: Rest day. Black columns: Represent daily water intake. Grey columns: Represent daily urine excretion. Dash black line: Represents mean water intake (4065 mL) under normoxic conditions at 16 m altitude. Round dotted black line: Represents mean water intake (4634 mL) under hypoxic conditions at 3860 m altitude. Dashed grey line: Represents mean urine excretion (3097 mL) under normoxic conditions at 16 m altitude. Round dotted grey line: Represents mean urine excretion (3644 mL) under hypoxic conditions at 3860 m altitude. Differences from water intake under normoxic conditions: * $p < 0.1$. Differences from urine excretion under normoxic conditions: ** $p < 0.01$. Differences between water intake and urine excretion under altitude conditions: *** $p < 0.001$.

Conclusions

Individualized hydration strategies to optimize hydration and re-hydration of wheelchair marathoners training at altitude should consider: 1) type and length of session 2) non-invasive assessment of hydration status variables such as SG and Na^+ 3) diuresis assessment 4) Guarantee 600 mL · h^{-1} or 10 mL · min^{-1} during training and 4 L of liquid per day. Moreover, an elite endurance athlete must prevent dehydration and overdrinking (± 2 % body mass), to maintain performance, reduce cardiovascular and thermoregulatory strain, and avoid hyponatremia during longer workouts.

12 PUBLISHED MANUSCRIPT VI

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(2018) and was ranked 50 out of 81 in the category of sports sciences.

Abstract

The purpose of this study was to analyze heart rate variability (HRV) oscillations before and after a marathon which involved trans-meridian air travel and substantial time zone differences in a professional wheelchair athlete with Charcot-Marie-Tooth (CMT) disease. The natural logarithm of the root mean square difference between adjacent normal R-R intervals (Ln rMSSD) was measured daily on the days before, including and following the race. Relative to baseline, small (-3.8 – -4.6 %) reductions in LnRMSSD were observed following relocation and on the race-day, indicating only minor effects of travel on cardiac-autonomic activity. On the morning following the marathon, a 23.1 % reduction in Ln rMSSD was observed, which returned to baseline by 48 h. The race time set by the athlete was the world-leading time in his class (T52). This case study showed that Ln rMSSD responses to marathon in an elite wheelchair athlete with CMT was similar to those previously reported among unrestricted endurance athletes.

Key words: Autonomic nervous system, athletics, Paralympic, cardiac autonomic modulations.

Introduction

The marathon is a physiologically demanding endurance racing event. [189] While the 42195 m completion times for elite runners are ~130 minutes, top wheelchair athletes (i.e. "wheelers") can finish the race within 90 minutes (for those with no upper-extremity impairments, sport classes T53-54) and in 115 minutes (for those with trunk and arm impairments, T51-52). Even though mechanical stress in wheelchair racing seems to be lower than in running, cardiovascular load is similar, while oxygen transport demands are quite lower, [204] possibly because there is less muscle mass involved in wheelers than in runners. [205] To the best of our knowledge, only one previous study [204] has analyzed heart rate (HR) dynamics in top-finisher marathon wheelchair athletes. The participants from this study maintained a high mean HR (171.6 ± 20.5 beats·min^{-1}) continuously throughout the race. In contrast, a group of elite runners with a personal best marathon time of ≤ 2 hours and 11 minutes had a mean HR of 167 ± 5 beats·min^{-1} during a 10 km test at marathon pace. [189] Classical physiological measurements of the function of the cardiovascular system, such as mean HR, are currently being complemented by new signal analyses -i.e., the heart rate variability (HRV) that provides more precise information on the autonomic control of HR. [140] The most commonly used parasympathetic index is the natural logarithm (ln) of the root mean square differences between adjacent normal beat to beat (R-R) intervals (Ln rMSSD) as it seems to be more reliable than other parasympathetic indices such as the high frequency (HF) component of R-R interval variability. [164] Furthermore, Ln rMSSD has been used in studies with elite athletes. [243] Ln rMSSD has been shown to display a coefficient of variation (CV) of 5 – 7 % in elite endurance athletes and ~10 % in

recreational runners. [166] Less day-to-day Ln rMSSD fluctuations (represented by CV) have been associated with more favorable adaptations to training among athletes. [160]

After strenuous training sessions, HR can be under sympathetic dominance, whilst recovery can be highlighted by the return of the parasympathetic modulation to baseline. [323] For example, 48 hours after a strenuous exercise like a marathon, there have been reported signs of sympathetic activation in runners. [324] Additionally, immediately after the completion of a half marathon, elevated sympathetic cardiac drive was seen. [325] In a study by Hynynen et al., [294] a decrease in vagal-related markers (rMSSD and HF) the night after completing a marathon were observed compared to values obtained following only moderate exercise.

Consequently, in elite sport, the assessment of autonomic activity using different indices of HRV have been used for different purposes such as: a) to determine the cardiac regulation during different phases of training, including tapering in disciplines such as rowing [162] or triathlon; [293] b) to determine the timing to prescribe intense training sessions when HRV reaches a reference value, considered as the optimal freshness condition for the athlete; [141,142] and c) to understand the physiological disturbance caused by training load near sea-level [326] or under stressful environmental conditions such as at altitude. [311] Another variable which could impair autonomic control of HR is transmeridian air travel with substantial time zone differences, [327] which is a common circumstance in elite sports competition.

To date, there are no studies with elite wheelchair marathon athletes that have reported the pre-post-race autonomic activity. The current case study reports the daily HRV responses to an eastward transmeridian flight (Alicante, Spain to Oita, Japan, 8-hour time difference) and a marathon in an elite wheelchair athlete affected by the Charcot-Marie-Tooth disease (CMT).

Methods

Subject

The athlete who participated in this case study was a 35- year-old male professional wheelchair marathon athlete with CMT, class T52 by World Para Athletics. CMT is the most common hereditary peripheral neuropathy, affecting up to 30 per 100000 people worldwide. [206] CMT totally affects distal muscle function and partially affects proximal function. [206] The athlete was a highly accomplished competitor with a silver medal at the 2000 and 2004 Paralympic Games and 107 victories in road events, including the Boston, Chicago, London and Oita Marathons. His main descriptive features are: height = 1.76 m; body mass = 52 kg; power output at second lactate threshold = 61 W; heart rate at second lactate threshold = 166 beats · min^{-1}; training 8000 km per year; former world record-holder in his sport class in 800 m (116 s), 1500 m (216 s), 5000m (757 s), half marathon (3028 s) and fourth best-ever time in marathon (6125 s). This study was set up at an international road race (Oita International Wheelchair Marathon, Oita, Japan) in which his finishing time (6481 s) was ranked as the world best season time in the sport class T52 at the International Paralympic Committee Athletes ranking.

The participant provided written informed consent to be a research subject in this case study. All the procedures were approved by the Ethics Research Committee of the Miguel Hernández University (Elche, Spain).

Study design

Ten days before the race date (RD) an incremental test was performed on a specific wheelchair ergometer in which steady conditions were maintained (temperature 22-24 °C, humidity 73-75 %). The protocol described by Polo-

Rubio [217] included a 20 min warm-up period at constant power (20 W). The athlete started the incremental test at a brake power of 6 W, maintaining a stroke frequency between 90 and 100 strokes · min^{-1}, increasing the power by 3 W every 60 s until the athlete was not able to maintain that frequency. Power output was considered as the ergometer braking power during the last completed step of the test. The same HR monitor used in the marathon was used to register HR and a telemetry system (K4 b2, COSMED, Rome, Italy) was used during wheelchair propulsion to measure O_2 uptake and CO_2 production. The recommendations by Chicharro et al. [237] were followed for calculating the second ventilatory threshold (Vt2). The Vt2 was estimated when the athlete generated 61 W, the O_2 uptake was 51 ml · kg^{-1} · min^{-1} and the HR reached 166 beats · min^{-1}. In the last step, in which the athlete was able to maintain the projected stroke frequency, he generated a power of 67 W, and the VO_{2max} was 57 ml · kg^{-1} · min^{-1}, reaching 176 beats · min^{-1} at that intensity. Six days before ($RD_{-6,-5,-4,-3,-2,-1}$) the marathon day in Oita, Japan and two days after racing ($RD_{+1,+2}$), the day-to- day HRV upon awakening in the supine position and after bladder-emptying were measured. For HR recordings (Polar RSCX 800, Kempele, Finland), a metronome was used to ensure breathing was consistent (15 Breaths · min^{-1}). Detection and correction were applied to the R-R intervals to remove ectopic beats. [238] The Ln rMSSD was calculated using Kubios HRV 2.0 (Kuopio, Finland, 2008), analysing the last five minutes of each 10-min recording. [238] Only natural logarithm (Ln) values were used in the statistical analyses. The rMSSD were chosen as the main vagal HRV index.

For HR recording during exercise, the same monitor for morning HRV recordings was used. During the race, a GPS (Polar, G3 GPS sensor, Polar Electro Oy, Kempele, Finland) was used to measure speed and record split

times every 5000 m (0-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30- 35, 35-40) and the last 2195 m (40-42.2).

The athlete flew from Spain (GMT + 1) to Japan (GMT +9) immediately after morning HRV assessment on RD_{-3}, so no measurements were taken on RD_{-2} because the trip took almost thirty hours. (Figure 14).

The Japanese Association of Athletics Federations officially approved the marathon course in Oita city. Race conditions were dry with temperatures ranging between 24-26 °C, 77 % humidity and 1.39 $m \cdot s^{-1}$ winds. The course was almost flat, with start and finish lines at the same altitude, so the only up and down course oscillations were short bridges.

During the 6481 s that the race lasted for the athlete of this study, some nutritional recommendations were followed to avoid a performance decline and to optimize HR response. [239] A solution of 1250 ml with 70 g of carbohydrate (CHO) was consumed at a comfortable rate, adhering to the minimum rate of 100 ml every 10 min to avoid dehydration. [77] Therefore, the athlete´s drinking-rate was 116 ml every 10 min. Because the participant did not report gastrointestinal issues in his career with the use of CHO gels, [321] at the 31 km mark he consumed a 42 g CHO gel (Glucose + Fructose). [229] The total CHO rate was 1.03 $g \cdot min^{-1}$ which is within the recommended range. [228]

Statistical analysis

Ln rMSSD was averaged across all days pre-travel (RD_{-6} – RD_{-2}) to serve as baseline (BL). The smallest worthwhile change (SWC) in Ln rMSSD was determined as ± 0.5 of the BL standard deviation. [242,243] Thus, all Ln rMSSD values obtained post-travel were compared to BL thresholds.

The distribution of marathon-derived exercise HR was examined using the Kolmogorov-Smirnov normality test. A repeated measures ANOVA was carried out for the HR variable, including the factor, Race Split, into levels 0-

5, 5- 10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40 and 40-42.2. A post hoc least significant difference (LSD) multiple range tests determined differences between factor levels. Statistical significance was set at $p < 0.05$, and all the analyses were conducted using the Statistical Package for Social Sciences (SPSS v. 22, Inc., Chicago, IL, USA).

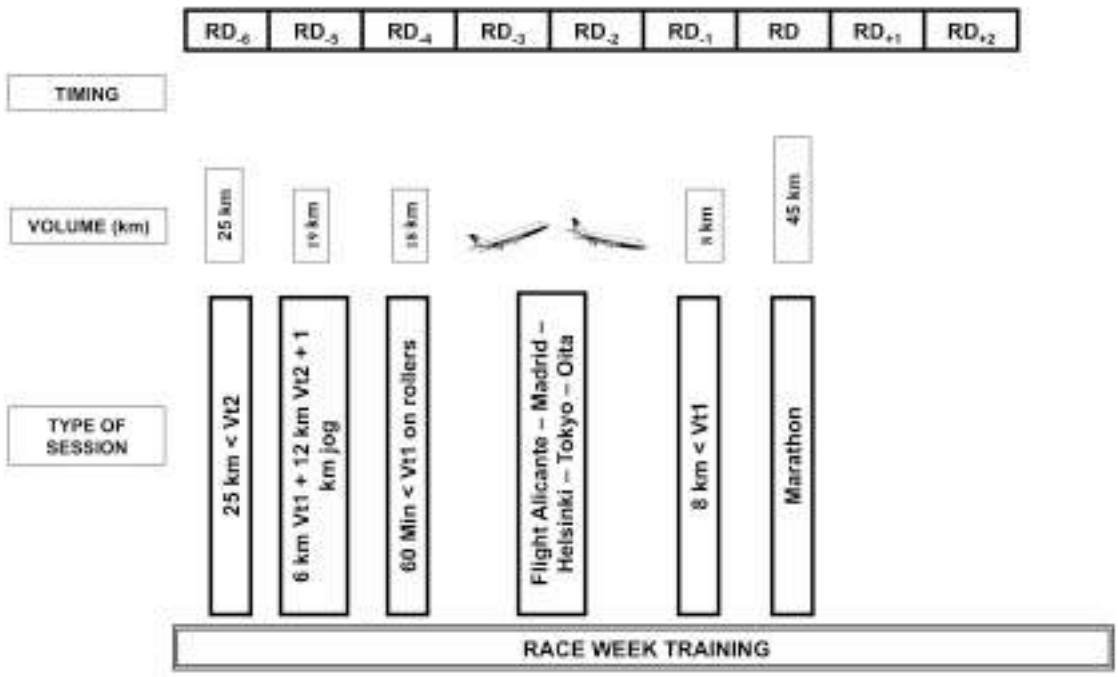

Figure 14. Race week training program.

Results

The average speed of the race was 6.51 m · s^{-1}, while the 5 km segment average of time was 770 s, being both very steady throughout the race. The km 25 to km 30 segment was the slowest (6.37 m · s^{-1}), while the fastest was from km 5 to km 10 (6.84 m · s^{-1}). The average time every 5 km was 770 s and it was very steady (see Table 18).

Differences were found between the first half of the marathon (start line to

21096 m) average HR and the second half (21096 to 42195 m) 163 ± 6 vs. 167 ± 6 beats · min^{-1}, $p < 0.001$. In the first half of the race, the average HR was slightly lower than the mean HR during the full race (165 ± 7 beats · min^{-1}), while the second half was slightly higher.

The athlete covered the first half of the race in 53 min and 22 s (3202 s) and the second one in 54 min and 39 s (3279 s). From the start of the race to the 30 km mark, the athlete raced at an intensity slightly below the HR at Vt2 (166 beats · min^{-1}), while from km 30 to the finish, line he raced at an intensity slightly higher than the HR at Vt2 (see Table 18).

The time set by the athlete was the world leading time in his class in his division in the Oita International Wheelchair Marathon.

Ln rMSSD values can be viewed in Figure 15. Compared to BL, Ln rMSSD negatively exceeded the SWC (-4.6 %) on the first day post-travel (RD_{-1}) and on RD (- 3.8 %). A greater reduction in Ln rMSSD (-23.1 %) was observed one day post-race (RD_{+1}). Ln rMSSD returned to within BL at two-days post-race (RD_{+2}).

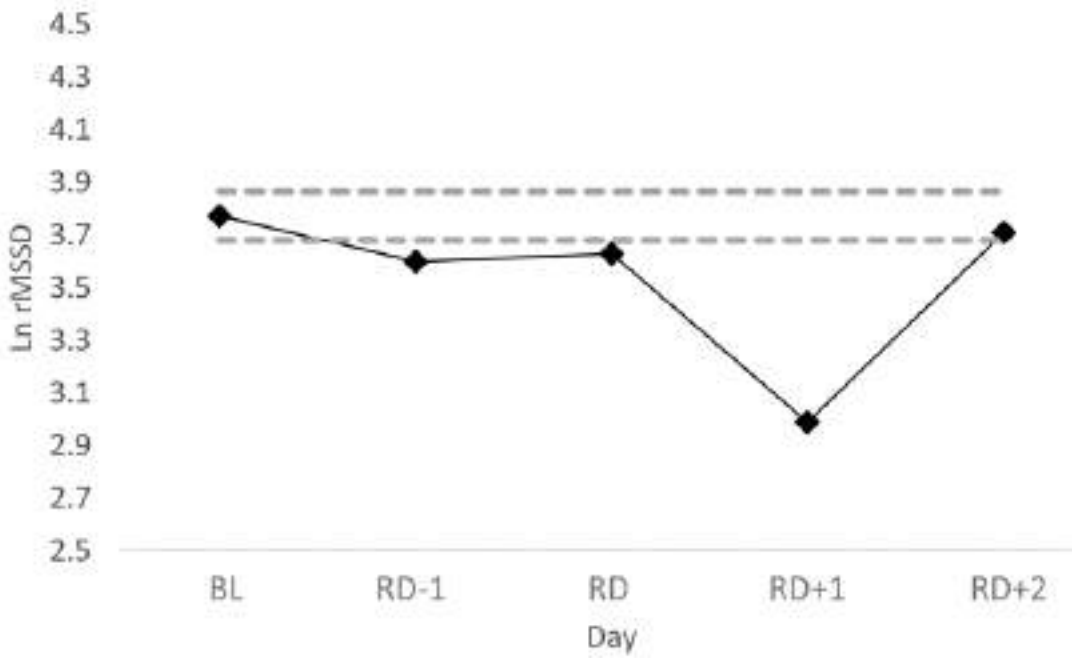

Figure 15. Baseline and daily natural logarithm of the root mean square differences between adjacent normal R-R intervals (Ln rMSSD).

BL = baseline; RD-1 = one day before race day; RD = race day; RD+1 = one day post-race; RD+2 = two days post-race. The area between the dotted lines represents the smallest worthwhile change in Ln rMSSD.

Table 18. Heart rate, speed and time during the race.

Km	0-5	5-10	10-15	15-20	20-25	25-30	30-35	35-40	40-42.2
HR	161±5	164±6*	162±6†	165±6*‡	163±7*	163±5*§	169±7*†‡§ ‖	170±5*†‡§ ‖	170±4*†‡§ ‖
SD_{Vt2}	- 5.9	- 2.3	- 3.8	- 1.4	- 2.6	- 3.1	+ 3.1	+ 4.1	+ 4.4
Speed	6.43	6.84	6.46	6.69	6.46	6.37	6.40	6.64	6.42
SD_{Vel}	- .08	+ .33	- .05	+ .18	- .05	- .14	- .11	+ .13	- .09
Time	779	732	774	747	777	786	782	755	348
SD_{Time}	+ 9	- 38	+ 4	- 23	+ 7	+ 16	+12	- 15	+ 23

HR (beats · min^{-1}), average heart rate in every 5 km segment; SD_{Vt2} (beats · min^{-1}), standard deviation of HR over ventilatory threshold of athlete; speed (m · s^{-1}), average speed in every 5 km segment; SD_{Vel} (m · s^{-1}), standard deviation of the speed of every segment over the mean race speed; Time (s), time of each 5 km segment; SD_{Time} (s), standard deviation of time of each 5 km segment over the mean race time every 5 km.
* Differences from 0-5 (p<.001); † Differences from 5-10 (p<.001); ‡ Differences from 10-15 (p<.001); § Differences from 15-20 (p<.001); ‖ Differences from 20-25 (p<.001); ¶ Differences from 25-30 (p<.001); ** Differences from 30-35 (p<.001); †† Differences from 35-40 (p<.001); ‡‡ Differences from 40-42.2 (p<.001).

Discussion

The aim of this study was to assess Ln rMSSD oscillations before and after travel and a marathon in an elite wheelchair marathon athlete with CMT. The main finding was that Ln rMSSD showed small reductions (-3.8 – -4.6 %) relative to baseline following travel, before the race. Ln rMSSD was further reduced (-23 %) after the marathon for one day and subsequently returned to baseline two days post-race.

Heart rate

The exercise HR was very similar from the starting line to km 30 at which point the athlete started to lead the race and his HR exceeded the Vt2 intensity (166 beats · min^{-1}). Even though temperature was high at that point of the race (24 °C), hydration status was maintained throughout the race and thus thermoregulation likely did not influence cardiovascular responses as it has been recently demonstrated by James et al. [239]

No differences were found regarding the cardiovascular response from top wheelchair athletes who were tested in the same marathon [204] and elite marathon runners. [189] Even though we did not measure the oxygen uptake during the race, the athlete analyzed in this study exhibited higher values (51 ml · kg^{-1} · min^{-1}) at marathon intensity (Vt2 = 166 beats · min^{-1} and power

output of 61 W) in a lab test than the athletes tested by Asayama et al. [204] but lower values than elite runners. [189]

Speed and time

Speed was steady during the whole race with little variation in the mean race speed (6.51 m · s^{-1}) for each segment. The second segment was the fastest with an increment of 0.33 m · s^{-1} compared to the mean race speed and the sixth segment was the slowest where the speed was 0.14 m · s^{-1} under the mean race score. These results are in concordance with Haney and Mercer [289] who concluded that slower marathon finishers had greater variability of pace compared with faster marathon finishers.

Time for every segment was very similar. It should be noted that in the last segment, the surface of the track changed when entering into the stadium compared to the road and could have influenced the rolling resistance. This explanation has been tested before in cycling, [290] decreasing the speed and increasing the time in this part of the race. Furthermore, the athlete stopped pushing the chair during last 20 m which is another factor that also may have affected this segment time.

HRV

Pre-race Ln rMSSD

Baseline Ln rMSSD (3.77 ± 0.19) was similar to that of a Paralympic swimmer with an undisclosed neuromuscular condition [328] and to that of recreational endurance runners. [166] The coefficient of variation of baseline Ln rMSSD (5 %) was consistent with that of elite endurance athletes during tapering. [166]

Ln rMSSD decreased relative to BL upon arrival to Japan. This response has been observed in a case study of an elite male decathlete who experienced

reduced vagally- mediated HRV relative to BL, following eastward travel across 6 time-zones. [291] A previous study involving a team of junior rowers observed reductions in vagally-mediated HRV only after three days of relocation across 5 time-zones. [161] Recent research has demonstrated that individual responses to air travel are related to both fitness and body composition. [292] This may explain why the highly fit and lean athlete in the current case study only experienced small reductions in Ln rMSSD in response to relocation.

Reduced Ln rMSSD in the days preceding competition have been attributed to pre-competitive excitement [213] or to hemodynamic changes such as a reduction in plasma volume due to reduced training volume from tapering. [164] The athlete in the current study only performed one workout (below the first ventilatory threshold) in the 3 days before the race due to travel and relocation. A decrease in Ln rMSSD on RD has also been observed in an elite triathlete across five competitions and was associated with an optimal competition performance status. [293] It is difficult to determine if the reduced Ln rMSSD on RD^{-1} and RD were a result of stress from travel, [161,291] effects of temporary training cessation [164] or precompetitive anxiety. [213] However, since the athlete performed very well in the race, the small decrements in Ln rMSSD before the race were possibly not fatigue-related. [215]

A 23 % reduction in Ln rMSSD relative to baseline was observed on the day after the race, indicating suppressed vagal modulation. Reduced cardiac-parasympathetic activity for ~24 hours in response to prolonged endurance exercise has been previously observed following a 75 km cross-country ski race [139] and a marathon run. [294] Additionally, a six- month study found that recreational marathon athletes showed a substantial decrease in vagally-mediated HRV when an internal load model (i.e. Training Impulse - TRIMP-) reached the highest values. [216] Further, a study with seven middle-distance

runners showed a decrease of 41 % in vagal related markers of HRV during three-weeks of heavy training. [214] Collectively, it appears that the wheelchair athlete in the current case study with CMT showed similar cardiacautonomic responses to prolonged endurance exercise as compared to unrestricted endurance athletes. [140]

Conclusion

Small reductions in Ln rMSSD were observed following relocation, indicating only minor effects from travel. A much larger reduction in Ln rMSSD was observed one day post-race, which returned to baseline by 48 hours. The race time set by the athlete was the world-leading time in his class. The cardiac-autonomic response to marathon competition observed in this elite wheelchair athlete with CMT was similar to previous findings among unrestricted endurance athletes.

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13 REFERENCES

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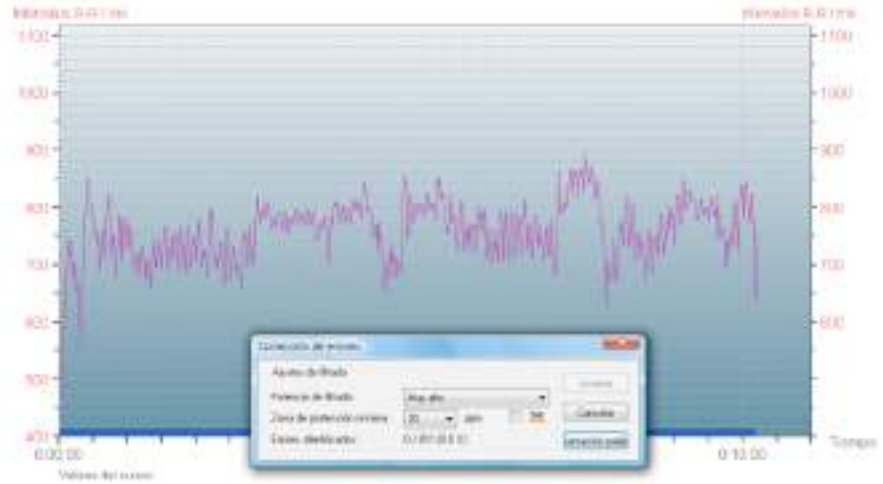
14. APPENDICES

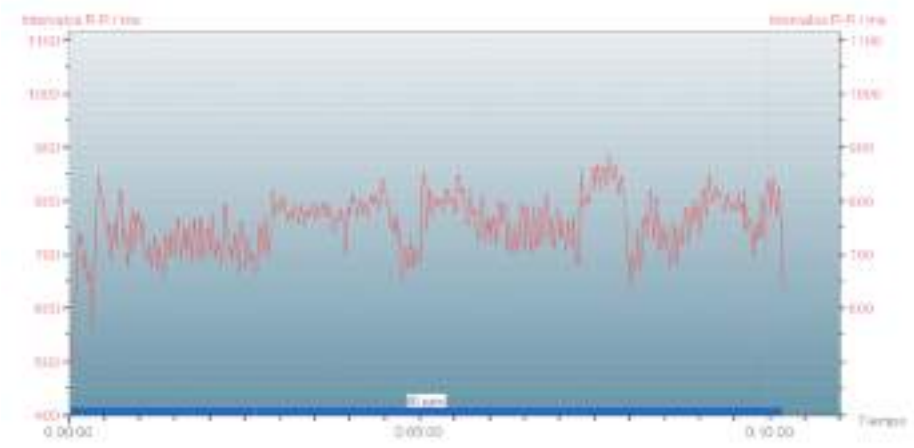
14 APPENDICES

14.1 RR raw data.

RR raw data from January 27Th 2016 (Wednesday W_3 at FP) before filtering, correction, and detrending ectopic beats. Analysis was done using software (Polar Protrainer 5.0, Polar Electro, Kempele, Finland).



RR raw data from January 27Th 2016 (Wednesday W_3 at FP). after filtering, correction, and detrending ectopic beats.

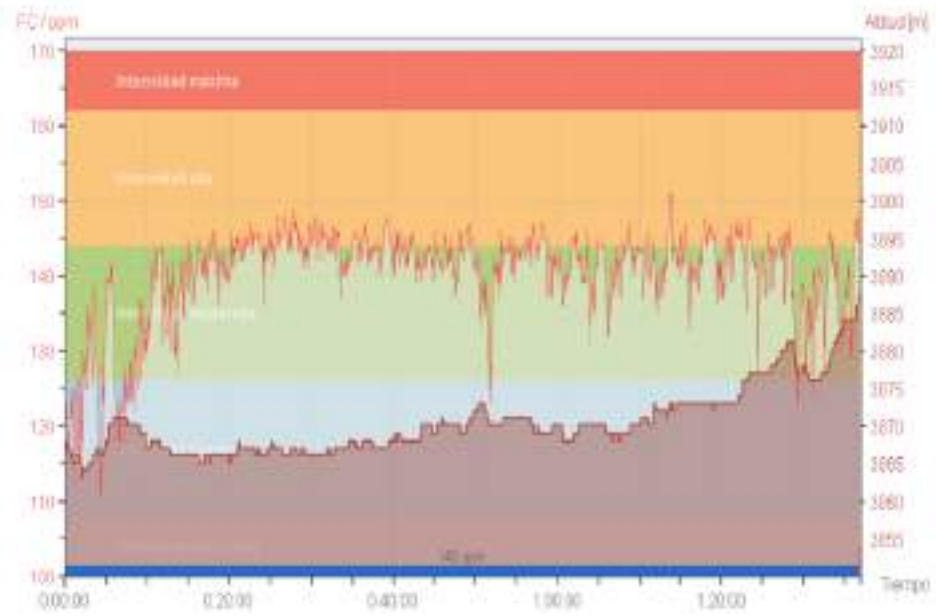


14.2 HRV data.

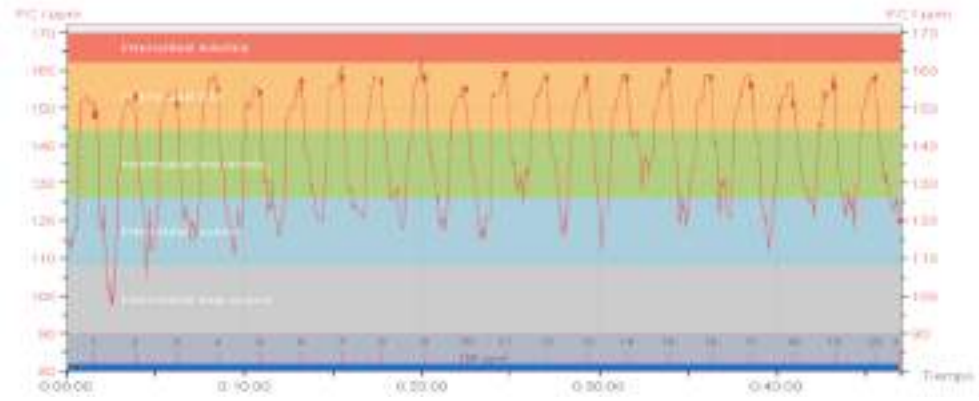
HRV data results from Kubios HRV 2.0. Data displayed from January 27Th 2016 (Wednesday W_3 at FP) from second 5-min segment of a 10-min recording sample.

14.3 HR data.

Heart rate data from B session (30.4 km VT1 in 1h36min40s) performed on January27Th 2016 (Wednesday W_3 at FP).

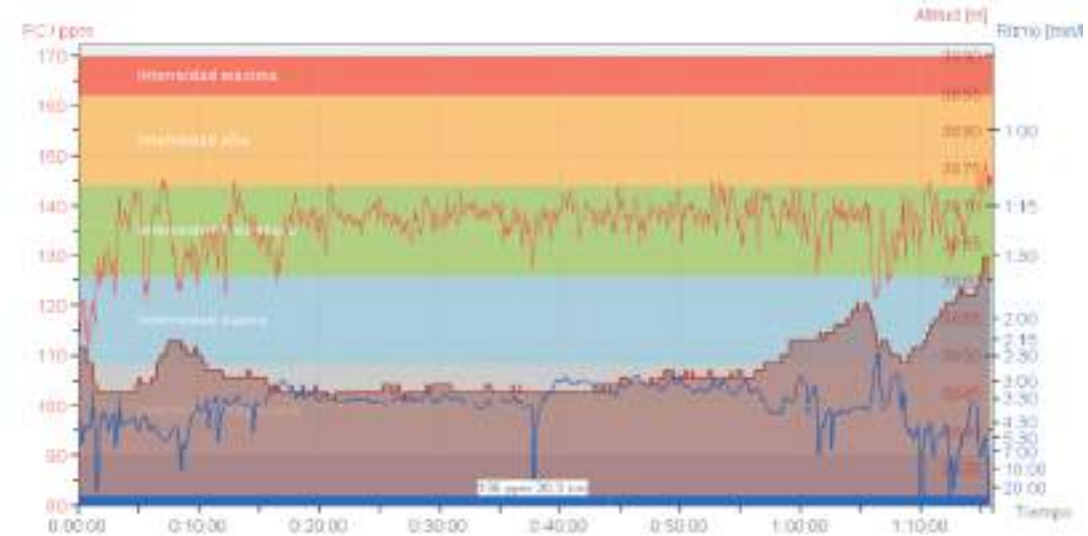


Heart rate data from A session (20 x 400m VT2 with 75 s recovery within repetitions) performed on January26Th 2016 (Tuesday W_3 at FP).



Heart rate data from C session (6 x 2000m VT2 with 120 s recovery within repetitions) performed on January 29Th 2016 (Tuesday W_3 at FP).

Heart rate data from first workout from FP altitude sojourn. 20 km <VT1 session in 75 min performed on January 13Th 2016 (Wednesday W_1 at FP).



14.4 Pictures of the athlete.

The athlete during the marathon monitoring study.

The athlete (front row left corner) during the marathon monitoring study.

The athlete winning during the marathon monitoring study.

The athlete training during IP in a B session on January 20Th 2015.

Scio me nihil scire – Sócrates

"I only know that I know nothing"

"Sólo sé que no sé nada"