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Una prueba de ultra trail por etapas: estudio de biomarcadores de daño muscular, óseo, renal e hiponatremia

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Tesis Doctoral

UNA PRUEBA DE ULTRA TRAIL POR ETAPAS: ESTUDIO DE BIOMARCADORES DE DAÑO MUSCULAR, ÓSEO, RENAL E HIPONATREMIA

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UNIVERSIDAD DE ZARAGOZA
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Tesis Doctoral en modalidad por compendio de publicaciones.

La Tesis Doctoral que se presenta titulada “UNA PRUEBA DE ULTRA TRAIL POR ETAPAS: ESTUDIO DE BIOMARCADORES DE DAÑO MUSCULAR, OSEO, RENAL E HIPONATREMIA” se ajusta a la normativa vigente en la actualidad en la Universidad de Zaragoza, según Extracto del Acuerdo de 25/06/2020 del Consejo de Gobierno de la Universidad de Zaragoza por el que se aprueba el Reglamento sobre Tesis Doctorales (Título IV, Capítulo III), en cuanto a la modalidad denominada como compendio de publicaciones. La Tesis Doctoral cumple con los requisitos solicitados para las publicaciones en cuanto al lugar del doctorando en los artículos, el factor de impacto de la revista, y el tipo de indexación en el Journal Citation Report (JCR) de la Web of Science. Los estudios se han publicado en revistas relacionadas con las ciencias de la actividad física y la salud, centrándose en el perfil temático de alteraciones de biomarcadores tras la realización de una carrera de ultra trail por etapas. A continuación, se relacionan los cuatro artículos publicados que componen la presente Tesis Doctoral:

1. Lecina, M., Castellar-ot, C., Isaac, L., Carrasco, L., & Pradas, F. (2022). Acute Kidney Injury and Hyponatremia in Ultra-Trail Racing : A Systematic Review. Medicna, 58(5), 1–18. **https://doi.org/10.3390/medicina58030340**

2. Lecina, M., López, I., Castellar, C., & Pradas, F. (2021). Extreme Ultra-Trail Race Induces Muscular Damage , Risk for Acute Kidney Injury and Hyponatremia : A Case Report. International Journal of Environmental Research and Public Health, 1–8. **https://doi.org/10.3390/ijerph182111323**

3. Lecina, M., Castellar, C., Pradas, F., & López-Laval, I. (2022). 768-km Multi-Stage Ultra-Trail Case Study-Muscle Damage, Biochemical Alterations and Strength Loss on Lower Limbs. International Journal of Environmental Research and Public Health, 19(2), 1–13. **https://doi.org/10.3390/ijerph19020876**

4. Castellar-Otín, C., Lecina, M., & Pradas, F. (2022). Bone Turnover Alterations after Completing a Multistage Ultra-Trail: A Case Study. Healthcare, 10, 798–808. **https://doi.org/10.3390/healthcare10050798**

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Que la Tesis Doctoral titulada “Una prueba de ultra trail por etapas: estudio de biomarcadores de daño muscular, óseo, renal e hiponatremia”, que presenta D. Miguel Ángel Lecina Monge. Al superior juicio del Tribunal que designe la Universidad de Zaragoza, ha sido realizada bajo mi dirección durante los años 2020-2022, siendo expresión de la capacidad técnica e interpretativa de su autor en condiciones tan aventajadas que le hacen merecedor del título de Doctor, siempre y cuando así lo considere el citado Tribunal.

Fdo. Carlos Castellar Otín

En Huesca, a 8 de junio de 2022

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Fdo. Francisco Pradas de la Fuente

En Huesca, a 8 de junio de 2022

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ABA	Abalakov Test
AKI	Acute Kidney Injury
AKIN	Acute Kidney Injury Network
KDIGO	Kidney Disease: Improving Global Outcomes
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BALP	Bone-Specific alkaline phosphatase
BDW	Body Weight
BMD	Bone Mineral Density
BT	Bone Turnover Process
BTM	Bone Turnover Markers
Ca^{2+}	Serum Turnover Calcium
CK	Creatine Kinase
CMJ	Countermovement Jump Test
CTX	C-Terminal Cross-Linking Telopeptide Type I
DEXA	Dual-Energy X-Ray Absorptiometry
EAH	Exercise-Associated Hyponatremia
EFG	Estimated Glomerular Filtrate
ER	Post-Exertional Rhabdomyolysis
EXP	Years Competing in Ultra-Trail Races
LDH	Lactate Dehydrogenase
$[Na^+]$	Sodium Serum Concentration
MB	Myoglobinuria
OC	Osteocalcin
OP	Osteoporosis
PRE	Baseline Values
POST	Just After Completing the Race
REC2	Two Days After Having Completed the Race
REC9	Nine Days After Having Completed the Race
RIFLE	Risk, Injury, Failure, Lost and End of Stage
SCR	Creatinine Serum
SF	Stress Fractures
SLO	Accumulated Elevation Trained
SJ	Squat Jump Test
UT	Ultra-Trail Races
VOL	Trained Volume
WHO	World Health Organization

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RESUMEN GENERAL

La práctica de Ultra Trail (UT) ha experimentado un boom en los últimos años, tanto en número de participantes como en eventos que se organizan anualmente. Estas pruebas deportivas sobrepasan los límites fisiológicos de los corredores atendiendo principalmente a dos factores: (i) la duración de las distancias de sus recorridos, por definición siempre superiores a la prueba de maratón, (ii) y los entornos naturales en los que tienen lugar, con grandes desniveles tanto positivos como negativos, que dificultan su realización. Múltiples estudios han demostrado los beneficios que la actividad física, practicada de manera regular, suponen para el organismo, aumentando las capacidades funcionales de diferentes órganos y sistemas, a la vez que disminuye la posible aparición de enfermedades no transmisibles y la reducción del riesgo de mortalidad general. Esta evidencia científica, se ha visto plasmada en forma de guías y recomendaciones de los organismos más prestigiosos en el campo de la salud pública como la World Health Organization (WHO), o la United Nations Educational, Scientific and Cultural Organization (UNESCO), prescribiendo a todas las personas la realización de ejercicio físico, independientemente de su edad, sexo o características individuales.

Sin embargo, la dosis eficaz de ejercicio para obtener los beneficios anteriormente mencionados, no pueden verse representados en estas carreras, ya que exceden los límites tolerables de muchos de los participantes, en particular atletas no profesionales sin la adecuada preparación física, técnica o psicológica para poder afrontar adecuadamente estas competiciones, provocando sobre el organismo un efecto opuesto plausible, con alteraciones más o menos marcadas en múltiples órganos y sistemas, que pueden ocasionar cuadros médicos de tipo agudo de diversa gravedad, e incluso, en algunas ocasiones, un daño crónico.

Teniendo en cuenta los potenciales efectos negativos de estas competiciones, así como el considerable aumento de corredores no profesionales que cada año participan en estas pruebas deportivas, resulta necesario estudiar las consecuencias que pueden tener sobre la salud de los corredores la realización de estas carreras extremas, con el fin de reducir la aparición de

condiciones médicas y patologías adversas, asegurando de este modo, su bienestar y su seguridad. En este sentido, el objetivo principal de esta Tesis Doctoral es analizar las principales alteraciones agudas de daño muscular, renal, óseo e hiponatremia que produce una prueba de UT sobre el organismo en corredores no profesionales, así como en el período de recuperación a los dos y nueve días, respectivamente.

Para poder dar respuesta al objetivo principal de esta Tesis Doctoral, en primer lugar, se revisaron los principales efectos negativos que pueden provocar estas carreras mediante una scoping review. Tras el análisis de los resultados obtenidos, se destacaron varias condiciones médicas de carácter nocivo para la salud como: daño renal agudo, daño cardíaco, daño muscular, hiponatremia, daño osteoarticular, además de alteraciones generales en serie roja y blanca. Se evaluó la evidencia científica en cada una de estas condiciones médicas (daño muscular, renal y óseo así como la hiponatremia) para determinar en cuáles de estas alteraciones existía en la actualidad una ausencia o escasez de estudios de relevancia. Tras este proceso, se decidió evaluar el daño renal agudo (AKI), la hiponatremia asociada al ejercicio (EAH), la rabdomiólisis post esfuerzo (ER) y finalmente, el remodelado óseo (BT). El estudio de estas alteraciones se llevó a cabo mediante el análisis de biomarcadores específicos para cada una de estas condiciones médicas, plasmándose los resultados obtenidos en cuatro artículos científicos publicados en revistas indexadas en JCR en los cuartiles Q1 o Q2.

El análisis de AKI y EAH se estudió de forma conjunta en una revisión sistemática que analizó la incidencia de estas dos patologías en función de la distancia de la carrera. Se obtuvieron tasas elevadas de AKI con mayor número de episodios en las carreras más cortas y en las de mayor duración, no observándose un aumento lineal de la incidencia de AKI en función de la distancia únicamente, sino también, en función de la velocidad de la prueba. Los cuatro sujetos incluidos en nuestros estudios de caso sufrieron alteraciones de SCR y EFG en post, pero en ningún momento llegaron a desarrollar AKI, al no cumplir los criterios diagnósticos. En cuanto al EAH, las variaciones en la concentración sanguínea de [Na^+] fueron mínimas y en ningún momento se detectó ningún episodio de EAH.

En cuanto al desarrollo de ER, se observaron aumentos considerables de todos los parámetros que analizan esta situación ((Creatinkinasa (CK), Lactodeshidrogenasa (LDH), aspartato aminotransferasa (AST) y alanina aminotransferasa (ALT)), tanto al finalizar la carrera (post), como en el periodo de recuperación a los dos y nueve días (rec2) y (rec9), respectivamente. Estas alteraciones en los biomarcadores sanguíneos de ER se asociaron a pérdidas funcionales en la capacidad contráctil de los músculos extensores de las piernas, medidas a través de varios test de salto de la batería de Bosco. Los tres saltos analizados en pre, post, rec2 y rec9 fueron: squat jump (SJ), contra movimiento (CMJ) y Abalakov (ABA). Todos ellos mostraron descensos bruscos en post volviendo a valores basales en rec9, siendo CMJ el salto más afectado en post y ABA el que sufrió un menor descenso al comparar pre vs. post. Por último, el BT sufrió un desajuste aumentando los marcadores de remodelado óseo (BTM) relativos a resorción ósea (C-terminal telopeptido del colágeno tipo I (CTX)) y calcio sérico (Ca^{2+}), y una disminuyendo los de formación ósea (Osteocalcina (OC)) y fosfatasa alcalina (BALP), así como de resorción ósea en post, re2 y rec9.

En conclusión, y considerando todos los resultados expuestos en estos cuatro estudios científicos, se puede concluir que las UT provocan una serie de alteraciones en varios órganos y sistemas que pueden ser evaluadas mediante el uso de biomarcadores específicos. El uso de estos biomarcadores por parte de los profesionales sanitarios, así como de los entrenadores y corredores, podrían resultar de gran utilidad como medida de prevención a la hora de afrontar estas carreras, detectando posibles problemas médicos de forma precoz, además de reducir la progresión de estas enfermedades al monitorizar los efectos negativos de estas pruebas en los corredores al finalizar la carrera y en los periodos de recuperación.

ÍNDICE

1. Introducción

En las últimas décadas, las comúnmente conocidas como UT, carreras de ultrafondo o carreras de ultra resistencia, han experimentado a nivel mundial un importante crecimiento tanto en número de competiciones como en participantes (Cejka et al., 2014; Knechtle et al., 2020). Dicho aumento ha sido exponencial y tan solo se ha visto frenado en los dos últimos años por las medidas de contención de la pandemia del COVID-19 (Stöhr et al., 2021), que impidieron la realización de la mayoría de estas competiciones. A nivel mundial, la federación internacional de ultra runners contabilizó más de 100.000 participantes en más de 1.000 pruebas realizadas en el año 2018 (Waśkiewicz et al., 2019). Este aumento se debe a participantes de edades avanzadas y al creciente número de féminas que se han visto atraídas a este tipo de pruebas (Scheer et al., 2019), cuando tradicionalmente los participantes eran solo varones de mediana edad, con amplia experiencia en pruebas de larga distancia como la maratón o media maratón (Knechtle et al., 2020).

Las UT se desarrollan en un entorno no estable (en diferentes espacios naturales por superficies variadas y no pavimentadas), hecho que provoca una mayor dificultad para llevar a cabo su delimitación, categorización y posterior estudio (Scheer et al., 2020). Como consecuencia de esta heterogeneidad existen una gran variedad de pruebas de UT en función de diferentes características como: los desniveles positivos y negativos sobre el nivel del mar, el número de etapas, la meteorología, los avituallamientos y la distancia acumulada, distinguiéndose entre UT media de 42 a 69 km, UT larga de 70 a 99 km y UT extra larga de 100 km o más (Urbaneja et al., 2018). A pesar de esta gran diversidad de pruebas, todas ellas poseen un factor común y es que, por propia definición, han de superar los 42,195 km en su recorrido, motivo por el cual a menudo son denominadas como “ultra maratones” o también pruebas de “ultra resistencia” (Scheer et al., 2020).

Paralelamente al progresivo aumento de estas pruebas también ha surgido un creciente interés científico, visible en el número de publicaciones y artículos científicos aparecidos en los últimos años (Knechtle et al., 2018). Tan solo en la base de datos Web of Science el número de

registros encontrados al buscar la palabra UT asciende a 74, sin tener en cuenta otras denominaciones como ultra endurance o ultramarathon, términos con los que a menudo son referidas indistintamente estas competiciones deportivas. Del total de registros encontrados, 43 de ellos se publicaron en los últimos 3 años. Este hecho demuestra el gran interés científico que despiertan estas pruebas de resistencia extrema. En las UT, al igual que en otras competiciones asociadas con el rendimiento y la competición, se han estudiado los factores que influyen en el éxito deportivo destacando los siguientes: el género (Scheer, 2019), la edad (Knechtle et al., 2020), factores de entrenamiento y preparación (Matos et al., 2020), e incluso la procedencia y nacionalidad de los participantes (Balducci et al., 2017). Sin embargo, el esfuerzo extremo que provocan este tipo de carreras, llevando hasta al límite fisiológico el organismo de los corredores, ha provocado que haya surgido una cierta preocupación por las alteraciones que estos eventos provocan en la salud de los participantes, y sus efectos perjudiciales tanto en el corto plazo como en el largo plazo (Scheer et al., 2021). Los efectos que se han ido documentando en la literatura científica al evaluar el impacto negativo padecido por los atletas tras la finalización de este tipo de carreras, comprenden una gran cantidad de órganos y sistemas (Knechtle et al., 2018), sin embargo, el daño renal (Hodgson et al., 2017; Rojas-Valverde et al., 2020), el daño cardíaco (Gajda et al., 2020), el daño muscular (Park et al., 2018) y los desórdenes en la homeostasis de electrolitos (Scotney & Reid, 2015) han sido los más evidenciados.

Varias hipótesis han sido formuladas con el fin tratar de explicar el origen fisiopatológico de estas alteraciones, sin embargo, todas confluyen en tres factores: la duración, la velocidad de desplazamiento de los atletas y los desniveles asociados a estas carreras, considerándolos como los principales responsables etiológicos de dichas alteraciones fisiológicas. En base a estas evidencias científicas se propone esta Tesis Doctoral por compendio de publicaciones, en la que se plantea estudiar los efectos renales agudos, la EAH producida por la alteración de la concentración sanguínea del ion sodio, la destrucción muscular y sus consecuencias funcionales en los niveles de fuerza, así como el desajuste en el remodelado

óseo en una prueba extrema por etapas. A continuación, se presentan los objetivos e hipótesis de estudio.

1.1 Hipótesis

La hipótesis general de esta Tesis Doctoral es que la participación en una prueba de UT provoca alteraciones agudas musculares, renales, óseas, así como en la concentración del ion sodio en post, en rec2 e incluso en rec9. Sin embargo, estos efectos negativos evaluables a través de biomarcadores, serían transitorios y reversibles, no provocando patologías medicas severas en estos corredores (AKI, BT, EAH, ER).

- HIPOTESIS A

La participación en una UT provocaría aumentos en SCR y descensos en EFG durante post, estas alteraciones en los parámetros SCR y EFG, volverían a su nivel basal sin cumplir los criterios diagnósticos de AKI en rec2 y rec9.

- HIPOTESIS B

La participación en una UT de categoría extrema por etapas provocaría alteraciones en [Na^+] pero estas serían leves y no alcanzarían el criterio diagnóstico de EAH.

- HIPOTESIS C

La participación en una UT de categoría extra y por etapas supondría alteraciones severas en CK, LDH, AST y ALT en post que volverían a niveles pre en rec2 y rec9 sin alcanzar el criterio diagnóstico de ER. Estas alteraciones, sin embargo, sí que se relacionarían con pérdidas de fuerza en los músculos extensores de las piernas de los corredores, pero igualmente se recuperarían en rec2 y rec9.

- HIPOTESIS D

La participación en una UT de categoría extra y con varias etapas provocaría un

desajuste en BT, aumentando la resorción ósea analizable en CTX y $Ca2+$ a la vez que disminuiría los marcadores de formación ósea (OC y BALP) en post que se normalizaría en rec2 y rec9.

1.2 Objetivos

El objetivo general de esta Tesis Doctoral es evaluar los potenciales efectos negativos que pueden sufrir los corredores en su organismo tras la realización de una carrera por etapas de UT, analizando los biomarcadores en suero y orina de cada una de las patologías más comunes en estas pruebas (daño renal, alteraciones de electrolitos, deshidratación, daño e inflamación muscular y remodelación ósea), relacionando las características técnicas específicas de estas carreras y la posible influencia en su aparición y desarrollo.

- OBJETIVO A

Determinar en una UT de categoría extra con 11 etapas la función renal aguda, mediante el análisis del biomarcador en sangre de creatinina sérica (SCR) y las correspondientes ecuaciones de filtrado glomerular (EFG), así como mediante la diuresis, al finalizar la prueba (post) y a los dos y nueve días en el periodo de recuperación (rec2 y rec9), respectivamente.

- OBJETIVO B

Evaluar tras finalizar una prueba de UT por etapas, la presencia de hiponatremia post ejercicio (EAH) y su gravedad medida mediante la concentración de ion sodio [Na^+], así como la dinámica de recuperación en rec2 y rec9.

- OBJETIVO C

Determinar la aparición de (ER) en post, rec2 y rec9, mediante el análisis de biomarcadores selectivos de daño muscular en sangre ((Creatinquinasa (CK), Lactodeshidrogenasa (LDH), aspartato aminotransferasa (AST) y alanina aminotransferasa (ALT)), y la disminución de fuerza de los músculos extensores de los miembros inferiores.

- OBJETIVO D

Valorar el BT mediante el análisis de biomarcadores de remodelado óseo (BTM) tanto del proceso de formación ósea (Osteocalcina (OC) y fosfatasa alcalina (BALP), como de resorción ósea (C-terminal telopeptido del colágeno tipo I (CTX) y calcio sérico (Ca^{2+})), en post, rec2 y rec9.

A continuación, se muestra en la tabla 1 un resumen de los diferentes Objetivos e hipótesis específicos de los cuatro artículos publicados.

Tabla 1. Relación de los diferentes artículos y objetivos de estudio.

Artículo	Objetivos e hipótesis	Tipo de estudio	Marcadores	Patología analizada	Tiempo de análisis
1	A, B	Revisión sistemática	Creatinina sérica. Filtrado Glomerular	AKI, EAH	Pre, post
2	A, B, C	Estudio de caso (n = 1)	Creatinina sérica, Filtrado Glomerular, Concentración sérica del ion sodio Creatinquinasa Lactodeshidrogenasa Serie roja y blanca	AKI, EAH	Pre, post, rec2, rec9
3	C	Estudio de caso (n = 4)	Creatinquinasa Lactodeshidrogenasa aspartato transaminasa alanina aminotransferasa	ER	Pre, post, rec2, rec9
4	D	Estudio de caso (n = 4)	Osteocalcina, Enlaces cruzados del colágeno óseo Tipo I Calcio sérico Fosfatasa alcalina.	BT	Pre, post, rec2, rec9

AKI, Daño agudo renal; EAH, hiponatremia asociada al ejercicio; ER, rabdomiólisis post esfuerzo; BT, BT.

1.3 Metodología y diseño de los estudios de caso

La selección de las metodologías empleadas en esta Tesis Doctoral de modalidad por compendio, se basó en los criterios de consecución de los objetivos planteados como objeto de estudio. Para el objetivo general, se planteó la realización de una revision sistemática de acuerdo a los criterios de The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA), que evaluase la incidencia de AKI y EAH de acuerdo a la duración, velocidad de carrera y los desniveles, tanto positivos como negativos incluidos en las diferentes UT. Para el estudio de los tres objetivos específicos se seleccionó la metodología de estudio de caso. La selección de este tipo de estudio es debida a la extrema dificultad, así como los límites implicados en este tipo de prueba, en donde se aconseja realizar una aproximación mediante un estudio exploratorio inicial. La bibliografía muestra estudios comparativos, descriptivos e incluso de tipo intervención con mayor muestra poblacional, pero siempre con menores desniveles y duraciones inferiores (etapas y duración total) de las carreras incluidas. El estudio de las variables de la distancia y del desnivel como factor etiológico de las alteraciones planteadas en las hipótesis de esta Tesis Doctoral requiere un tipo de prueba extrema y, por tanto, no sería lo más adecuado incluir una gran muestra en el estudio, debido a su extrema dificultad especialmente asociada a los riesgos para la salud de estos corredores. Cabe destacar que la carrera incluida en los estudios de caso recogidos en esta Tesis Doctoral, GR11 en 11, no es una prueba deportiva con miles de participantes, sino que fue un reto diseñado específicamente para el análisis de estas hipótesis de estudio. Si bien, la fortaleza y el grado de evidencia podría considerarse como menor, la extrema dificultad y singularidad de la prueba incluida ofrecería datos muy reveladores sobre hasta qué punto existe una relación lineal entre la dificultad de la prueba (distancia y desnivel), y el grado de estas alteraciones. Del mismo modo, aunque es un número bajo de sujetos, la muestra sí que sería representativa de este reto, ya que incluyó el total de los participantes en esta prueba. El amplio número de condiciones médicas evaluadas a través de diferentes biomarcadores selectivos incluidos en las hipótesis de estudio, posibilitó la obtención de una amplia visión en conjunto del organismo del corredor, incluyendo las alteraciones más comunes descritas por la evidencia científica en este tipo de pruebas.

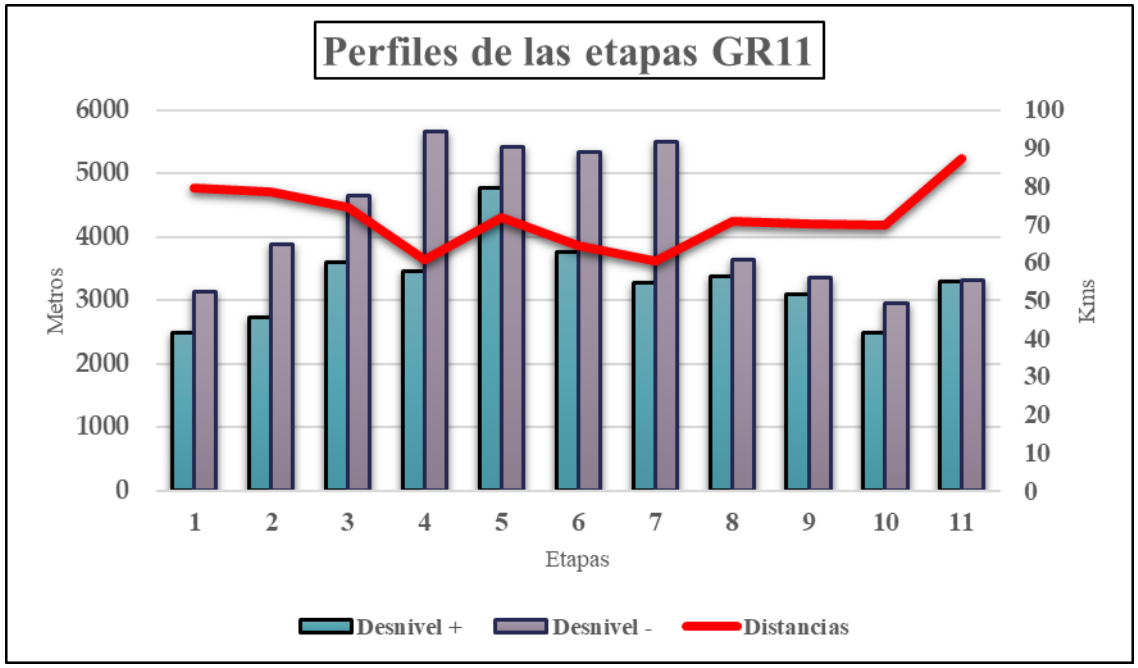
Cabe destacar que, a pesar de que el limitado número de sujetos incluidos impide la realización de una estadística paramétrica, esta muestra sí que posibilita el estudio mediante estadística descriptiva e incluso algunas pruebas inferenciales no paramétricas, analizando las posibles asociaciones entre los parámetros analíticos y múltiples variables del entrenamiento, así como la repercusión funcional de dichos parámetros en la fuerza del corredor. Estos datos aportarían el uso de posibles marcadores para prevenir la aparición de las patologías estudiadas, así como para mejorar el rendimiento de los corredores mediante la inclusión de componentes de carga de entrenamiento específico. A la luz de los datos obtenidos en esta Tesis Doctoral, se podrían diseñar futuros estudios con otros diseños que incluyesen una mayor muestra y aportasen mayor grado de evidencia.

1.4 Características de la carrera

El estudio consistió en una serie exploratoria de estudios de caso en los que participaron 4 sujetos que completaron el recorrido "GR11 Challenge Trail" en 11 etapas consecutivas. La información de la prueba puede ser consultada en el sitio web http://gr11en11.org/. GR11 en 11 es una UT de varias etapas, que une las costas mediterránea y atlántica a lo largo de los Pirineos, cubriendo 786 km en un total de 11 etapas.

La prueba se realizó siguiendo el recorrido GR11 con un total de 786 kilómetros y con un desnivel positivo de 47.500 metros. El GR11 poseyó una dificultad añadida por los valores de temperatura y humedad registrados (13,08±7,86ºC y 70,87±9,25% humedad relativa), que dificultaron su realización. En los 11 días de carrera consecutivos los corredores recorrieron 71,49±8,2 km por etapa/día con un desnivel medio de 3.209±1.013,78 m positivos y 3.248±619 m negativos. El protocolo de hidratación fue *ad libitum*. A continuación, en la figura 1 se muestran los perfiles de las etapas de la prueba de UT.

Figura 1. Perfiles de las etapas de la prueba GR11 en 11.



1.5 Características de la muestra

Un total de cuatro deportistas varones, sin patologías y con experiencia previa en UT participaron de manera voluntaria en esta investigación. Los sujetos fueron informados sobre los objetivos del estudio antes de su inicio. El protocolo fue revisado y aprobado por el Comité Ético de Investigación Clinica de Aragón (CEICA) con el número de referencia del protocolo 18/2015 a fecha de 11 noviembre de 2015 siguiendo las pautas establecidas por la declaración Ética de Helsinki y actualizadas en el World Medical Assembly en Fortaleza de 2013 para estudios que incluyan seres humanos. Los criterios establecidos para participar en esta investigación fueron los siguientes: (a) varón; (b) sujetos sanos; (c) no sufrir ninguna lesión osteo articular en miembros inferiores ni superiores tas completar la carrera; (d) mayores de edad; (e) experiencia en competición de UT. Las principales características de la muestra en referencia a datos morfológicos, variables fisiológicas y de entrenamiento, se presentan en la tabla 2.

Tabla 2. Características de la muestra.

Parámetros	MD ± SD
Edad (años)	38,08±4,11
$VO_{2máx}$ (ml·kg^{-1}·min^{-1})	61,17±8,96
$Fc_{máx}$ (latidos·min^{-1})	187,35±8,54
Velocidad Aeróbica Máxima (km·h^{-1})	16,91±0,83
Talla (cm)	175,72±3,65
Peso (kg)	70,09±9,05
IMC	22,70±2,05
Experiencia (años)	5,63±1,26
Volumen (horas·semana^{-1})	11,61±2,22
Desnivel (metros anuales)	116.615±37.462

2. Revisión de la literatura

2.1 Las carreras de resistencia a pie

Las pruebas de resistencia definen a todos aquellos deportes o actividades físicas que requieren de esfuerzos físicos prolongados a lo largo del tiempo (Bompa et al., 2019). El auge de las carreras a pie en todas sus modalidades en los últimos años ha supuesto un aumento tanto de participantes como de eventos organizados (Ehrensperger et al., 2013; Knechtle et al., 2020). Tradicionalmente, estas carreras coincidían con las distancias que comprendían las pruebas establecidas por la Federación Internacional de Atletismo (antigua IAF), sin embargo, en los últimos años este tipo de pruebas ha trascendido el ámbito del deporte profesional y competitivo para alcanzar a mayores segmentos de población, ampliando las distancias, la elevación sobre el nivel del mar y diversificando los entornos donde se llevan a cabo.

Paralelamente al desarrollo de las pruebas han surgido multitud de nombres para estas carreras, denominándose como ultra maratón, carreras de montaña, ultra endurance, travesías, cross, ascensiones, etc. Todas estas denominaciones se usan indistintamente para referirse a las carreras de larga duración que se realizan fuera de pistas o terrenos pavimentados, y que se realizan en el medio natural en entornos más o menos montañosos.

Sin embargo, y a pesar de estas tres características comunes, estas pruebas poseen particularidades técnicas específicas que obligan a establecer diferentes categorías para facilitar su estudio.

2.2 Las carreras de ultra trail o ultra maratón

De acuerdo con la World Athletics, antigua IAAF (International Association of Athletics Federations), las categorías existentes, incluyendo las pruebas oficiales de carreras de resistencia a pie, se presentan en la tabla 3. (Athletics, 2021).

Tabla 3. Características de los diferentes tipos de carreras de resistencia a pie.

Designación	Distancia	Terreno y desnivel	Organismo Internacional
Sprint	100 m/200 m /400 m	Pista Oficial	World Athletics
RDM, RDL	800 m/1.500 m/5.000 m/10.000 m	Pista oficial	World Athletics
Road Running	Media maratón/ Maratón	Recorridos pavimentados con desniveles mínimos	World Athletics
Race Walks	20 km y 50 km	Recorridos pavimentados sin desniveles.	World Athletics
Cross Country	4 – 10 km	Circuito en campo. Vueltas de 2 km. Desnivel máximo 10 m por vuelta.	Prueba oficial de la WA, Fuera del calendario pista cubierta y aire libre
Mountain Running	Hasta 42,195 km	Sin senderos ni caminos, terrenos técnicos Desniveles < 20%	WMRA. Aceptado IAAF
Ultra Running	Cualquier distancia	Desde 50 km hasta 100 km o por tiempo > 6 horas.	Na
Trail Running	Cualquier distancia.	Sin límite de desnivel. Terreno asfaltado o pavimentado < 20-25%	ITRA aceptado WA
Ultramarathon Running	> 42,195 km	Cualquier tipo.	IAU, ITRA aceptado WA
Fell Running or Hill running	< 10 km sin límite.	Terreno pavimentado > 20% hasta el 40%	FRA sin reconocimiento por WA

Sky running	5 a 99 km en tres categorías	Recorridos en lugares a más de 2.000 m sobre nivel del mar o 6% de desnivel	ISF no reconocida por WA
Obstacle Course Racing	Cualquiera	Cualquier terreno natural y/o urbano	FISO no reconocida por WA
Orienteering	Por tiempo de 12 a 100 minutos.	Sin senderos, cualquier terreno.	IOF no reconocida por WA

WA: World of Athletics. ITRA: International Trail running Association; WMRA: World Mountain Running Association; IAU: International Association of Ultramarathon; FRA: Federation of Fell Running; IOF: International Federation of Orienteering; FISO: Federation International de Sports d´Obstacles.

Mientras las pruebas de cross-country o de media maratón y maratón han sido reconocidas e incluidas en los calendarios oficiales del deporte del atletismo, tanto en campeonatos oficiales como en los propios Juegos Olímpicos, las pruebas que se realizan fuera de estas modalidades continúan en pleno desarrollo y no forman parte del calendario olímpico. Una característica común es que todas ellas consisten en recorrer distancias superiores a la maratón (42,195 km), sobre superficies rocosas montañosas y sin pavimentar. Sin embargo, esta característica no es suficiente para definir al conjunto de pruebas que cumplen este criterio. Dentro de la definición de ultra maratón nos encontramos con pruebas como las carreras por montaña, las UT, trail running, ultra maratón y marchas por montaña. Si bien, estos términos son a menudo empleados de manera indistinta, existen una serie de características y de organismos que regulan su práctica y que las hace diferentes (Scheer et al., 2020). Además de esta complejidad semántica, existen otra serie de características que hacen referencia el nivel de profesionalización de estas carreras, tales como amateur, recreacional, entrenado, expertos, profesional, etc., que dificultan establecer unas categorías formales e impiden estandarizar su estudio.

En este sentido, podemos afirmar que el número de carreras de resistencia en el medio natural ha aumentado en popularidad en los últimos años. Este incremento implica una gran cantidad de eventos diferentes con múltiples términos y nomenclaturas a menudo contradictorios y poco precisos (tabla 3). A pesar de esta heterogeneidad de pruebas, todas ellas

comparten un factor común que es la gran cantidad de esfuerzo que suponen para el organismo del corredor que participa en ellas. A consecuencia de este estrés, han surgido investigaciones centradas en averiguar qué efectos negativos para la salud de los corredores producen estas pruebas sobre los diferentes órganos y sistemas de los corredores, y cómo poder prevenir estos efectos aumentando la seguridad en este tipo de competiciones deportivas extremas.

2.3 Características recomendadas para definir una carrera de ultra trail

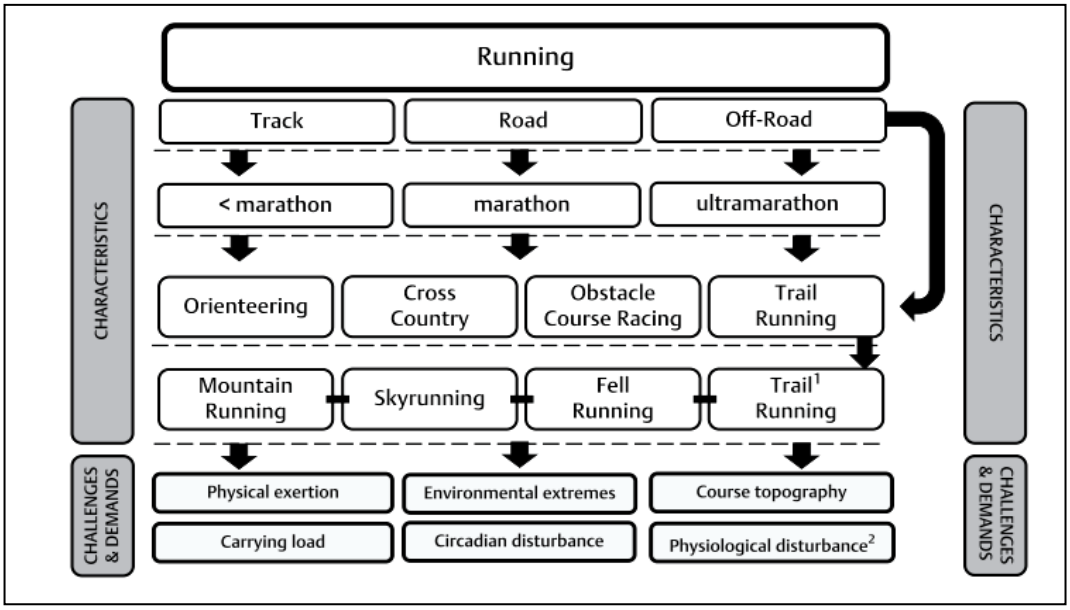
A continuación, y tras haber revisado la gran variedad existente de carreras de UT, se detallan las características que deberían incluir todas estas pruebas para su estudio y categorización (Scheer et al., 2020):

1. **Distancia.** Se precisará la distancia preferiblemente en el sistema métrico (km).
2. **Superficie.** Tipos de pavimentos y superficies, así como el porcentaje de cada uno de ellos respecto a la distancia total de la carrera (por ejemplo, hierba 15% o senderos rocosos 20%, etc.).
3. **Desnivel sobre el nivel del mar.** Se deben precisar los desniveles positivos y negativos totales sobre el nivel del mar, preferiblemente en metros. Si la prueba consistiese en la repetición de vueltas, estas deberían poseer el mismo desnivel tanto positivo como negativo.
4. **Continua o por etapas.** La prueba se realiza sin ningún tipo de interrupción (continua), o consiste en varias etapas con descansos intercalados, en este caso se deberían especificar el número de etapas, así como la duración de cada una de ellas en kilómetros.
5. **El tipo de avituallamientos.** Estas pruebas, por su larga duración, requieren avituallamiento de bebidas que aseguren la hidratación de los corredores. Existen tres tipos: (i) si el corredor se encarga totalmente de todo este proceso se denomina auto abastecidas, (ii) si es realizado por la organización totalmente abastecidas y, (iii) en último lugar en caso de que se realice por ambas, se denomina semi auto abastecidas.

Además de indicarse la hidratación en cada carrera, se deberían especificar las cantidades distribuidas, así como la composición química de cada una de las bebidas.

6. **Las condiciones ambientales.** La temperatura mínima y máxima medida en grados Celsius o Fahrenheit, así como la humedad relativa medida en porcentaje relativo.
7. **Nombre de la prueba y calendario.** Se debería especificar la denominación oficial de la prueba, así como la fecha cuando se realiza. El evento debería facilitar el enlace a internet en donde esté disponible toda esta información.
8. **Terminología y descripción del tipo de carrera.** El término off-road debería incluir el tipo de superficie y el porcentaje de la carrera. El término "ultra trail" no debería ser utilizado sin haber especificado las características descritas en los puntos anteriores. El término ultra maratón solo hace referencia a la distancia, con lo cual no sería de igual modo suficiente para definir completamente la carrera.
9. **Entidad organizadora y nivel de la prueba.** Se debería citar la entidad que organiza la carrera y qué organismo oficial soporta el evento. Del mismo modo, se debería describir el nivel de profesionalismo de la prueba (amateur, semi profesional, campeonato, etc.).

Figura 2. Cuadro resumen de las diferentes pruebas de carreras de a pie en función de la superficie de la prueba, la distancia y las características técnicas (Scheer et al., 2020).



2.4 Efectos que provocan las carreras de ultra trail sobre el organismo

La práctica de actividad física de manera regular está recogida en multitud de guías y recomendaciones de organismos públicos vinculados con la salud pública (World Health Organization., 2018; Tremblay et al., 2011). Si el ejercicio cumple una serie de características en cuanto a volumen e intensidad del esfuerzo, se obtienen una serie de adaptaciones orgánicas y funcionales que mejoran la salud, ayudando a prevenir el desarrollo de enfermedades no transmisibles en la población en general (Thalau et al., 2007). Oja et al. (2015), evaluaron en una revisión sistemática la reducción del riesgo de desarrollar enfermedades cardiovasculares en varios deportes. Solamente las carreras de resistencia y el fútbol demostraron una reducción significativa de dicho riesgo. El éxito de una UT no solo se basa en estas recomendaciones científicas, si no que existen otras razones que han colaborado para convertir estas pruebas en un verdadero evento deportivo. La facilidad de la técnica deportiva, la accesibilidad, el contacto con el medio natural y el aspecto motivacional que implican estas carreras ha atraído a miles de personas de cualquier nivel, edad y sexo (Waśkiewicz et al., 2019). Sin embargo, los beneficios de la práctica deportiva son inversamente proporcionales a la intensidad y duración de los esfuerzos. En este caso, las UT suponen un verdadero reto para el organismo del corredor, sufriendo múltiples alteraciones y en ocasiones originando graves enfermedades (Scheer & Murray, 2011). Estas alteraciones fisiológicas a menudo cursan de manera breve y remiten tras un periodo adecuado de recuperación, sin embargo, estos esfuerzos no prolongados pueden llegar a provocar efectos negativos no solo agudos sino también crónicos (Scheer et al., 2021). En función de la gravedad de estas alteraciones fisiológicas, los corredores pueden sufrir enfermedades y condiciones orgánicas que requieran atención médica justo al finalizar la prueba e incluso varios días después (Dawadi et al., 2020; Runacres et al., 2021; Scheer & Murray, 2011). A continuación, se describen los efectos y condiciones médicas más comunes que se desarrollan al participar en una UT y que fueron los analizados en nuestros estudios de caso.

2.4.1 Daño renal agudo y biomarcadores asociados

Según diferentes estudios, los efectos más descritos que padecen los corredores que participan en UT son los de tipo renal, tanto por su incidencia como por la gravedad del cuadro clínico provocado (Irving et al., 1991; Noakes, 1990). La incidencia de AKI, hallada en estudios en pruebas de UT, es muy heterogénea variando desde el 15% hasta el 49% (Hodgson et al., 2017; Rojas-Valverde et al., 2020). El AKI ha sido definido de diferentes formas en función de la clasificación empleada para su diagnóstico (Lopes & Jorge, 2013), motivo por el cual existe una gran variedad en la incidencia de esta enfermedad. De acuerdo al sistema de definición KDIGO (Kidney Disease: Improving Global Outcomes), AKI se define como un descenso abrupto en el EFG con el consecuente aumento en la concentración de productos nitrogenados en la sangre, con o sin oliguria asociada (Seijas et al., 2014). Existen tres clasificaciones principales para su diagnóstico: KDIGO, AKIN y RIFLE. A pesar de basarse todas las clasificaciones en la variación del incremento del marcador SCR, la disminución del EFG o la disminución de la producción de orina, los estadios de gravedad de AKI diagnosticados no son uniformes (Salgado et al., 2014). AKI incluye la posibilidad de la aparición de una lesión estructural como una deficiencia o pérdida de la función renal (Makris & Spanou, 2016), que podría asociarse con un importante incremento en la morbi-mortalidad tanto a corto como a largo plazo (Hodgson et al., 2017; Rojas-Valverde et al., 2020).

Estas clasificaciones, provenientes del uso clínico y médico, han sido utilizadas en el campo de las UT, obteniendo tasas de incidencia de AKI muy diversas y en ocasiones infra diagnosticando a corredores con cuadros realmente graves (Cuthill et al., 2009). Sin embargo, la mayoría de los cuadros de AKI reflejados en este tipo de pruebas son leves, asintomáticos y se resuelven de manera natural, sin requerir tratamiento ni hospitalización (Lipman et al., 2016), a pesar de haberse comprobado que en ocasiones puede mantenerse durante varios días (Chlíbková et al., 2015; Irving et al., 1986; Knechtle & Rosemann, 2015). Estas alteraciones en los marcadores de daño renal continúan, por tanto, sin ser precisos, ni fiables en la detección de AKI en las UT (Little et al., 2019), motivo por el cual se han utilizado nuevos marcadores que

ayudan a detectar de manera más precoz el daño renal. Teniendo en consideración lo expuesto hasta el momento, resulta fundamental determinar qué características de las pruebas (duración, desnivel y presencia o no de varias etapas), pueden suponer un factor predisponente a la aparición de AKI. En este sentido, se decidió llevar a cabo una revisión sistemática en donde se tratara de evaluar la incidencia de AKI en función de las categorías de las pruebas analizadas (Objetivo A) (Lecina et al., 2022).

2.4.2 La hiponatremia post ejercicio y la sobrehidratación

Varios estudios han evaluado las alteraciones presentes en el balance de electrolitos durante y después de finalizar una UT (Kłapcińska et al., 2013; Sanchis-Gomar et al., 2016). El hierro (Fe^{2+}), el sodio [Na^{+}], el potasio [K^{+}] y el calcio [Ca^{2+}], sufren variaciones significativas en un alto índice de corredores tras completar este tipo de competiciones de resistencia extrema (Waśkiewicz et al., 2012). Las alteraciones que más comúnmente se han encontrado han sido las ocurridas en la concentración sérica del ion [Na^{+}] (Lipman et al., 2021). Esta condición médica ha sido descrita como hiponatremia asociada al ejercicio (EAH) y se define como una concentración sérica de [Na^{+}] por debajo de 135 mmol/L durante o a las 24 horas tras finalizar la actividad física (Spasovski et al., 2017). La severidad de esta condición médica depende del grado de disminución de [Na^{+}], pudiendo alcanzar hasta tres grados diferentes: media (130–135 mmol/L), moderada (125–129 mmol/L) y severa (< 125 mmol/L) (Hew-Butler et al., 2015).

La EAH puede presentar una sintomatología muy confusa y con síntomas poco específicos (Spasovski et al., 2017), incluyendo náuseas, mareos, incremento de la presión intracraneal, pérdida de consciencia e incluso la muerte (Shephard, 2011), por lo que a menudo es infradiagnosticada a pesar de tener un criterio diagnóstico relativamente sencillo y fiable. En muchas ocasiones es la responsable de desfallecimientos y colapsos de muchos corredores, que erróneamente son atribuidos a golpes de calor o lipotimias, ya que la sintomatología es similar (Siegel et al., 2009).

La elevada incidencia hallada de EAH en este tipo de carreras, es debida a que las UT se desarrollan en entornos naturales en condiciones ambientales de temperatura y humedad extremas, con grandes variaciones durante el día y la noche, lo que implica una correcta hidratación para reponer las pérdidas de líquido que sufren estos corredores (Sawka et al., 2007).

Existen diferentes estrategias de hidratación utilizadas en las UT, pero básicamente se dividen en dos grandes tipos: planificada y *ad libitum* o *ad thirst* (Scotney & Reid, 2015). Estos métodos se basan en dejar que el corredor ingiera tanto líquido como desee hasta saciar su sed (*ad libitum o ad thirst*), o hidratarse en función de un cálculo previo establecido en el que las sensaciones del corredor no son tenidas en cuenta a la hora de ingerir líquidos durante el desarrollo de la prueba.

La incidencia de EAH en estas carreras es altamente variable con porcentajes que oscilan desde el 0% (Knechtle et al., 2011), hasta otros que han alcanzado valores del 46,66% (Cairns & Hew-Butler, 2016). Las últimas investigaciones apuntan a la excesiva duración de estas pruebas como factor etiológico de la aparición de EAH (Martínez-Navarro et al., 2020). Otro factor decisivo es el ritmo de carrera, asociando un menor ritmo con mayores aumentos de peso (BDW) y por tanto con mayores tasas de EAH (Cejka et al., 2012). De acuerdo a esta característica, dos han sido los mecanismos fisiopatológicos propuestos como las principales causas de la aparición de EAH: la excesiva hidratación y el consecuente aumento BDW (Brge et al., 2011), además de otras alteraciones hormonales principalmente de la hormonas vasopresina y aldosterona. Sin embargo, el papel exacto que estas hormonas juegan en la patogénesis de EAH no ha podido ser completamente probado obteniéndose resultados contradictorios (Bongers et al., 2018; Knechtle et al., 2012). Por tanto, teniendo en consideración las investigaciones referidas anteriormente, se estableció de interés y relevancia estudiar el análisis de la aparición de EAH mediante la realización de una revisión sistemática y un estudio de caso (objetivo B) a través del análisis de $[Na^+]$ en post, rec2 y rec9.

2.4.3 La rabdomiólisis post esfuerzo

La ER es definida como un daño muscular debido a un esfuerzo intenso que provoca la liberación de proteínas en el torrente sanguíneo a raíz de la rotura de las miofibrillas musculares (Lippi et al., 2019). En el ámbito hospitalario la ER muestra la denominada triada sintomatológica: mialgia, debilidad y mioglobinuria (MB) (Bäcker et al., 2020). En el ámbito deportivo a esta triada sintomatológica se uniría una pérdida de la función contráctil, y supondría por tanto una disminución de los niveles de fuerza muscular de los corredores, sobre todo en los músculos extensores de las extremidades inferiores, disminuyendo la eficacia de la carrera y por tanto el rendimiento (Knechtle & Nikolaidis, 2018). Numerosas investigaciones han relacionado la duración e intensidad del esfuerzo como principales mecanismos fisiopatológicos de la aparición de ER en las UT (Rojas-Valverde et al., 2019; Skenderi et al., 2006).

El diagnóstico de ER se realiza mediante el análisis de ciertos biomarcadores de daño y destrucción muscular, de acuerdo a la evolución de cada sujeto al comparar los valores previos al inicio de la carrera y al final (Rojas-Valverde et al., 2019). Los marcadores más utilizados son CK, LDH (Martínez-Navarro et al., 2020), AST y ALT (Bäcker et al., 2020; Le Goff et al., 2020). CK y LDH son enzimas musculares que poseen una gran sensibilidad para el diagnóstico de ER (Cacelín-Garza & Díaz-Gutiérrez, 2013). El aumento de CK ha sido documentado en la maratón con incrementos de hasta 35 veces el valor basal (Lee et al., 2016). El desnivel de la prueba también ha demostrado una relación directa con el incremento de CK, aumentando el incremento de los valores de CK al finalizar la prueba (Kim et al., 2007).

Tradicionalmente la ER se diagnostica cuando se superan valores cinco veces superiores al valor basal del biomarcador CK. Sin embargo, el uso de CK como único marcador diagnóstico posee un alto margen de error, lo que obliga a añadir pruebas complementarias para su detección precoz (Torres et al., 2015).

La MB, además de LDH y los marcadores de daño hepático (AST y ALT), sirven de refuerzo diagnóstico a la hora de detectar la presencia de ER (Mielgo-Ayuso et al., 2020). Las cinéticas de eliminación de estos marcadores son más lentas que la MB, lo que ayudaría a detectar daños musculares de forma más fiable incluso días después (Knapik & O'Connor, 2016; Sanchis-Gomar et al., 2016). La CK, a diferencia de la MB, no alcanza su pico máximo hasta 3-5 días después del final del episodio que genera la elevación, y puede tardar hasta 6-10 días en normalizarse.

Además de los efectos negativos que provoca la ER, se han descrito efectos en el rendimiento motor de los corredores, afectando a la eficiencia técnica de la carrera (Marcora & Bosio, 2007), así como al tiempo y marca al completar la prueba (Millet et al., 2003). Se ha evaluado la fatiga neuromuscular a través de diferentes pruebas (electroestimulación, electromiografía, potenciales evocados, etc.), pero la más utilizada y fácil de aplicar en el campo del deporte es el test de Bosco (González Lorenzo & Garrido Chamorro, 2004). Esta prueba usa una plataforma de contacto conectada a un ordenador y es capaz de medir el tiempo de vuelo y la altura de los saltos del deportista. El test incluye diferentes saltos y un protocolo que especifica la forma correcta de ejecución de dichos saltos (González Lorenzo & Garrido Chamorro, 2004). Dos estudios revisados han evaluado la pérdida de fuerza mediante test de Bosco al completar una UT (Martínez-Navarro et al., 2020; Pradas et al., 2021) y, en ambos, se evidenciaron pérdidas visibles de altura en los saltos de los corredores tras completar la prueba. Estos descensos fueron mayores en CMJ que en ABA o SJ.

A partir de estas investigaciones se plantea evaluar la ER (objetivo C) en el estudio de caso, analizando los marcadores más usados y fiables para la detección de ER (CK, LDH, AST y ALT), además de contrastar la relación de estos incrementos con la pérdida de fuerza muscular en piernas, evaluándolo a través de la altura alcanzada en diferentes saltos del test de Bosco (SJ, CMJ y ABA).

2.4.4 Los efectos de las carreras de ultra trail en la remodelación ósea

La práctica de actividad física de forma regular ha demostrado ser efectiva para la salud en general, y el sistema músculo esquelético en particular, hasta el punto de ser incluida en las guías oficiales de salud de la WHO y de la Canadian Society for Exercise Physiology (CSEP) (World Health Organization., 2018; Tremblay et al., 2011). El tejido óseo aumenta su densidad al ser sometido a cargas externas, como ocurre en la práctica de actividad física, evitando la aparición de enfermedades óseas como la osteoporosis (Benedetti et al., 2018). Sin embargo, no todo ejercicio provoca estas adaptaciones óseas positivas, y algunos tipos de deportes como la natación, al no soportar el peso en el tejido óseo, no aumenta la densidad mineral ósea (BMD) (Vicente-Rodríguez, 2006). De igual modo, cuando el ejercicio es muy intenso o continuado se pueden producir fracturas de estrés (SF), que son ocasionadas por una sobrecarga debida a cargas submáximas sobre el tejido óseo (Merete et al., 2015). Se han reportado fracturas por estrés en cadera o fémur hasta en un 0,5% de los participantes en pruebas de UT (M. J. Page et al., 2021), y de hasta un 1-3% en los varones atletas frente a un 12% en mujeres atletas (Torstveit & Sundgot-Borgen, 2005). Esta diferencia entre el número de fracturas por estrés entre sexos ha sido justificada por la denominada “triada” que sufren las atletas féminas, que es definida por la American College of Sports Medicine (ACSM), por una eficiencia energética con o sin trastornos de conducta alimentaria, asociados a una disfunción en el ciclo menstrual y un bajo BMD (Torstveit & Sundgot-Borgen, 2005).

Sin embargo, recientes estudios han demostrado que pueden ocurrir fracturas sin reducción de BMD (Garnero et al., 1996). La WHO destaca que el diagnóstico de osteoporosis se basa en valores límites estandarizados (T-score $\leq -2{,}5$ veces menores que una persona joven de mismo sexo), aunque este punto de corte no representa más que una mayor probabilidad de sufrir FE. La reducción de BMD es analizada mediante técnicas de imagen (DEXA) en diferentes puntos anatómicos (fémur, vértebra lumbar, etc.), en función del protocolo seguido (Adami et al., 2008).

Estas técnicas diagnósticas no parecen ser suficientemente sensibles para la predicción de SF, con estudios que han demostrado una relación directa entre las pérdidas de BMD y SF (Tian et al., 2019), y otros que por el contrario no han encontrado ningún tipo de valor predictivo de la reducción de BMD y SF (Yanovich et al., 2013). A partir de estos resultados, se han propuesto diferentes biomarcadores de BT como alternativa diagnóstica y factor predictivo de posibles SF, al ofrecer de una forma sencilla la posibilidad de monitorizar el estado del BT en un momento completo, y ofrecer así una visión completa del estado del tejido óseo más allá de una simple medición (Torres et al., 2003). El BT consiste en un proceso vivo de reconstrucción y destrucción óseo a lo largo de la vida, siendo la remodelación ósea un factor esencial a la hora de valorar la microarquitectura ósea (Gómez-Bruton et al., 2013). Existen diferentes marcadores en suero y orina de los dos procesos de BT: formación y destrucción (Tian et al., 2019). A continuación, se muestran los biomarcadores más comunes en suero y orina (figura 3).

Figura 3. Principales biomarcadores de BT. Extraído de Romero Barco et al. (2012)

Marcadores de formación	Marcadores de resorción
Suero	**Suero**
Fosfatasa alcalina total (FA)	Fosfatasa ácida tartrato-resistente (TRAP)
Fosfatasa alcalina ósea (FAO)	Telopéptido C-terminal del colágeno tipo I (ICTP)
Osteocalcina (OC)	β-CrossLaps (β-CTX)
Propéptido C-terminal del protocolágeno tipo I (PICP)	Telopéptido N-terminal del colágeno tipo I (NTX)
Propéptido N-terminal del protocolágeno tipo I (PINP)	
	Orina
	Excreción urinaria de calcio
	Hidroxiprolina
	Piridinolina (Pir)
	Deoxipiridinolina (Dpir)
	Telopéptido C-terminal del colágeno tipo I (ICTP)
	α-CrossLaps (α-CTX)
	Telopéptido N-terminal del colágeno tipo I (NTX)

El BT, aparte de la formación de tejido óseo, posee una función reguladora de la homeostasis de la glucosa, que es vital para la práctica de los deportes de resistencia al utilizar de forma exhaustiva las vías aeróbicas y anaeróbicas (Rubert & De la Piedra, 2021).

En la literatura científica aparecen múltiples metaanálisis que corroboran la relación existente entre el aumento de fracturas óseas en población en general con los marcadores de remodelación ósea (Johansson et al., 2014; Tian et al., 2019; Yanovich et al., 2013), pero hay un evidente déficit de estudios que aborden la incidencia de este tipo de fracturas en las UT. Tan sólo se han hallado tres estudios que hayan evaluado las alteraciones sufridas en los biomarcadores de BT en UT (Kerschan-Schindl et al., 2009; Mouzopoulos et al., 2007; Park et al., 2018), con distancias menores y diferentes medidas de los biomarcadores. Por este motivo, se plantea en el último estudio de caso (objetivo D), la necesidad de evaluar las consecuencias que pueden tener el participar en una prueba de UT por etapas sobre los principales marcadores de formación ósea, osteo calcina (OC) y fosfatasa alcalina especifica de hueso (BALP), junto con marcadores de destrucción ósea como el calcio sérico (Ca^{2+}) y ctx telopeptídeo c-terminal del colágeno tipo I (CTX), al acabar la carrera y a los dos y nueve días después.

3. Artículos Originales

3.1 Artículo 1

Lecina, M., Castellar-ot, C., Isaac, L., Carrasco, L., & Pradas, F. (2022). Acute Kidney Injury and Hyponatremia in Ultra-Trail Racing : A Systematic Review. Medicna, 58(5), 1–18. **https://doi.org/10.3390/medicina58030340**

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MDPI

Systematic Review

Acute Kidney Injury and Hyponatremia in Ultra-Trail Racing: A Systematic Review

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Abstract: *Background and objectives*: Ultra-trail races can cause episodes of acute kidney injury (AKI) and exercise-associated hyponatremia (EAH) in healthy subjects without previous renal pathology. This systematic review aims to review the incidence of these two syndromes together and separately taking into account the length and elevation of the ultra-trail race examined. *Materials and Methods:* A systematic review was conducted through electronic search in four electronic databases (PubMed, EBSCO, Web of Science and Alcorze). *Results:* A total of 1127 articles published between January 2006 and December 31, 2021 were included, 28 of which met the inclusion criteria. The studies were categorized according to the length and stages of the race in four categories: medium (42 to 69 km), long (70 to 99 km), extra (>100 km) and multi-stage if they included various stages. A total of 2950 runners (666 females and 2284 males) were extracted from 28 publications. The AKI incidence found was 42.04% (468 cases of 1113), and 195 of 2065 were diagnosed with EAH, accounting for 9.11%. The concurrence of both pathologies together reached 11.84% (27 individuals) from a total of 228 runners with AKI and EAH simultaneously analyzed. Sorted by race category, the AKI+EAH cases were distributed as follows: 18 of 27 in the extra (13.63% and n = 132), 4 in the large (5.79% and n = 69) and 5 in the medium category (18.15% and n = 27). *Conclusions:* According to these results, extra and medium races showed a similar incidence of AKI+EAH. These findings underline the importance of the duration and intensity of the race and may make them responsible for the etiology of these medical conditions. Due to their variable incidence, EAH and AKI are often underdiagnosed, leading to poorer prognosis, increased condition seriousness and hindered treatment. The results of this review urge participants, coaches and race organizers to take measures to improve the early diagnosis and urgent treatment of possible EAH and AKI cases.

Keywords: ultra-endurance; acute kidney injury incidence; diagnosis; biomarkers; hyponatremia

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1. Introduction

Ultra-trails, also known as ultra-endurance races, or simply ultra-races, have recently experienced significant worldwide growth both in the number of competitions and in the number of participants [1,2]. In the USA alone, a total of 546 races were held in 2016, and this number increased to 1073 in 2019. The number of participants also increased from 46,739 to 109,810. This number is only an estimate and could account for just 20–30% of the total in this country [3]. Ultra-trail races take place in unstable and unpredictable environments, which complicates their delimitation, categorization and study [4]. They can be classified based on elevation gain and loss, number of stages, weather, aid stations and total distance, with medium ultra-trails ranging from 42 to 69 km, long ultra-trails from 70 to 99 km and extra-long ultra-trails in the range above 100 km [5]. Research around

ultra-trails has sparked great interest in recent years, with a focus mainly on analyzing success with regard to performance-influencing variables [6]. However, due to the increased popularity that these events have been experiencing [7], it is of great interest to understand their consequences on participants' health not only in the short [8,9] but also in the long term [10]. This could even include analyzing the incidence of medical conditions induced by the races and the type of medical care provided during and after the race [11,12].

The impact of such races on the human body has been investigated from a broad perspective. Studies have focused on describing how ultra-trail races affect the physiological response of different systems, and have analyzed musculoskeletal [13], cardiac [14] and bone damage [15] and even other minor aspects such as chafing [16]. A key aspect to be considered in ultra-trail races is that athletes must be able to self-feed and self-hydrate. In this regard, factors such as experience, sex, pace and environmental temperature can influence the risk of nutrition- and hydration-related mistakes. Inadequate feeding and drinking can lead to increased extracellular water and therefore increased body weight [17]. This occurrence—overhydration—is commonly seen in ultra-races [18,19] and it entails a risk of developing exercise-associated hyponatremia (EAH). EAH is defined by a below-normal serum sodium concentration (<135 mmol/L) during or up to 24 h after physical activity [20]. It has been classified in three grades of severity basing on [Na+]: mild (values from 130 to 135 mmol/l), moderate (values from 125 to 129 mmol/L) and severe (values < 125 mmol/l) [21]. It can present very diverse symptomatology [22], including nausea, dizziness, increased intracranial pressure, loss of consciousness and even death [23]. The incidence of EAH varies depending on the characteristics of the race, with rates ranging from 4 to 51% for ultramarathon runners [24]. EAH has been widely researched, and there is strong evidence that overhydration is the primary cause of its onset in ultra-trail runners [25]. However, hyponatremia may be also euvolic (60% of all cases in hospitalized patients are euvolic) or hypovolemic when it is associated with low plasma volume [26]. Apart from EAH, fluid replacement and fluid loss suffered by ultra-runners during the effort may also result in acute renal impairment [27]. In the Comrades marathon, Boulter et al. found four runners who suffered EAH and acute kidney injury (AKI) together, and all of them were overhydrated and had taken calcium supplements during the race (452 mg) [28]. When ultra-runners suffer EAH due to overhydration, their glomerular filtration rate deteriorates and their ability to dilute urine and excrete free water decreases [29]. Equally, dehydration is another major cause of AKI [30]. AKI is defined as an abrupt (within hours) decrease in glomerular filtration, with a consequent increase in the concentration of nitrogenated products in the blood, with or without associated oliguria [31], which encompasses both injury (structural damage) and impairment (loss of function) [32]. Despite the different classifications—RIFLE (Risk, Injury, Failure, Loss, End-stage), AKIN (Acute Kidney Injury Network) or KDIGO (Kidney Disease: Improving Global Outcomes)—AKI is always diagnosed when comparing serum creatinine (SCR) changes (a 1.5 fold increase in serum creatinine or >25% decrease in glomerular filtration (eGFR) rate for RISK stage) or urine output (<0.5 mL/kg/h for 6 h). However, the stage and severity of AKI varies according to the criteria used [33].

AKI includes the possibility structural injury such as a decreased kidney function [32], which could be associated with a significant increase in morbidity and mortality in both the short and the long term [34]. Episodes of combined AKI and EAH have been described in hospitalized patients with frequencies ranging from 5% to 42% and varying degrees of severity [35,36]. Nevertheless, no data have evaluated the presence of AKI+EAH together in the sports field, let alone in ultra-trail races.

Considering the nature of these races and their growing popularity, it seems necessary to gather information on their impact on runners' bodies. Thus, the main objective of this systematic review was to determine AKI and EAH incidence individually and the concurrence of both medical conditions, studying their incidence in the different race categories and finally evaluating their effect and severity in the ultra-trail context.

2. Materials and Methods

2.1. Criteria for Study Search and Selection

A systematic search of the relevant literature was conducted following the PRISMA method [37]. Selected articles were related to AKI and EAH in ultra-trails and published in the last fifteen years, with a deadline of 31 December 2021. A structured search was performed in different sources of high-quality information in the field of health and sport sciences using the following information databases: PubMed, EBSCO, Web of Science and Alcorze. The search terms and keywords included a mix of medical subject headings (MeSH) and some free-text words for ultra-trail sport. The keywords used in the next equation of the search were: "ultra-endurance," OR "ultra-trail," OR "ultramarathon, "OR "off road race," AND "kidney damage," OR "renal impairment," OR "acute kidney injury," OR "renal injury," OR "renal damage biomarkers," AND "hyponatremia," OR "exercise associated hyponatremia," OR "electrolyte disorder," OR "sodium concentration,". In the Appendix A section, all the equations used are included.

2.2. Inclusion Criteria

1. Case studies, observational studies, longitudinal or prospective studies.
2. Adult population of both sexes.
3. Races longer than 42 km with one or more stages, held in a natural setting.
4. Analysis of biomarkers related to the prevalence of AKI.
5. Analysis of biomarkers related to EAH.
6. Articles written in English or Spanish.

2.3. Exclusion Criteria

1. Patients with previous chronic renal pathology.
2. AKI or EAH was not assessed.
3. Combined sports (triathlon or biathlon), short duration (<42 km) or track-like environments without elevation gain (>500 m).

2.4. Data Extraction

Two authors identified papers through database searching (M.L. and F.P.). During the review process, the following information was collected from each of the studies: year of publication; author; participant-related data (number, mean age and sex); type of event (distance, elevation gain and number of stages); environmental conditions (temperature and degree of relative humidity); body weight loss; pre and post serum creatinine (SCR); pre and post estimated glomerular filtration rate (eGFR); AKI criterion used; RIFLE (Risk, Injury, Failure, Loss, End-stage) kidney disease classification, AKIN (Acute Kidney Injury Network), or KDIGO (Kidney Disease: Improving Global Outcomes); degree and severity of AKI, pre and post EAH ($[Na^+]$ values); EAH severity. Once the inclusion criteria and exclusion were applied to each study, the aforementioned data were extracted independently by two authors (M.L. and F.P.) using a spreadsheet (Microsoft Inc, Seattle, WA, USA). Disagreements were resolved by discussion until a consensus was reached.

2.5. Overall Quality of Included Studies

Included papers were selected by two independent (M.L. and L.C.) reviewers according to the established inclusion and exclusion criteria. Mendeley Desktop®v.1.18.9 (Elsevier, Amsterdam, The Netherlands) was used to remove duplicate articles and analyze the titles and abstracts. When necessary, a further full-text analysis was conducted. Decisions were always approved by both reviewers. However, in case of doubt or discrepancy, a third reviewer was consulted to solve the disagreement. The research and analysis process lasted a total of three weeks. The procedure followed in the review is described in detail in the flowchart (Figure 1).

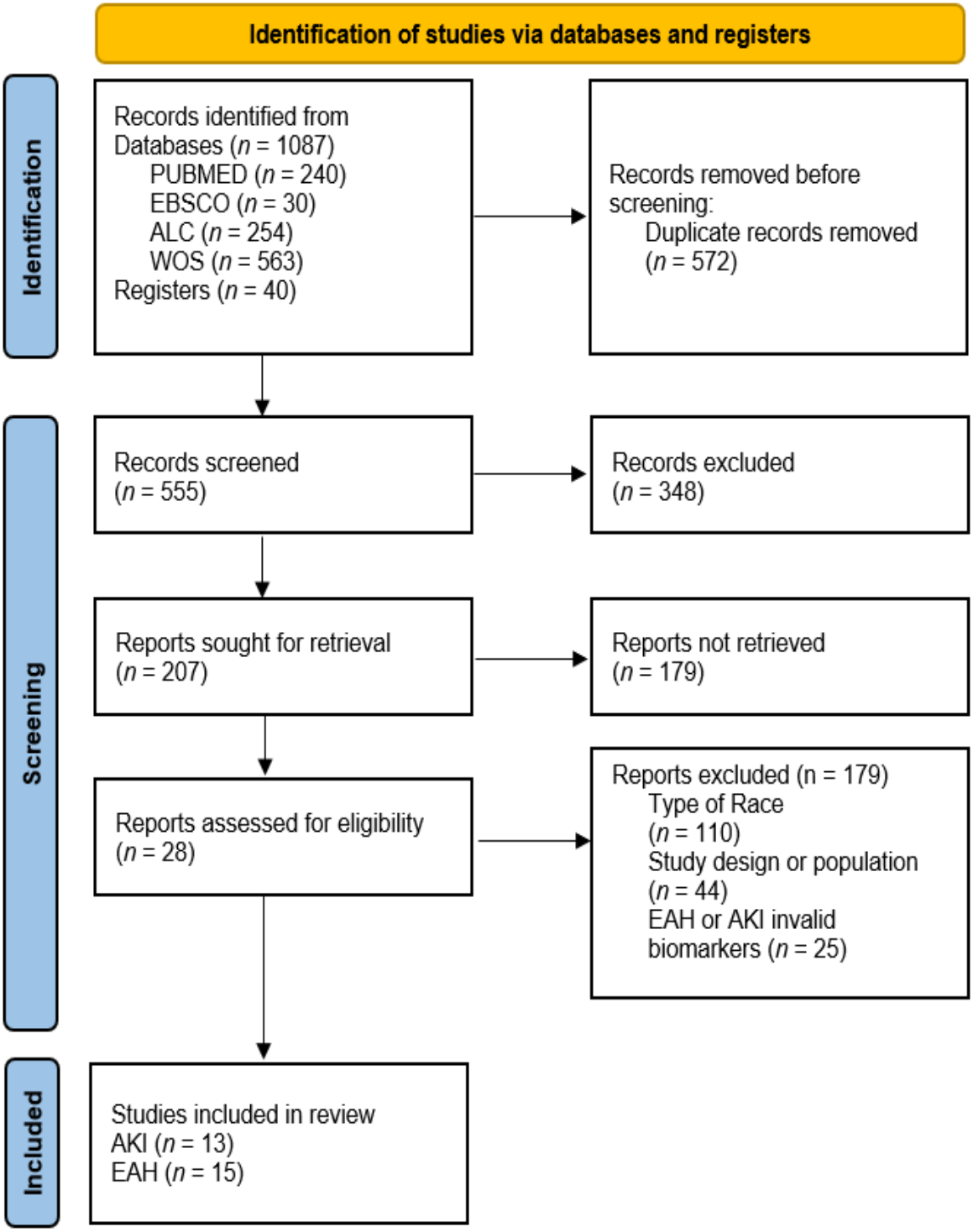

Figure 1. Flow chart describing the selection process of the included studies [38].

2.6. Risk of Bias Assessment and Methodological Quality

Episodes of AKI and EAH have been evaluated in prospective descriptive, cohort and even comparative studies, but no articles were found that met the inclusion criteria with trial or intervention-type designs in ultra-trail races. Descriptive studies are not assessable with methodologically validated tools such as PEDro [39] or Cochrane Collaboration's tool [40]. The tools used for the quality assessment of observational descriptive studies specify that their use should be solely illustrative and should only offer qualitative criteria [41]. Regardless, it was decided that the degree of evidence of the included studies should be

evaluated using two tools. Firstly, the Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies of the National Heart, Lung and Blood institute [42] was used. According to these specific tools, two authors (L.C and I.L) independently assessed the risk of bias in the included studies. This scale has 14 items that can be responded to with yes, no, Na (not applicable), Nr (not reported) and Cd (cannot be determined), and the scale rates studies as good, fair or poor. All 14 items were evaluated for each article by two different authors (L.C and I.L). Inter-reviewer disagreements were resolved by consensus. Arbitration by a third reviewer was used for unresolved disagreements.

The tool does not use the number of "yes" answers to establish each of the categories but rather leaves the decision of defining cutoff values for each category up to the user. Articles were classified as good when 11 or more items were marked "yes", fair for 7 to 10 items and poor when only 6 or fewer items were marked "yes". Secondly, the Rosenbrand et al. [41] tool for level of evidence was used, where studies are evaluated with four letters (A, B, C, D).

3. Results

A total of 1127 potentially relevant studies were identified for the review (Figure 1). Forty more articles were added after their selection from reference lists in other studies, identified through other media. After reviewing titles and abstracts, duplicates were excluded, and the number of articles was thinned out to 219. These selected articles were fully reviewed and evaluated to verify whether they met the inclusion criteria. A total of 28 articles were accepted for final review, 13 of which focused on AKI (Table 1) [28,43–54] and the remaining 15 on EAH (Table 2) [24,27,55–67]. Nine of the studies reported combined results of AKI and EAH [24,27,28,44,45,49,51,52,58]. The distribution of AKI cases in the different categories of ultra-trail races are fully shown in Table 3. EAH incidence sorted by race categories are expressed in (Table 4). The concurrence of AKI+EAH are summarized in (Table 5).

Table 1. Included studies examining AKI as a primary variable in subjects participating in ultra-trail races.

Author; Year; Reference	Population; Number; Sex; Age	Race; Length, Stages, Elevation	Temperature (Range); Humidity (% Relative)	AKI Biomarkers Pre, Post and Rec SCR (mg/dL) eGFR (mL/min/m^2)	EAH Biomarkers [Na+] (mmol/L); Body Weight Loss	AKI Criteria (Stage; *n*; %) EAH Criteria (Stage; *n*; %)
Lipman, 2014 [43]	30; 7 F, 23 M; 39.6 ± 10 yrs	250 km; 6; -	-	↑SCR Pre 1 ± 0.25; Post 1.4 ± 1.3	-	RIFLE; Risk (8, 57%) Injury (1, 7%)
Poussel, 2020 [44]	24; 1 F, 23 M; 36.5 yrs	120 km; 1; 5700+ m	8.6–11.1 °C; 89–99%	↑SCR Pre 8.6 Post 8.9 ↓ eGFR Post (−29.2%)	-	KDIGO; Risk (1, 4.2%) Injury (0, 0%)
Hoppel, 2019 [45]	8; 0 F, 8 M; 41.5 yrs	67 km; 1; 4500+ m	17–37 °C; 54%	↑SCR Pre 0.9 ± 0.05 Post 1.54 ± 0.29 Rec 1.03 ± 0.14 ↓ eGFR Pre 106.23 ± 0.05 Post 58.53 ± 14.33 Rec 93.23 ± 13.06	↓[Na+] Pre 140.13 ± 1.64 Post 137.5 ± 42 Rec 139.5 ± 2.83 BDWL (−0.8 kg)	AKIN; Stage 1 (0, 0%) Stage 2 (2, 25%) EAH; Mil (3, 26.66%) Mod (1, 12.5%)
Jouffroy, 2019 [46]	21; -; 43 ± 7 yrs	80 km; 1; 1500+ m	-	↑SCR Pre 0.89 ± 16.1 Post 0.68.8 ± 21.4 ↓ eGFR Pre 90 ± 14 Post 119 ± 33	↓[Na+] Pre 143 ± 5 Post 141 ± 7 Rec(9 d) 141 ± 5 -	KDIGO; Risk (1, 6%) Injury (0, 0%)

Table 1. *Cont.*

Author; Year; Reference	Population; Number; Sex; Age	Race; Length, Stages, Elevation	Temperature (Range); Humidity (% Relative)	AKI Biomarkers Pre, Post and Rec SCR (mg/dL) eGFR (mL/min/m^2)	EAH Biomarkers [Na+] (mmol/L); Body Weight Loss	AKI Criteria (Stage; *n*; %) EAH Criteria (Stage; *n*; %)
Hoffmann, 2016 [47]	627; 187 F, 440 M; 41.36 yrs	161 km; 1; 5500+, 7000− m	-	↑SCR Group Risk 1.46 (1.39–1.61) Group Injury 1.98 (1.85–2.57)	-	RIFLE; Risk (227, 36.2%) Injury (31, 4.9%)
Pradas, 2021 [53]	20; 0 F, 20 M; 42.45 yrs	108 km; 1; 5800+ m	14.4 ± 4.4 °C; 57 ± 16.1%	↑SCR amateur Pre 90.1 ± 13.1 Post 120.8 ± 23.4 ↑SCR high lvl Pre 85 ± 10 Post 133 ± 11	↓[Na+] amateur Pre 140 ± 2 Post 140 ± 2 ↓[Na+] high lvl Pre 140 ± 1 Post 141 ± 3	RIFLE; Risk (0, 0%) Injury (0, 0%) EAH; Mil (0, 0%)
Khodaee, 2021 [54]	37; 8 F, 29 M; 43 yrs	161 km; 1; 2800+ m	2–22 °C; 57%	↑SCR Pre 0.99 ± 0.15 Post 68.8 ± 21.4	-	RIFLE Risk (18, 49%) Injury (0, 0%)
Le Goff, 2020 [48]	33; 0 F3 M; 45.8 ± 8.7 yrs	64.2 km; 1; 1400+ m	-	↑SCR Pre 9.14 ± 1.05, Post 12.46 ± 2.09 Rec (3 h) 11.03 ± 2.0	-	AKIN; Risk (0, 0%)
Lipman, 2016 [49]	128; 36 F, 92 M; 39.6 ± 9 yrs	250 km; 6; Nr	-	↑SCR Pre 0.72 ± 0.13 Post 1.28 ± 0.34	-	RIFLE; Risk (62, 48.4%) Injury (36, 28.1%)
M. Navarro, 2020 [50]	65 km race 4 F,15 M; 107 km race 17 F, 26 M 41 ± 6 yrs	107 km and 65 km; 1; 5604+, 4356− m	-	↑SCR ↑107 km >↑65 km Post, Post 24 h ↓ eGFR 65 km = 107 km Post, Post 24 h	↓[Na+] 65 km race BDWL (−6, +9) kg	RIFLE; Risk (32, 51.6%) Injury (0, 0%) EAH: Mil (3, 4.83%) Mod (2, 3.22%) Sev (0, 0%)
Belli, 2018 [51]	6; 0 F,6 M; 47 ± 5 yrs	217 kms; 1; 12,200 m;	-	↑SCR Pre 1.00 ± 0.03, Post 1.33 ± 0.08 ↓ eGFR Pre 89 ± 5 Post 65 ± 5	↓[Na+] Nr; BDWL (−4.1 ± 0.7%)	RIFLE Risk (0, 0%)
Cairns, 2016 [52]	15; 3 F,12 M; (40.7–50.6) yrs	100 km y 174 km; -	17.3–21.6 °C -	↓ eGFR EAH mod-31, −43 y-8 ↓ eGFR EAH mil −26,−25 y-7 ↓ eGFR No EAH −11,−20 y-1	-	RIFLE; Risk (0, 0%) EAH; Mil (3, 20%) Mod (7, 46%)
Boulter, 2011 [28]	4; 0 F,4 M; 35 ± 6 yrs	89 km; 1; -	14-(−24 °C); 63%	↑SCR Post (656–1139)	↓[Na+] Post (131–136) BDWL (−6 a +9) kg	RIFLE; Risk (0, 0%) Injury (4, 100%)

F: female; M: male; yrs: years; Km: kilometers; m: meters of elevation; $^+$: slope positive; −: slope negative; ±: positive and negative elevation mixed; AKI: acute kidney injury; EAH: exercise-associated hyponatremia; ↑SCR: elevation in creatinine serum from baseline; ↓eGFR: decrease in estimated glomerular filtrate; BDWL: body weight loss; -: not reported or not available; RIFLE and KDIGO AKIN criteria; EAH stages. Mil: mild; Mod: moderate; Sev: severe; Pre: baseline; Post: after race; Rec: recovery days after the race; ↓[Na+]: decrease in sodium serum concentration from baseline.

The results of the review are shown in Tables 1–5. For better understanding, the results are presented in 5 sections. The first, Section 3.1, describes the types of races and the sample characteristics of the different studies. Section 3.2 describes the cases of AKI and the markers used for its diagnosis. Section 3.3 describes the cases of EAH. Section 3.4 presents the cases of combined EAH and AKI. Section 3.5 assesses the methodological quality of the studies included in the systematic review.

Table 2. Included studies examining EAH as the main target in subjects participating in ultra-trail races.

Author; Year; Reference	Population; Number; Sex; Age	Race; Length, Stages, Elevation	Temperature (Range); Humidity (% Relative)	AKI Biomarkers Pre, Post and Rec SCR (mg/dL) eGFR(mL/min/m^2)	EAH Biomarkers [Na+] (mmol/L); Body Weight	AKI Criteria (Stage; *n*; %) EAH (Stage; *n*; %)
Page, 2007 [55]	123; 23 F, 97 M; -	60 km; 1; 1340+ m	8–14 °C; 60%	-	↓[Na+] (130–134); BDWL +1.32 kg	EAH; Mil (5, 4%)
Scotney, 2015 [27]	44; 8 F, 36 M; 39.5 yrs	82 km; 1; 1000+ m	6–15.6 °C; 79%	↑SCR Post 96.6 ± 20	↓[Na+] (132–147); BDWL 1.75 ± 1.36 kg (2,42–1.85%)	AKI; Risk (0, 0%) EAH Mod (2, 5%)
Chlíbkovál, 2019 [56]	20; 6 F, 14 M; 39.5 yrs	24 horas; 1; 764+ m	(−7.9)–20.6 °C; 88.3%	-	↓[Na+] (137–147); BDWL (−0.9%)	EAH Mil (0, 0%)
Hoffman, 2013 [57]	669; 229 F, 440 M 41.36 yrs	161 km; 1; 5500+, 7000− m	20–38 °C;	-	↓[Na+] (137–147); BDWL = 34.9% BDWL (0->3%) 46.6% BDWL (>3%)18.5%	EAH Mil (88, 13.2%) Mod (13, 1.9%)
Winger, 2013 [58]	207; 40 F, 167 M; 43.0 ± 9.6 yrs	161 km; 1; 5500+, 7000− m	-	-	↓[Na+] Post (131–134);	EAH; Mil (12, 5.8%)
Bracher, 2012 [59]	50; 0 F, 50 M; 47.8 yrs (45.4–50.3)	100 km; 1; 645+ m;	15.6–21.7 °C; 52–69%	↑SCR Pre 77.8 (74.5–81.1) Post 100.4 (93.3–107.5)	↓[Na+] Pre 136.6 (135.4–136.7) Post 100.4 (93.3–107.5)	AKI; Risk (0, 0%) EAH; Mil (0, 0%)
Knechtle, 2011 [60]	120; -; 44.5 ± 7 yrs	350 km; 7; 11,000+/− m	15.6–21 °C; 62%	-	↓[Na+] Pre 138.2 Post 138.49 BDWL (−0.2%)	EAH; Mil (7, 8%)
Hew-Butler, 2008 [61]	82; 24 F, 58 M; 43.7 ± 1.1 yrs	56 km; 1; 1000+ m	-	-	↓[Na+] Pre 139 ± 0.3 Post 138± 10.4 BDWL (−3.8%)	EAH, Mil (0, 0%)
Cuthill, 2009 [24]	4; 3 F,1 M; 41.5 yrs	152.88 km; 1; 4267 m	-	↑SCR Post 350.75 (114–761)	[Na+] Post 127.5 (120–134)	AKI; Injury (2, 50%) Failure (1, 25%) EAH; Mil (4, 100%)
Shepard, 2012 [62]	145; 0 F, 145 M;	100 km; 1; >500+ m	-	-	↓[Na+] Post (130–135)	EAH; Mil (7, 4.8%)
Costa, 2013 [63]	74; 28 F, 46 M; 41.8 yrs	225 km; 5; 2200+ m	32–40 °C; -	-	↓[Na+] - BDWL (−1.2%)	EAH; Mil (8, 42%)
Cejka, 2012 [64]	76; 0 F, 76 M; 47.1 yrs	100 km; 1; 645+ m	12–21 °C; -	-	[Na+] Pre 137.0 (2.7) Post 138.6 (2.6) BDWL (−1.8%)	EAH; Mil (4, 5.3%)
Knechtle, 2012 [65]	219; 219 M, 0 F; 46.2 ± 9.3 yrs	100 km; 1; 1050+ m	15–8 °C; -	-	[Na+] Pre 137.7 ± 2.3 Post 138.6 ± 2.7 BDWL	EAH; Mil (0, 0%)
Schenk, 2021 [66]	69 km race 0 F,11 M; 121 km race 0 F, 7 M 41.2 yrs	121 km and 69 km; 1; 7554+ m, 4260+ m	22–30 °C; -	-	↓[Na+] 69 km Pre 142.9 ± 1.9 Post 143.7 ± 2.1 ↓[Na+] 121 km Pre 142.0 ± 1.7 Post 142.6 ± 4.2 BDWL 69 km −3.1%; 121 km −2.7%	EAH 69 km Mil (0, 0%) Mod (0, 0%) EAH 121 km Mil (0, 0%) Mod (0, 0%)
Khodaee, 2021 [67]	84; 69 M, 15 F 42.1 ± 9 yrs	161 km; 1; 2800+ m	2–22 °C; 57%		↓[Na+] Pre 138.4 ± 1.7 Post 135.8 ± 3	EAH: Mil (17, 20%) Mod (1, 1.19%)

F: female; M: male; yrs: years; km: kilometers; m: meters of elevation; +: slope positive; −: slope negative; +/−: positive and negative elevation mixed; AKI: acute kidney injury; EAH: exercise-associated hyponatremia; ↑SCR: elevation in creatinine serum from baseline; ↓eGFR: decrease in estimated glomerular filtrate; BDWL: body weight loss; -: not reported or not available; RIFLE and KDIGO criteria; AKIN criteria; EAH stages. Thousand: mild; Mod: moderate; Sev: severe; Pre: baseline; Post: after race; Rec: recovery days after the race; ↓[Na+]: decrease in sodium serum concentration from baseline.

Table 3. AKI cases sorted by race category.

Race Category (Number of Studies)	Length (km)	Elevation (m)	Population; Gender; Age	AKI Cases (*n* and %)			
				Risk	Injury	Failure	Total
Medium (3)	65.33 ± 1.52	3630 ± 1950	60 (4 F,56 M) 42.98 ± 2.45	34 56.67%	0 0%	0 0%	34 53.67%
Large (3)	83.66 ± 4.75	1166 ± 288	69 (8 F,61 M) 39.60 ± 4.05	1 1.44%	4 5.80%	0 0%	5 7.24%
Extra (10)	136.22 ± 39.59	4851 ± 3440	826 (219 F, 607 M,) 42.92 ± 3.38	287 34.74%	34 4.11%	1 0.12%	304 38.98%
Multistage (2)	2500 ± 0	2000 ± 0	158 (43 F,115 M) 39.6 ± 2.1	70 44.30%	37 23.41%	0 0%	108 68.35%
Total (18)	127.82 ± 61.56	3560 ± 2951	1113 (274 F, 839 M)	392 35.22%	75 6.73%	1 0.1%	468 42.04%

Table 4. EAH cases sorted by the race category.

Race Category (Number of Studies)	Length (km)	Elevation (m)	Population; Gender, Age	EAH Cases (*n* and %)			
				Mild	Moderate	Severe	Total
Medium (7)	61 ± 8.30	2501 ± 1974	482 (422 F, 60 M) 42.98 ± 2.45	11 2.28%	2 0.41%	0 0%	13 2.69%
Large (3)	83.66 ± 4.75	1166 ± 288	69 (8 F, 61 M) 39.06 ± 4.05	4 5.79%	2 2.90%	0 0%	6 8.69%
Extra (11)	124.63 ± 27.82	3746 ± 2613	1320 (307 F, 1013 M) 42.68 ± 2.95	142 10.75%	19 1.43%	0 0%	161 12.19%
Multistage (2)	287.50 ± 88.38	7067 ± 5562	194 (52 F, 142 M) 43.15 ± 1.90	15 7.73%	0 0%	0 0%	15 7.73%
Total (19)	114.08 ± 67.38	3319 ± 2792	2065 (427 F, 1638 M) 42.1 ± 1.90	172 7.73%	23 1.11%	0 0%	195 9.44%

Table 5. AKI+EAH cases sorted by race category.

Race Category (Number of Studies)	Length (km)	Elevation (m)	Population; Gender; Age	AKI+EAH (*n* and %)
Medium (2)	66 ± 1.41	4750 ± 353	27 (4 F, 23 M) 41.55 ± 0.07	5 18.51%
Large (3)	83.7 ± 4.72	1166 ± 288.67	69 (8 F, 61 M) 39.60 ± 4.05	4 5.79%
Extra (5)	113 ± 21.90	3342 ± 2366	132 (23 F, 109 M) 43.59 ± 2.47	18 13.63%
Total (10)	95 ± 25.2	2971 ± 2094	228 (35 F, 193 M) 41.99 ± 3.12	36 11.84%

3.1. Types of Ultra-Trail Races and Participant Data

A total of 30 different ultra-trail races were analyzed in 28 studies, all of which were observational with the exception of two comparative studies [50,66]. The study by Martinez Navarro et al. compared two ultra-trail races of different distances [50]. On the other hand, Knechtle et al. evaluated a stage race and a 100-plus km race, in addition to other ultra-endurance sports, such as triathlon or cycling, which were not considered in the AKI and EAH data review as they did not meet the inclusion criteria [60]. Races were divided into four major categories according to the length and the existence or not of stages: twenty studies were single-stage and four were multi-stage. The latter category included two 250 km races with six stages [43,49], one 225 km race with five stages [63] and one 350 km race with seven stages [65]. The one-stage category included races ranging from 45 to 217 km. Single-stage races were classified into three different groups according to length [5]: 14 extra-long races (≥100 km) [24,44,47,50–54,57–59,62,64,66,67], 3 long ones (70–99 km) [27,28,45] and 8 medium ones (>42.195–69 km) [44,47,49,54,55,60,64,65]. The cumulative elevation gains and losses ranged between 645 m [59] and 12,000 m [51]. The population sample included 2284 men (77.42%) and 666 women (22.57%), which matches the results from other studies that assess participation by sex in this type of race [68,69]. The average age was 42.01 ± 2.95 years, also similar to the data shown in other similar studies [7,70].

3.2. AKI

The total number of AKI cases found in the present systematic review was 468 and the incidence was 42.04% (n = 1113). AKI cases were classified according to severity using the AKIN, KDIGO or RIFLE criteria [31,33]. AKI cases were distributed as follows: 392 subjects met the risk-stage criteria (35.22%) [16,44–47,49,50,52,54], 75 subjects suffered AKI in the injury stage (6.73%) [24,28,42,46,48,53] and only one subject met the criteria for failure (0.01%), but he did not require renal replacement therapy. This case occurred in a 95-mile ultra-trail [24]. Five of the reviewed studies evaluating AKI found no cases [27,47,52,58,67].

A total of 107 cases (67,72%) were found in the multi-stage races (n = 158) [43,49], of which 70 were classified in the risk stage and 37 in the injury stage. No runner reached failure-stage AKI. In the single-stage races, 361 cases of AKI were found (n = 955): 322 in the extra-long races (n = 826) [24,43,46,49,51,53], five in long races (n = 69) [28,46] and only 34 in the medium-distance races (n = 60) [45,50]. All AKI cases categorized by severity and race category are fully shown in Table 4. Two biomarkers were used for AKI diagnosis: pre and post-race SCR level, used in 14 of the 15 studies that assessed AKI [28,43–51,53], and eGFR through the use of SCR-derived equations [44–46,50–52,54]. One of the highlights was the innovative method based on the analysis of Cystatin C (Cys C) that was used in one of the studies [44]. Previous data reveal a greater use of the SCR biomarker compared to the eGFR one, despite the latter being considered the gold standard in clinical practice. The values of both markers were collected in Tables 1 and 2 (pre-race and post-race), when available. Mean pre-race SCR values ranged between 0.72–1 mg/L. Post-race SCR values multiplied between 1.03 [44] and 1.71 [45] when compared to pre-race values. eGFR decreases ranging from 29% [44] to 47.7% [45] were reported in all studies.

3.3. EAH

A total of 195 cases of EAH were found (n = 2065) with an incidence of 9.44%, of which 172 (8.33%) were diagnosed as mild [24,28,44,46,49,51,54,57,61–64,66] and 23 (1.11%) as moderate [27,49,51,56,66]. No runners were reported as having symptoms of severe EAH. In addition, no cases of hospitalization or medical treatment were found. Seven of the analyzed studies did not report any cases of EAH [45,52,55,58,60,64,65]. Fifteen mild cases were found among the multi-stage races (n = 194) [63,65]. Single-stage extra-long races made up 157 out of the total 195 registered cases [24,49,51,56,57,61,63]. A total of six cases were reported among the long category races (n = 69) [27,28], and 13 cases among the medium category ones (n = 471) [44,49,54]. Races with no EAH cases were found in

all categories. All EAH cases categorized by severity and race category are fully shown in Table 5.

3.4. Concurrence of AKI and EAH

Nine studies evaluated simultaneous AKI and EAH [24,27,28,44,45,49,51,52,58]. In 27 cases, the subjects suffered both pathologies (12.98%). Of the total ultra-trail runners included in this review, EAH and AKI were studied (n = 228). Of the 11 studies whose main objective was AKI, 6 studies also evaluated EAH episodes [28,44,45,49,52,67]. A total of 18 cases of AKI+EAH (13.63%) were found in extra-long races (n = 132) [24,49,51,52], 4 subjects suffered both AKI and EAH (5.79%) in long ones (n = 69) [28] and 5 (18.51%) cases were reported in medium races (n = 27) [45,50].

3.5. Level of Evidence from Included Individual Studies

The descriptive design of the included studies limited the use of standardized and validated tools, as indicated in Section 2.4. However, the level of evidence of each study was assessed using the tool of Rosenbrand et al. [41] and the National Heart and Lung and Blood Institute [42]. Results are shown in Table 6.

Table 6. Methodological quality assessment of included studies using two assessment tools.

Study	Quality Assessment Tool [1] [41]															Level of Evidence [2] [42]
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	Q	
Hoffman, 2013 [57]	yes	yes	no	no	yes	yes	yes	yes	yes	yes	yes	na	na	no	Good	A
Winger, 2013 [58]	yes	yes	yes	nr	no	no	yes	yes	no	no	yes	na	no	no	Good	A
Bracher, 2012 [59]	yes	yes	yes	yes	no	yes	yes	na	yes	no	yes	na	yes	yes	Good	A
Knechtle, 2011 [60]	yes	yes	nr	na	yes	yes	yes	yes	yes	no	yes	nr	nr	yes	Good	A
Hew-Butler, 2008 [61]	yes	yes	nr	nr	yes	yes	yes	yes	yes	no	yes	na	yes	yes	Good	A
Lipman, 2014 [43]	yes	yes	yes	no	no	yes	yes	yes	yes	no	yes	na	yes	na	Fair	B
Poussel, 2020 [44]	yes	yes	yes	yes	yes	yes	yes	no	yes	no	yes	na	no	na	Fair	B
Hoppel, 2019 [45]	yes	yes	no	yes	no	yes	yes	na	yes	yes	yes	na	no	na	Fair	B
Jouffroy, 2019 [46]	yes	yes	yes	yes	no	yes	yes	na	yes	yes	yes	na	yes	yes	Fair	B
Hoffmann, 2015 [47]	yes	yes	no	no	no	no	no	yes	yes	no	yes	na	no	yes	Fair	B
Le Goff, 2015 [48]	yes	no	no	yes	no	yes	yes	na	yes	no	yes	na	nr	yes	Fair	B
Lipman, 2016 [49]	yes	yes	yes	no	no	yes	yes	yes	yes	no	yes	na	yes	na	Fair	B
M. Navarro, 2020 [50]	yes	yes	yes	na	yes	yes	yes	na	yes	yes	yes	na	yes	yes	Fair	B
Khodaee, 2021 [54]	yes	yes	yes	no	no	yes	yes	yes	yes	no	yes	na	yes	na	Fair	B
Khodaee, 2021 [67]	yes	yes	yes	no	no	yes	yes	yes	yes	no	yes	na	yes	na	Fair	B
Pradas, 2021 [53]	yes	yes	yes	yes	yes	yes	yes	no	yes	no	yes	na	no	na	Fair	B
Schenk, 2021 [66]	yes	yes	yes	yes	yes	yes	yes	no	yes	no	yes	na	no	na	Fair	B
Belli, 2018 [51]	yes	yes	no	yes	yes	yes	yes	na	yes	yes	yes	na	yes	yes	Fair	B
Cairns, 2016 [52]	yes	yes	yes	yes	no	no	yes	yes	yes	yes	yes	na	no	yes	Fair	B
Boulter, 2011 [28]	yes	yes	nr	no	na	na	yes	na	yes	na	yes	na	na	yes	Fair	B
Page, 2007 [55]	yes	yes	nr	yes	no	yes	yes	yes	yes	no	yes	na	nr	yes	Fair	B
Scotney, 2015 [27]	yes	yes	yes	yes	yes	no	yes	yes	yes	no	yes	na	yes	yes	Fair	B
Chlíbková1, 2019 [56]	yes	yes	yes	no	yes	yes	yes	no	yes	no	yes	na	no	yes	Fair	B
Cuthill, 2009 [24]	yes	yes	na	yes	na	na	yes	yes	yes	no	yes	na	na	yes	Poor	C
Shepard, 2012 [62]	yes	nr	nr	no	yes	yes	yes	no	nr	no	yes	na	nr	no	Poor	C
Costa, 2013 [63]	yes	yes	nr	no	no	yes	yes	yes	yes	yes	yes	na	yes	yes	Poor	C
Cejka, 2012 [64]	yes	yes	yes	nr	no	yes	yes	yes	yes	no	yes	na	nr	yes	Poor	C
Knechtle, 2012 [65]	yes	yes	nr	yes	no	yes	yes	yes	yes	no	yes	nr	yes	yes	Poor	C

[1] Level of Evidence; [2] Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies; nr, not reported; na, not applicable; cd, cannot be determined, C1–C14, check list criteria; Q, quality.

4. Discussion

The presence of AKI and EAH in ultra-endurance sports has been studied in previous systematic reviews that separately analyzed both markers [30,34]. However, this systematic review jointly addresses AKI and EAH incidence specifically in ultra-trail races and analyzes the concurrence of AKI+EAH. Consequently, three sections are included: in the first one, AKI and its risk factors and diagnostic biomarkers are assessed; the second section assesses EAH; the last section describes the simultaneous development of AKI and EAH in ultra-races.

4.1. AKI

The main incidence of AKI episodes found in this systematic review was 42.04%, and 468 cases (n = 1113). Considering the category of the races, a great heterogeneity exists, with AKI incidence ranging from 7.24% in the long races to 56.66% in medium races. These results are similar to those described in other systematic reviews [30,34]. Despite the high incidence, only one case met the failure stage, which reinforces previous findings suggesting that the alterations suffered by runners are transitory and resolve days after with no medical intervention required [53]. Hernandez et al. analyzed in a case study the possibility of completing a 62 km race after two kidney transplants. No alteration was found in either biomarker or kidney function [71]. It has been hypothesized that longer distances may increase the AKI incidence; however, in our study, medium races showed higher values than longer races. This finding underlines the importance of running speed as an important factor for generating AKI. In moderate exercise, renal flow may fall to 25%; however, in extreme exercise, eGFR may decrease by up to 50% [34].

Nevertheless, it should be highlighted that AKI can be challenging to diagnose in its early stages and that it is often syndromic and asymptomatic [72]. AKI is diagnosed through SCR, eGFR and urine analysis. The most widely used biomarker was SCR, which was used in 14 studies [24,27,28,43–51,59]. The eGFR method was only used in six studies [44–46,50–52,54], despite being considered the gold standard for AKI diagnosis [73]. In the study conducted by Wołyniecet et al. during a 100 km track race, it was found that eGFR did not vary, while SCR increased by more than 24.53% [74]. In this review, some studies that assessed both markers showed opposed diagnoses. For instance, some subjects who met AKI criteria according to SCR levels did not meet the criteria when using the eGFR marker [50]. Furthermore, other subjects did meet the criteria according to eGFR yet did not when assessing SCR [44]. Two studies were found in which both markers reflected the same diagnosis [46,50]. Though it is obvious that athletes will experience a rise in SCR at some point during the race [49,75], and despite the existing evidence, the real impact of high SCR on kidney injury remains unclear. It is also unknown whether it constitutes a real pathological entity or whether it is just a sign of the body's stress response [44].

Multi-stage races show higher declines in eGFR values compared to single-stage races. In multi-stage races, eGFR stabilizes at 24–72 h, with drops in SCR and eGFR observed in rest days between stages. This recovery is linked to rehydration, kidney recovery and the slower running pace [76] that is common in multi-stage races [77]. Conversely, data collected in this review showed higher rates of AKI in multi-stage races [43,49] than in single-stage ones. This could be explained by the extreme heat in which the former took place, given that electrolyte and body water loss can lead to EAH, dehydration and eventually AKI. Both races studying AKI were held in a desert [43,49]; temperatures were thus extremely hot during the day and cold at night. Another factor that has been proposed for explaining the high incidence of AKI in multi-stage races is the running pace at which these races are completed; the recovery between stages allows athletes to maintain a faster race pace than in large races, where they have to complete longer distances in a row [78,79].

Regarding single-stage races, Martínez Navarro et al. [50] investigated the incidence of AKI based on race distance in two ultra-trail races: one 107 km-long one (n = 43) and another 65 km one (n = 19). The results only showed significant SCR level increases in the 107 km race, with no differences in AKI incidence between the two races either immediately post-race (65 km: 44%; 107 km: 56%; p = 0.42), or 24 h later (65 km: 7%; 107 km: 0%; p = 0.1). Belli et al. [51] considered elevation gain to be another interesting variable but found no cases of AKI after analyzing a 217 km-long race with 12,200 m of cumulative elevation gain. However, an observational study by Hoffmann et al. [47] found that 36% of participants developed risk of AKI (n = 227) and 4.9% were injured (n = 36) in a 161 km race with a cumulative elevation gain of 12,500 m. It therefore seems clear that the elevation gain and distance are not the only factors that can influence AKI development.

Hoffman et al. [47] studied AKI recurrence in 627 runners who participated in a 161 km race. The sample was divided according to whether athletes had previously suffered AKI

episodes in other ultra-trails. Three groups were created following the RIFLE criterion for SCR: no risk, risk and injury. Runners who had previously reached a state of failure were not included. Post-race SCR values were higher in the injury group than in the risk group (1.46 vs. 1.98). Additionally, 227 cases of risk-stage AKI and 31 cases of the most severe stage were found. By contrast, Poussel et al. [44] only found one among 24 study subjects who was diagnosed with the RIFLE risk stage in a 120 km-long race. Although temperatures were moderate (8,6–11 °C), the humidity was extreme (89–99%) in this race, which may be why so few AKI episodes occurred. Similarly, Cairns et al. [52] used the RIFLE diagnostic criterion and found no AKI cases in a sample of 15 runners who participated in a 100 km race with lower humidity and milder temperatures (17.3–21 °C) than the previous case.

4.2. EAH

Cases of EAH were found in 195 athletes (9.44%) of a sample of 2065 runners, fewer than in other similar studies [22,80]. The EAH incidence according to race category was more homogeneous, ranging from 2.69% of 482 subjects in medium-distance races to 12.19% in extra ones (n = 1209). Research shows that factors associated with EAH are: exercise duration [36], slow running pace [81], female sex [21], low body weight [82], excessive pre-race hydration, hydration protocols using hypotonic drinks according to athletes' experience [83], NSAID use [84] and extreme heat or cold conditions [63,85]. The results here obtained contrary to AKI incidence seem to support the idea that longer races seem to increase the risk of suffering EAH.

- Types of ultra-trail races and development of EAH

As was indicated above, exercise duration is considered another important factor when it comes to EAH [18,21,85]. Several papers have explored whether race duration increases the frequency and severity of EAH. In medium-distance races, some mild EAH cases were found [44,49,54], but no moderate ones were detected. As for long-distance races, Boulter et al. [28] found four (100%) mild EAH cases, while Jouffroy et al. [46] found none. A higher number of cases were found among extra-long races. In one of the most relevant papers, Hoffman et al. [57] found 88 cases of mild EAH and 13 cases of moderate EAH among the total 669 subjects. Similar results were described by Winger et al. [58], who found 12 cases of EAH in a sample of 207 participants. Three other studies also reported EAH cases, with incidences of seven, eight and four cases, respectively [24,61,63]. By contrast, Bracher et al. [59] found no participants suffering from EAH. As for the multi-stage races, Knechtle et al. [65] identified 7 mild cases among 120 participants and Costa et al. [63] found 8 mild cases among 74 participants.

- Hydration and body weight

Body weight and excessive consumption of beverages with or without [Na^+] supplementation are two relevant factors for developing EAH [17,82,86]. However, there is still no consensus in the ultra-trail hydration guidelines regarding how much liquid should be consumed or how much body weight athletes should loose during the race [21,83]. BDWL seems to be an effective measure for preventing EAH. However, neither the exact percentage of weight that runners could lose during the race nor the body composition measurement methodology are fully agreed on for ultra-races [86]. Cejka et al. [64] suggested an alternative to BDWL for the measurement of overhydration, based on the increase in runners' arm and leg volume. They proved that an increase in liquid consumption had a direct effect on runners' feet volume (r = 0.54, $p < 0.0001$), and was inversely related to the [Na+] decrease ($r = -0.28$, p = 0.0142) and to EAH. However, this method has not been standardized and BDWL continues to be used in research on a regular basis.

Though some guidelines recommend a 2% BDWL [21], several papers in this review reported cases of EAH with equal or even higher BDWL values. This may occur as a result of efficiency in runner's metabolic pathways, achieved through adaptation to training. By obtaining more water during the oxidation of energy substrates, they produce greater amounts of endogenous water [87]. These physiological adaptations should be helpful for

the avoidance of EAH risk. However, most guidelines indicate that dehydration begins at the 3% BDWL mark. In fact, dehydration, extreme sweating and BDWL are all cited as causes of EAH. Thus, excess BDWL could be directly linked to dehydration. In the absence of another more reliable measure, BDWL is still the most widely used in research. Several studies found weight loss of less than 2% in runners who did not suffer from EAH [27,28,49,55,56,59,62,63].

- Environmental race *conditions*

Environmental conditions have also been studied as a risk factor associated with the occurrence of EAH in long mountain races, especially when temperatures are extremely cold or warm [23,88,89]. Chlibkoval et al. [56] found no cases of EAH in an observational study conducted in extreme cold (−7.96 to −20 °C), but with a small elevation gain (764 m). In addition, participants barely lost body weight (−0.8 kg). These results contradict most research suggesting that a weight loss of at least 2% during the race is necessary to avoid EAH [21]. Similarly, Costa et al. [63] found 8 mild EAH cases among 74 runners who participated in a 225 km-long stage race with high temperatures (32–40 °C). The average weight loss was only 1.2%.

4.3. AKI and EAH

The concurrence of AKI+EAH (11.87% of 228 subjects) was significantly higher in medium races and in extra-long ones (18.51% vs. 13.63%) than in the larger races (5.79%). These results fall in line with other previous studies that found AKI+EAH incidence ranging from 5–42% [35,36]; however, these studies took place in a clinical setting. The incidence found is relevant and supports the idea that the etiology of these pathologies could be related to their appearance. Three main factors increase the risk of developing AKI in ultra-trail races: rhabdomyolysis [30,76,90] NSAID use [84] and EAH [36]. The simultaneous onset of EAH and AKI has been less studied than the rhabdomyolysis and AKI combination, which has produced several publications and even a systematic review [30]. This may be because the relationship between AKI and EAH can seem vague. However, the simultaneous appearance of these two conditions is quite common, according to several studies [24,28,44,49,51].

4.3.1. Overhydration and Rhabdomyolysis

The pathophysiological mechanism of EAH is closely linked to excessive hypotonic fluid consumption and weight loss. Other key factors are sex—with women being at higher risk than men—and lack of racing experience [53], which can lead to overhydration and EAH. On the other hand, excessive fluid intake combined with pathophysiological defects such as the inability to suppress antidiuretic hormone activity [19] or defects in the mobilization of intracellular [Na^+] into the blood stream could favor the accumulation of intracellular water. This intracellular fluid increase would generate weakness in the myocyte cell membranes and promote their rupture and rhabdomyolysis, releasing myoglobin and other elements into the bloodstream [88]. To our knowledge, only one study focused on the concurrence of AKI and EAH. Cairns et al. [52], in a comparative study, evaluated the relationship between pre-race EAH status and eGFR values. Only runners with moderate EAH showed larger decreases in eGFR, in the five measurement points during the 174 km race and even 24 h after the end of the race. These data prove that higher [Na^+] concentrations produce larger eGFR reductions. However, none of the athletes met AKI criteria despite the reductions in eGFR. Therefore, it is impossible to state that EAH causes AKI. It does, nonetheless, cause a decline in eGFR.

4.3.2. Race Elevation Gain and Increased AKI and EAH

Another factor that has been highlighted to explain the association between EAH and AKI through rhabdomyolysis in these races is the large cumulative elevation gains [89]. Race mechanics involving a major eccentric component of muscle contraction could result in rhabdomyolysis through the increase in CK and other inflammation markers and muscle

destruction. Laboratory ergometer studies have shown that the eccentric component in descents produces greater motor unit recruitment. Muscle fiber rupture stemming from these situations is assessable through rises in CK [90]. However, CK increases were not seen in all the analyzed ultra-trail races, the values were very variable, and the peaks were found at different moments during the races, even in very flat events. The absence of muscular damage markers and rhabdomyolysis could be due to a slower race pace, which would compensate for the eccentric component of steep descents [89]. An example which illustrates that greater elevation gains/losses do not imply greater increases in SCR or CK is the observational study by Belli et al. [51], carried out in a single-stage 217 km race and with an elevation gain of 12,200 m. Rises in CK were found and AKI, SCR and eGFR markers were altered, but no athletes suffered AKI. In a race with only 645 m of elevation gain, Bracher et al. [63] obtained very similar results in SCR and [Na^+] reduction but did not meet the criteria for AKI or EAH. The explanation why hillier races do not necessarily involve greater CK or SCR rises than flatter ones lies in speed. Despite the greater muscle rupture and motor unit recruitment occurring in the more mountainous races, the slower pace maintained in these events allows for the avoidance of higher concentrations of CK and SCR. Martínez Navarro et al. [50] examined two ultra-trail races with similar elevation gains but different distances. They found contradictory results, with higher SCR levels in the athletes from the 107 km race, and higher EAH in athletes who ran the 65 km race. This might indicate that the pace is more linked to EAH than to AKI. A very recent investigation showed an association between CK values and EAH. One study compared runners with and without exercise-associated hyponatremia in the same 100-mile ultra-marathon. Runners with exercise-associated hyponatremia had less experience over 100 miles and a higher CK after completing the race and even during the recovery period [58].

Limitations

The present review excluded studies that were not in Spanish or English. Therefore, a language bias might exist. Another limitation in this review stems from the very nature of AKI. The different diagnostic criteria (AKIN, RIFLE and KDIGO) and different biomarkers (SCR, eGFR and urinary volume) complicated the homogenization of results and made a meta-analysis impossible. The lack of this type of analysis significantly limits the drawing of conclusions with a greater degree of evidence. As for study design, the largest bias lay in the absence of intervention-type randomized studies with control groups. However, the studies were evaluated with two different tools to ensure individual quality and degree of evidence quality, increasing the external validity of the systematic review. Lastly, the variety of ultra-trail races and the terminological confusion regarding their categorization and definition poses another significant risk of bias. Although the inclusion criteria set a lower limit of 42.195 km and natural environments, there was still a wide range of distances among the races, as shown in Tables 1 and 2.

5. Conclusions

Ultra-trail races might cause AKI and EAH, but the incidence is low in healthy individuals without previous pathology. These syndromes often go unnoticed and naturally re-solve without complications. However, the severity and potential repercussions on runners' health should lead participants to focus on preparation. In addition, raising awareness about the severity of this type of condition among organizers, coaches and medical staff is important. Hydration, NSAID consumption, race pace, runners' morpho functional characteristics, sex and route details are determining factors in the development of both conditions. Certain pathophysiological mechanisms common to AKI and EAH might favor their simultaneous development, with rhabdomyolysis and overhydration playing a key role in this connection. New studies on ultra-trail races in which EAH markers are systematically assessed should be conducted. Additionally, there is a need for tools that allow for precise AKI diagnosis and biomarkers that enable early detection of kidney damage.

Current guidelines and recommendations serve as references for races and participants, but they cannot be considered standards due to the great heterogeneity that exists in the field.

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Appendix A

Search strategies defined for every database:

WOS: ((Ultramarathon) or (ultra-trail)) and ((kidney damage) or (hyponatremia))

Pubmed:((ultra-trail) OR (ultramarathon)) AND ((hyponatremia) OR (acute kidney damage))

ALCORZE:((ultra-trail) OR (ultramarathon)) AND ((hyponatremia) OR (acute kidney damage))

EBSCO:((ultra-trail) OR (ultramarathon)) AND ((hyponatremia) OR (acute kidney damage))

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3.2 Artículo 2

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Case Report

Extreme Ultra-Trail Race Induces Muscular Damage, Risk for Acute Kidney Injury and Hyponatremia: A Case Report

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Abstract: A case study involving a healthy trained male athlete who completed a 786 km multi-stage ultra-trail race. Several markers were analyzed in blood and urine samples: creatinine (SCR) for kidney damage, sodium ([Na^+]) for hyponatremia, creatine kinase (CK) for exertional rhabdomyolysis, as well as other hematological values. Samples were taken before and after the race and during the recovery period (days 2 and 9 after the race). Results showed: SCR = 1.13 mg/dL, [Na^+] =139 mmol/L and CK = 1.099 UI/L. Criteria for the determination of acute kidney damage were not met, and [Na+] concentration was above 135 mEq/L, indicating the absence of hyponatremia. Exertional rhabdomyolysis was suffered by the athlete (baseline CK increased fivefold), though this situation was reverted after 9 days of recovery. Ultra-trail races cause biochemical changes in athletes, which should be known about by healthcare professionals.

Keywords: multi-stage endurance sport; renal impairment; fluid replacement; electrolyte disbalance; muscle damage

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1. Introduction

Endurance events have increased their popularity over the last decade [1]. In the USA, 546 races took place in 2016, and this number increased to 1073 in 2019 [2]. One of the most extreme of these sports is ultra-trail races [3]. These events involve considerable efforts, great elevation slopes, both positive and negative, and often severe weather conditions [4], which can develop serious health issues in some runners [5–7]. Acute kidney injury (AKI), exertional rhabdomyolysis (ER), and exercise-associated hyponatremia (EAH) are among the effects on runners' health when research focuses on this sport [8,9].

These races, particularly extra-long ones set in extreme situational conditions, can cause serious health effects on runners, such as the onset of acute kidney injury (AKI), which may or may not be accompanied by EAH. AKI includes structural damage such as a decreased kidney function [10], which could be associated with a significant increase in morbidity and mortality both in the short term and the long [9]. The incidence of AKI episodes found in ultra-trail races is very heterogeneous, varying between 0% [11–14] and 76% [15]. This wide range of results is due to the different criteria and biomarkers developed to diagnose this illness. The diagnostic of AKI is based on serum creatinine (SCR) or estimated glomerular filtration rate (eGFR) or diuresis [16]. All these markers are compared with baseline, and their increases after completing the race imply acute damage on the kidney of the runners [15,17] and sometimes chronic damage that, ultimately, may even require medical treatment or hospitalization [18].

Contrary to AKI, EAH is easily diagnosed by the concentration of sodium ion [Na^+] in blood [19], with values under 135 mEq/L set as EAH. Bodyweight is widely recognized as one of the most relevant factors linked to EAH and excessive consumption of beverages with or without [Na^+] supplementation is the main cause of EAH [20,21]. However, there

is still no consensus in the ultra-trail hydration guidelines regarding how much liquid should be consumed during the race, nor much bodyweight athletes should lose during the race [21,22]. By decreasing their bodyweight, runners seem to prevent from suffering EAH. However, neither the exact percentage of weight that runners must lose during the race nor the best hydration protocol for ultra-races has been defined [23].

Another key factor in the development of AKI and/or EAH [24] is post-exertional rhabdomyolysis (ER) [25]. ER is defined as the damage in the muscle characterized by myocellular morphological alterations, and as a result, protein leakage can occur [26,27]. The destruction of the myocyte is often related to any sport, especially those which imply long duration and strenuous intensity because of the mechanical damage and metabolism alterations produced by eccentric exercise [28]. In a systematic review, Rojas et al. [25] studied the cases of ER in different endurance sports, including ultra-trail. Identified cases were classified according to activity type as follows: walking = 1 (0.13%), swimming = 1 (0.1%), spinning = 30 (3.8%), combined activities = 90 (11.4%), cycling = 138 (17.4%), and running = 533 (67.2%). From the total of 130 cases reported with ER + AKI, 96.9% of participants were runners. Another study that compared ultra-trail to other endurance sports was [29], where running and cycling were compared. The cases of EAH were very similar: 6.7% cycling vs. 14.4% in ultra-trail.

The high negative elevation that defines most ultra-trail races offers the opportunity to research the relationship between eccentric exercise and ER. Several studies has proved that some biomarkers related to ER, increase their values during the race and even some days after completing the test [30,31]. The main markers related to ER in scientific reports are Lactodeshidrogenasa (LDH) and creatine kinase (CK) among liver alterations and inflammatory biomarkers such as leucocytes and protein c-reactive [32–34]. The diagnosis of ER remains unclear though, with many different values existing to match ER criteria [24].

This case study outlines the main variations in some urine and blood-determined biomarkers relevant to the three aforementioned conditions, as well as hematological and biochemical changes after a 786 km multi-stage ultra-trail race of 11 consecutive stages.

2. Case Report

A 42-year-old male athlete with 5 years of experience in ultra-trail races (172 cm, 77.3 kg, 8.14% body fat and 25.6 BMI) took part in this study. The subject is a non-professional runner but with broad experience in ultra-trail races (5 years of expertise). The runner did not present any medical condition or pathology which could interfere with the practice of ultra-trail running. His diet was balanced, and his sleep patterns were totally normal. The subject's maximum oxygen uptake (VO2max) was 50.71 $mL \cdot kg^{-1} \cdot min^{-1}$ and his average weekly training volume was 11 h of running, with 3500 m of cumulative elevation gain. No previous relevant medical history or chronic conditions that limit physical exercise existed. He completed the multi-stage ultra-trail race, which joins the Mediterranean and Atlantic coasts along the Pyrenees, covering 786 km in a total of 11 stages. The race had a warm temperature, with values ranging from 13.08 to 17.69 °C, and the humidity was (60.16–70.87%). It took the athlete 152 h 41″ at an average speed of 5.2 km/h (equivalent to 51% of VO2max). The average stage/day consisted of 71.49 km (SD ± 8.2) and 6457 m (SD ± 663.73) of elevation gain. In-race hydration was ad libitum. Body weight was measured before and immediately after each stage and was recorded both as absolute values and as percentages of body weight loss. Total weight loss was 1.9 kg − 1.8% of body weight. The plasma volume of the subject was 4.830 liters before the race and after completing the race 4690 liters. The volume plasma shift was calculated using the equation revisited of Dill and Costill revisited [35]. Using the following parameters: hemoglobin (pre and post), hematocrit (pre and post), and the plasma volume (pre and post), we estimated that the volume shift that the runner suffered was −1.88%.

Blood and urine samples were taken one day before the race (pre), at the end of the race (post), and on days 2 (rec2) and 9 (rec9) of the recovery periods. Blood samples were

collected in two 5 mL Vacutainer tubes (Vacutainer, beliver industrial state, plymouth PL6 7BP, United Kingdom) without anticoagulant for serum isolation and in two 5 mL tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Once collected, blood samples were coagulated for 25–30 min at room temperature and then centrifuged at 2500 rpm for 10 min to remove the clots. Serum samples were aliquoted into Eppendorf tubes (Eppendorf AG, Hamburg, Germany), previously washed with diluted nitric acid, and conserved at −80 °C until the biochemical analysis. For the determination of muscle damage markers and hydration status, a 2 mL blood sample was used. The analyzed biomarkers were serum creatinine (SCR) for AKI, creatinine kinase (CK) for ER, and sodium ion concentration ($[Na^+]$) for EAH. Urine sediment analysis was performed with a focus on erythrocytes, leukocytes, and proteinuria. The main hematological and biochemical markers were also analyzed. Results obtained after the GR-11 are displayed as tables. Table 1 shows the blood results and Table 2 the urine results.

Table 1. Blood and urine parameters before (baseline) and after race (post-exercise day 2 and post-exercise day 9).

Parameter Blood	Before-Race Pre (Baseline) Value	Post-Race Post (Post-Exercise) Value (% Difference)	Day 2 (rec2) Value (% Difference)	Day 9 (rec9) Value (% Difference)
Hemoglobin (g/dL)	144	131 (−9.03)	132 (−8.33)	146 (+1.58)
Hematocrit (%)	42%	39% (−7.14)	39% (−7.14)	43% (+2.38)
RBC (10^6/mL)	4.42	4.06 (−8.14)	4.05 (−8.37)	4.49 (+1.58)
MCV (fL)	95.6	95.4 (−0.21)	95.6 (0.0)	96.7 (+1.15)
MCH (pg)	32.6	32.4 (−0.61)	32(−1.84)	32.5 (−0.31)
MCHC (g/dL)	341	340 (−0.29)	340 (−0.29)	336 (−1.47)
RDW (%)	12.8	13.5 (+5.47)	13.7 (+7.03)	13.8 (+7.81)
Platelet count (10^6/mL)	242	315 (+30.17)	309 (+27.69)	391 (+61.57)
Platelet volume (fL)	8.1	7.6 (−6.17)	7.7 (−4.94)	7.3 (−9.88)
Leukocytes (10^3/mL)	3	9.4 (+213.33)	6 (+100.00)	6.8 (+126.00)
Neutrophils (10^3/mL)	3	6.5 (+116.67)	3.6 (+20.00)	3.7 (+23.33)
Neutrophils (%)	52.2	68.7 (+31.61)	60.9 (+16.67)	53.7 (+2.87)
Lymphocytes (10^3/mL)	2.1	1.7 (−19.05)	1.4 (−33.33)	2.3 (+9.52)
Lymphocytes (%)	35.3	17.8 (−49.58)	23.5 (−33.43)	34.5 (−2.27)
Monocytes (10^3/mL)	0.6	1 (+66.67)	0.5 (+16.67)	0.6 (0.00)
Monocytes (%)	10	10.4 (+4.0)	9 (−10.00)	8.6 (−14.00)
Eosinophils (10^3/mL)	1.8	0.2 (−89.89)	0.3 (−83.34)	0.1 (−94.44)
Eosinophils (%)	1.8	2.4 (+33.33)	5.3 (+194.44)	2.1 (+16.67)
Basophils (10^3/mL)	0.0	0 (+0.10)	0 (+0.10)	0 (+0.10)
Basophils (%)	0.7	0.7 (0.00)	1.3 (+85.71)	1.1 (+57.14)
Erythroblasts (10^3/mL)	0.0	0 (0.00)	0 (0.00)	0 (0.00)
Erythroblasts (%)	0.0	0 (0.00)	0 (0.00)	0 (0.00)
SCR (mg/dl)	0.88	1.13 (+28.41)	0.98 (+11.36)	0.84 (−4.55)
AST (UI/L)	21	66 (+214.29)	45 (+114.29)	86 (+309.52)
ALT (UI/L)	14	39 (+178.57)	33 (+135.71)	99 (+607.14)
Lipase (UI/L)	13	18 (+38.46)	27 (+107.69)	13 (0.0)
Urea (mg/dL)	33	64 (+93.94)	46 (+39.39)	35 (+6.06)
Uric Acid(mg/dL)	5.2	5 (−3.85)	4.7 (−9.62)	5.2 (0.00)
HDL Cholesterol (mg/dL)	90	86 (−4.44)	80 (−11.11)	94 (+4.44)
Total Cholesterol (mg/dL)	233	193 (−17.17)	194 (−16.74)	292 (+25.32)
Triglycerides (mg/dL)	79	80 (+1.27)	130 (+64.56)	88 (+11.39)
Na^+ (mmol/L)	136	139 (+2.21)	140 (+2.94)	137 (+0.74)
K^+ (mmol/L)	4.5	5.2 (+15.56)	5.5 (+22.22)	4.9 (+8.89)
Cl^+ (mmol/L)	102	107 (+4.90)	106 (+3.92)	99 (−2.94)
Ca^{2+} (mg/dL)	9.9	9.1 (−8.08)	9.1 (−8.08)	9.8 (−1.01)
Mg^{2+} (mg/dL)	2	2.1 (+5.00)	2 (0.00)	2.2 (+10.00)
P^+ (mg/dL)	2.9	3.5 (+20.69)	2.9 (0.00)	3.4 (+17.24)
Glucose (mg/dL)	92	99 (+7.61)	73 (−20.65)	95 (+3.26)

Albumin (g/dL)	4.3	3.89 (9.53)	3.64 (−14.35)	4.29 (−0.23)
CK (UI/L)	94	1099 (+1069.15)	478 (408.51)	109 (+15.96)
LDH (UI/L)	152	571 (+275.66)	422 (+177.63)	254 (+67.11)
Total Proteins (g/dL)	6.8	6.5 (4.41)	6.1 (−10.29)	7 (+2.94)
Urea (mg/dL)	33	64 (+93.94)	46 (+39.39)	35 (+6.06)

Data are expressed as absolute value and as ± percentage from baseline values AST, aspartateaminotransferase; ALT, alanineaminotransferase; CK, creatine kinase; HDL, high-density lipoprotein; K^+, ion potassium LDL, low-density lipoprotein; LDH, lactate dehydrogenase; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell; RDW, red blood cell distribution width; SCR, creatinine.

Table 2. Urine Parameters Before (Baseline) and After Race (Post-Exercise Day 2 and post-exercise Day 9).

Parameter Urine	Before-Race	Post-Race		
	Pre (Baseline) VALUE	Post (Post-Exercise) Value (% Difference)	Day 2 (rec2) Value (% Difference)	Day 9 (rec9) Value (% Difference)
Proteins (mg/dL)	0	0 (0.00%)	0 (0.00%)	0 (0.00%)
Density (Kg/L)	1021	1021 (0.00%)	1018 (−0.29%)	1014 (−0.69%)
PH	6.5	6 (−7.69%)	7 (+7.69%)	7 (+7.69%)
Glucose (mg/dL)	0	0 (0.00%)	0 (0.00%)	0 (0.00%)
Nitrites	0	0 (0.00%)	0 (0.00%)	0 (0.00%)
Ketonic Bodies (mg/dL)	0	0 (0.00%)	0 (0.00%)	0 (0.00%)
Leucocytes	0	0 (0.00%)	0 (0.00%)	0 (0.00%)
Erythrocytes	0	0 (0.00%)	0 (0.00%)	0 (0.00%)
Microalbumin (mg/dL)	<0.19	36 (+18,847%)	<0.19 (0.00%)	<0.19 (0%)
Bilirubin (mg/dL)	0	0 (0.00%)	0 (0.00%)	0 (0.00%)
Urobilinogen (mg/dL)	1	1 (0.00%)	1 (0.00%)	0.2 (−80.00%)

Data are expressed as absolute value and as +. − percentage from baseline value.

Renal function evaluated through SCR was found to be elevated compared with basal levels from post (+28.41%) through to the 2nd recovery day (rec2 = +11.36%) and dropped below the basal value on recovery day 9 (rec9 = −4.55%). ER measured through CK increased significantly post (+1069.15%) and returned to normal values on rec9. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT), both linked to ER, remained well above baseline levels on recovery day 9 (rec9 AST = +309.52%; rec9 ALT = +607.14%). The electrolyte balance ($[Na^+]$), associated with EAH, remained above 135 mEq/L throughout all the recovery phases. This situation did not meet the diagnostic criteria for EAH. Urine sediment analysis did not show evidence of hematuria or proteinuria, despite having found high values of microalbumin post (+18.84%). Values returned to normal during the recovery period (rec2 and rec9). Lastly, hematocrit dropped post (−7.14%) and rec2 (−7.14%), and leukocytes increased by 213.3%, 100%, and 126% for post, rec2, and rec9, respectively.

3. Discussion

Renal function assessed through SCR (the main marker for AKI) did not meet the criteria for considering kidney damage since SCR did not increase by 50% [10]. The elevated post-SCR value (+28.41%) returned to normal during recovery and even dropped below basal values. This recovery pattern has been previously reported in other similar studies [36,37] and allows for the determination that nine recovery days are enough for SCR normalization without medical treatment. However, other studies have found several cases of AKI reaching failure stage in one runner (25%) [38]. Due to the seriousness that AKI implies for runners' health, some studies have addressed the probability of suffering AKI after a previous episode. In this study, 16 runners met the criteria at the first race; the subsequent race caused less increase in SCR concentration and decrement in

estimated glomerular filtration rate than the first race. This pattern confirms that usually, runners recover from the increase in SCR after some days [11,13,39,40]

A comparison between multi-stage races and single-stage races shows that multi-stage races may help runners to recover from the efforts [15,39] reducing the number of episodes diagnosed in these kinds of ultra-trail races; this result may be due to the slower pace that runners use to cover higher distances [40]

One of the reasons behind EAH development in long-distance runners is a poorly planned hydration strategy [41]. Not only does this directly affects EAH, but it has also been described in cases of kidney damage and even ER, due to myoglobin released into the bloodstream following sarcolemma rupture [42,43]. Athlete weight loss should be considered for the evaluation of this aspect, and the current case study results show a weight loss of 1.8% of pre-body weight. This result contrasts with other research that suggests larger weight losses are needed to avoid EAH [44]. This inconsistency regarding EAH diagnosis can be solved by evaluating [Na^+] concentration, with values above 135 mg/dl positively indicating EAH. In this case, a bodyweight loss below 2% would not be significant for the diagnosis of EAH in such a long duration effort if the [Na^+] value remains below 135 mEq/L.

ER is the pathological condition involving muscle cell necrosis and the release of CK and myoglobin from muscle cells into tissues, finally carrying to the kidney. There is no general agreement as to exactly how the substances released cause AKI [26,45]. In addition to molecules leaked into blood, vasoconstriction and ischemia seem to be behind the etiology of AKI [10]. Myoglobin and hemoproteins filtered from glomeruli may cause damage to the tubular system affecting the perfusion of the kidney [46–48].

As far as the diagnosis of ER is concerned, many blood markers (CK, LDH, AST y ALT) and urine (hematuria, proteinuria) biomarkers have been tested and used to examine ER in sports. All of them have been linked to muscle tissue damage and necrosis [37,49]. However, CK is the most widely used molecule in ER studies [24] and the one which enables its detection and diagnosis [25].

Ultra-trail races imply a huge amount of eccentric muscle contraction because of the great elevation both positive and negative that define these kinds of races [4]. In comparison, the pace and the speed of the runners is slower than in marathon or in half-marathon however the apparition of cases of ER is higher [25]. This could be explained not only by the eccentric effort but also because of hydration issues ,which lead to unexperienced runners to EAH and subsequently to ER [29,48].

As a conclusion, the alterations found in this case are similar to other studies, which included races as long and extreme as included in this study [36,47]. Similarly, the subject recovered baseline values after nine days of recovery. The alteration in other makers such as AST or ALT would increase the chances of ER development and also potentially alter liver function, but it is not a criteria for ER [25]. Urine analysis did not show proteinuria or hematuria, which proves that ER is compatible with no visible abnormalities in urine markers [18] (see Table 2).

4. Conclusions

This paper shows that ultra-trail races produce alterations in AKI, EAH y ER—specific biomarkers. The number of diagnostic criteria, biomarkers, and the confusing symptomatology often led to these alterations not being adequately considered and regarded as simply adaptation derived from physical effort. Overhydration, intense exercise and eccentric muscle contractions create a perfect storm which can lead to some runners suffering from ER, AKI and EAH. The seriousness of these pathologies, alone or combined, should raise awareness to runners, coaches, and organizers of these races of EAH, AKI, and ER.

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3.3 Artículo 3

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Case Report

768-km Multi-Stage Ultra-Trail Case Study-Muscle Damage, Biochemical Alterations and Strength Loss on Lower Limbs

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Abstract: A series of case studies aimed to evaluate muscular fatigue in running a 768-km ultra-trail race in 11 days. Four non-professional athletes (four males) were enrolled. Muscle damage blood biomarkers (creatine kinase (CK), lactodeshydrogenase (LDH), aspartate transaminase (AST) and alanine aminotransferase (ALT) and lower limb strength were evaluated by using Bosco jumps test; squat jump (SJ), countermovement jump (CMJ) and Abalakov jump (ABA) were assessed before (pre), after the race (post) and for two and nine days during the recovery period (rec2 and rec9), respectively. Results showed: pre-post SJ = −28%, CMJ = −36% and ABA = −21%. Values returned to basal during rec9: SJ = −1%, CMJ = −2% or even exceeded pre-values ABA = +3%. On the contrary, muscle damage blood biomarkers values increased at post; CK = +888%, LDH = +172%, AST = +167% and ALT = +159% and the values returned gradually to baseline at rec9 except for AST = +226% and ALT = +103% which remained higher. Nonparametric bivariate Spearman's test showed strong correlations (Rs ≥ 0.8) between some jumps and muscle damage biomarkers at post (SJ-LDH Rs = 0.80, SJ-AST Rs = 0.8, ABA-LD H Rs = 0.80 and ABA-AST Rs = 0.80), at rec2 (SJ-CK Rs = 0.80 and SJ-ALT Rs = 0.80) and even during rec9 (ABA-CK). Similarly, some parameters such as accumulated elevation and training volume showed a strong correlation with LDH values after finishing the ultra-trail race. The alteration induced by completing an ultra-trail event in the muscle affects lower limb strength and may in some circumstances result in serious medical conditions including post- exertional rhabdomyolysis.

Keywords: ultra-endurance; strength loses; lower limb fatigue; muscle damage; neuromuscular fatigue

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1. Introduction

Sport events considered extreme or long-lasting have increased considerably in the last decade [1], generating a boom of trail-running races that defines the exponential growth in participation in this type of event [2,3]. The realization of extreme ultra-trail competitions requires on the part of the runner, a series of physical and energetic resources that guarantee the overcoming of the long-distance imposed, as well as the slope of accumulated elevation and climatic conditions that are sometimes extreme.

These characteristics induce risks in the runner's health [4] and have made them become an exceptional model for the evaluation of physiological response to one's load of effort and stress generated by fatigue [5]. Acute fatigue induced by physical demand of this type of effort is associated with a relevant modification of biochemical parameters that could result in inaccurate diagnosis [6]. The fact that this type of sport discipline requires participants to repeat cyclical movements for very long periods means that episodes of elevation of markers typical of worrying physiological situations have been

described, such as acute kidney damage or post-exertion hyponatremia [4,7]. The biochemical alterations should also be taken into account related to myocellular morphological modifications associated with protein breakdown [8], which in addition to generating a biochemical alteration, causes the loss of contractile function and a decrease in the levels of muscle strength in the runner [9].

Post-exertional rhabdomyolysis (ER), a name that describes this situation, is characterized by the destruction of the myocyte associated with eccentric exercises that are long in duration and involve strenuous intensity [10]. From a diagnostic point of view, this episode is diagnosed when certain biomarkers of muscle damage (creatine kinase (CK)) and acute inflammation in blood or urine (myoglobinuria (MB) appears to increase after the completion of the effort) [11]. CK, unlike MB that disappears at 24 h due to renal clearance, increases its value above baseline from 2 to 12 h after exertion [12,13], and it does not reach its maximum peak until 3–5 days after the end of the episode generating the elevation, and can take up to 6–10 days to normalize [14,15]. Concerning the quantitative value of different diagnostic values of ER, there is no established unanimity [16]. Sometimes it is diagnosed on a variable reference value of CK (1000 $U \cdot L^{-1}$ to 10,000 UL^{-1}) [16,17], while other authors agree that CK elevation 5 times the upper limit of normal is the defining biochemical abnormality for this condition [16,18–20]. Due to this variety in the diagnostic criteria of ER from CK [18,19], other markers such as lactate dehydrogenase (LDH) [21], aspartate transaminase (AST) and alanine aminotransferase (ALT) [14,22] are analyzed to assess its severity. These markers present a linear relation with both CK and MB increasing when comparing basal with post-race values [23].

In relation to long-term tests and ER, Rojas et al. attributed in a recent review that 67.2% of the diagnosed cases corresponded to trail-running athletes compared to other endurance sports [16]. The high negative elevation that defines this type of racing offers an opportunity to investigate the relationship between possible alterations in the ability to produce muscle strength and its possible relationship with ER. Several studies have shown that some biomarkers related to ER considerably increased their value during the race compared to baseline and are even maintained a few days after the end of the effort [19], in addition, they have a clear relationship with strength loss in lower limbs by the athlete [7,21,24]. Peake et al. determined that this loss in strength capacity is due to pathophysiological alterations such as modifications in contractile muscle activation, muscle morphology of the athlete or the type of contraction to which the athlete is subjected to [25].

Previous investigations related to this sport discipline demonstrates an evident post-race neuromuscular deficiency [24] translated into a curvilinear relationship between volume and a decrease in the runner's contractile capacity [9]. According to these authors, it seems that the loss in strength levels is inversely proportional to the duration of the event. When comparing possible changes in contractile capacity of the runner after different distances (65 km vs. 107 km), Martínez-Navarro et al. observed a greater decrease in flight height after races of greater distance (10% vs. 35%; losses of contractile capacity depending on the running distance) [21]. These conclusions have been proven by the research group of Temesi et al., who reinforced the idea of a meaningful deterioration of the contractile property of the muscle in very long-distance competitions [24]. In addition, Martínez-Navarro et al. tried to observe possible alterations of some post-race biochemical parameters and determined a significant elevation of both CK ($p < 0.01$; d = 0.9) and LDH ($p < 0.01$, d = 2.3), in addition to the loss in flight height described above [21].

Therefore, concerning the conclusions of some authors who determine the duration and intensity of exercise as variables associated with the degree of muscle fatigue [26], characteristics are also strongly related to episodes of ER [10]. It could be hypothesized that the completion of an ultra-trail event entails increased blood biomarkers related to muscle damage, and they could be related to decreases in the strength of lower limbs not only after finishing the race but also during the recovery period. Accordingly, the aim of the present study was to assess possible biochemical alterations related to muscle damage

and their consequences in runners' strength in lower limbs after completing a 768-km ultra-trail race.

2. Materials and Methods

2.1. Experimental Design

The study consists of a exploratory series of case studies involving 4 participants of the GR11 Challenge Trail. GR11 is a multi-stage ultra-trail race, which joins the Mediterranean and Atlantic coasts along the Pyrenees, covering 786 km in a total of 11 stages. The investigation was conducted in accordance with the Declaration of Helsinki and approval for the project was obtained from by the Ethics Committee of the Department of Health and Consumption of the Government of Aragon (Spain) (protocol code 18/2015).

2.2. Subjects

Four adults volunteered to participate in this study after an email invitation. All runners were experienced males (5 ± 1.26 years), highly trained (11.61 ± 2.22 h·week^{-1}) and accumulated large amounts of elevation trained (116,615 ± 37,462 m). All of them were non-smokers and were not under medical, pharmacologic or dietary treatment. The participants were informed of the purpose, procedures and risks of this study, and they provided prior personal written informed consent to participate. They were allowed to withdraw from the study at will at all times. The main inclusion criteria were as follow: (a) adults (>18 years); (b) completed at least two previous ultramarathons (>42 km); and (c) free of chronic medical condition or medical treatment on regular basis. All subjects also completed a questionnaire on basic demographics and pre-race training. The characteristics of the participants are found in Table 1.

Table 1. Characteristics of population included (n = 4).

Parameters	MD SD
Age (years)	38 ± 4.11
VO_{2max} (mL/kg/min^{-1})	61.17 ± 8.96
HR_{max} (beats·min^{-1})	187 ± 8.54
Maximal aerobic speed (km·h^{-1})	16.91 ± 0.83
Height (cm)	175.72 ± 3.65
Weight (kg)	70.09 ± 9.05
BMI	22.70 ± 2.05
Fat mass (%)	8.13 ± 0.68
Muscle mass (%)	46.75 ± 6.27
Experience (years)	5 ± 1.26
Weekly training load (hours)	11.61 ± 2.22
Annual slope accumulated (meters)	116,615 ± 37,462

BMI: body mass index; HR_{max}: maximum heart rate; VO_{2max}: maximum oxygen consumption.

2.3. Procedures

The GR-11 route joins the Mediterranean and Atlantic coasts along the Pyrenees, covering 786 km in a total of 11 stages. The average stage/day consisted of 71.49 km (SD ± 8.2), the average positive elevation was 4260.45 ± 1063.26 and average negative elevation was 4258.63 ± 989.13. The race had a warm temperature, with values ranging from 13.1 to 17.6 °C, and the humidity was (60.1–70.9%). In-race hydration was ad libitum. Training parameters were recollected by using a previous questionnaire including training volume (hours·week^{-1}), total positive and negative elevation accumulated (Slo) and years of experience in ultra-trail races (Exp). The characteristics of GR-11 are fully shown in Table 2.

Table 2. Characteristics of the extreme ultra-trail.

Stages	Duration (Km)	Positive Elevation (m)	Negative Elevation (m)
1	78.5	3136	3024
2	72.3	3886	3458
3	72.1	4655	4044
4	68.1	5660	4581
5	72.6	5411	6336
6	76.1	5344	4788
7	63.7	5492	5163
8	66.1	3641	4576
9	66.1	3361	3841
10	66.5	2958	2934
11	83.4	3321	4100
Total	784.91	46,865	46,845
MD	71.35	4260.45	4258.63
SD	±6.00	±1063.26	±989.13

2.4. Anthropometry

The different measures that made up the anthropometry measurement were height, weight and skin folds. All of them were measured 2 h prior to the start of the race and after completing stage 11 following the same order. Height measurement was made to the nearest 0.1 cm using a wall-mounted stadiometer (Seca 220, Seca, Hamburg, Germany), body weight was measured barefoot to the nearest 0.01 kg on calibrated electronic digital scales (Seca 769, Seca, Hamburg, Germany), skin folds were used a compass accurate to ±0.2 mm (Seca 212, Seca, Hamburg, Germany) and a tape with an accuracy of ±1 mm was employed. Six skin folds were taken: abdominal, suprailiac, subscapular, tricipital, thigh and leg and perimeters; arms and legs were in a relaxed 90° position. The equations of Yushaz were used to calculate the percentage of fat [27], and the equation according to Lee was used to determine the percentage of muscle [28].

2.5. Physical Performance Assessment

In order to determine corresponding physical performance values, a progressive and maximum laboratory test was performed on a treadmill (Pulsar, h/p/cosmos®, Nussdorf, Germany). The test was run on a 1% slope and began at a speed of 8 km/h, which increased 1 km h^{-1} every minute. Before the test began, the participants warmed up for 5 min on the treadmill operating at a speed of 6 km h^{-1} [29]. Respired gases were collected with an Oxycon Pro analyzer (Erich Jaeger GmbH, Hoechberg, Germany). A pulsometer was used to evaluate the maximal heart rate (Vantage M, Polar, Finland).

2.6. Lower Limb Strength Assessment

The assessment of lower limbs strength was conducted using Bosco jumping protocol [30], on a platform that was placed on a contact mat connected to a digital timer (Chronojump Boscosystems, Barcelona, Spain). The jumping tests included were as follows: squat jump (SJ), countermovement jump (CMJ) and Abalakov jump (ABA). Four different measures were taken: before the race start (pre), after completing the race (post), two days after (rec2) and, finally, nine days after completing the challenge (rec9) of every one of the four jumps. The protocol for establishing the height of every measure was based on Bosco et al. [30]. The highest jumps that did not have a difference greater than 1.5 cm were validated, and the average all of them was considered. The result was expressed in flight centimeters. In order to perform SJ, the participants started in a squatting position with knees bent at 90° and arms on hips to avoid influencing the jump. A goniometer was used to verify the knee angle. The participants had to remain in this squatting position for

3 s before performing SJ. For CMJ, the subjects started from an upright standing position with hands on hips to avoid any arm movement. Then as a single sequence, they made a swift downward movement, followed immediately by a rapid vertical movement to jump as high as possible [31]. Finally, during the ABA test, the participants had to begin by squatting and flexing their knees 90°, followed by swinging their arms to help them to jump as high as possible.

2.7. Blood Samples and Analysis

Twenty milliliters of venous blood (antecubital vein) were withdrawn from each participant in both pre, post, rec2 and rec9 evaluations (90 min before and 10 min after finishing the race, two days and nine days in the morning). Blood samples were collected in two 5-mL Vacutainer tubes (Vacutainer, beliver industrial state, plymouth PL6 7BP, United Kingdom) without anticoagulant for serum isolation and in two 5-mL tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Once collected, blood samples were coagulated for 25–30 min at room temperature and then centrifuged at 2500 rpm for 10 min to remove the clots. Serum samples were aliquoted into Eppendorf tubes (Eppendorf AG, Hamburg, Germany), previously washed with diluted nitric acid, and conserved at −80 °C until the biochemical analysis. In order to facilitate the interpretation of data, the change in analytical parameters was measured as follows: post, rec2 and rec9 days less pre, respectively.

2.8. Stadistical Analysis

Statistical analyses were carried out using the Statistical Package for The Social Sciences software (IBM SPSS Statistics for Windows, version 26.0, 64 bits Edition, IBM Corp., Armonk, NY, USA). Descriptive analysis was carried out in all variables, and average, median and standard deviation were calculated. Normal distribution of the variables was verified by using Kolmogorov-Smirnov and Shapiro-Wilk tests, but normality criteria were not met because of the low number of subjects. Consequently, a non-parametric test was performed. Bivariate inferential analyses using Spearmen correlation were performed to contrast the association between the change of strength tests and analytical parameters and between these variables and those that measure the volume of training. The nonparametric correlation coefficient was applied because normality was not reliable. A confidence level of 95% was established, and Spearmen´s rank correlation (Rs) was used to describe the relation between variables. Five different ranges were set according to Fowler et al. criteria to evaluate the strength between the strength variables and blood parameters [32]: (0.00 to 0.19) very weak; 0.20 to 0.39 weak; (0.40 to 0.69) moderate correlation; (0.70 to 0.89) strong; and (0.90 to 1.00) very strong. *p*-value was calculated but, due to the low number of subjects included, its value were not considered for final analysis.

3. Results

All subjects who accepted participating in the study completed the race (n = 4). The average total time for finishing 11 stages was 154 h 43 min (SD ± 23 min), with an average speed of 5.11 ± 0.46 km h^{-1} and an average pace 11 min 46 s (SD ± 3 min 4 s). No subject required medical treatment or was hospitalized after completing the race. Blood biomarkers analyzed are shown in Table 3. Range values were expressed for CK, LDH, AST and ALT according to age, gender and race [33]. All parameters increased their values when comparing pre vs. post and continued above the basal line, both rec2 and rec9, except for CK which descended above the basal line after the last measurement (CK pre = 98.5 UI/L vs. CK rec9 = 88.00 UI/L). Conversely, AST and ALT continued above 100% from baseline at rec9 (AST rec9 = 103% and ALT rec9 = 226%).

Table 3. Blood parameters before (baseline) and after race (post-exercise day 2 and post-exercise day 9).

Parameter Blood (Reference Values for Age and Gender)	Before-Race Pre (Baseline) Value	Post-Race Post (Post-Exercise) Value (% Difference)	Post-Race Day 2 (rec2) Value (% Difference)	Post-Race Day 9 (rec9) Value (% Difference)
AST (0–35 UI/L)	23.75 ± 3.20	63.50 ± 9.68 ↑ (+167.00)	44.50 ± 8.74 ↑ (+87.00)	48.25 ± 27.45 ↑ (+103.00)
ALT (0–45 UI/L)	17.25 ± 3.59	44.75 ± 12.44 ↑ (+159.42)	37.25 ± 8.80 ↑ (+115.94)	56.25 ± 34.25 ↑ (+226.08)
CK (20–215 UI/L)	98.51 ± 24.53	974.00 ± 402.66 ↑ (+888.85)	474.85 ± 1 85.70 ↑ (+382.00)	88.00 ± 16.27 ↓ (−7.72)
LDH (66–170 UI/L)	172.75 ± 14.71	470.30 ± 104.80 ↑ (+172.20)	316.00 ± 70.88 ↑ (+82.90)	208.00 ± 31.55 ↑ (+20.40)

Data are expressed as absolute value and as ± percentage from baseline values AST, aspartateaminotransferase; ALT, alanineaminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase.

SJ, CMJ and ABA decreased when comparing pre vs. post. During recovery period (rec2 and rec9), previous values were returned in three measurements. CMJ showed the largest decreases in all measurements, whereas ABA kept their values closer to baseline (see Table 4).

Table 4. Neuromuscular function before (baseline) and after race (post-exercise Day 2 and post-exercise Day 9).

Bosco Test	Before-Race Pre (Baseline) Value	Post-Race Post (Post-Exercise) Value (% Difference)	Post-Race Day 2 (rec2) Value (% Difference)	Post-Race Day 9 (rec9) Value (% Difference)
SJ (cm)	30.68 ± 2.46	22.05 ± 8.59 ↓ (−28.12)	27.01 ± 3.12 ↓ (−11.96)	30.15 ± 2.06 ↓ (−1.72)
CMJ (cm)	34.75 ± 3.98	22.00 ± 7.30 ↓ (−36.69)	29.43 ± 5.91 ↓ (−15.30)	34.00 ± 5.20 ↓ (−2.15)
ABA (cm)	39.48 ± 4.95	30.93 ± 7.63 ↓ (−21.65)	36.30 ± 8.69 ↓ (−8.05)	40.85 ± 4.54 ↑ (3.47)

Data are expressed as absolute value and as +. − percentage from baseline value. ↓ decrease from baseline value. ↑ increase from baseline value.

Strong correlations were observed between pre-post blood parameters and strength loses (SJ-LDH R_s = 0.80, SJ-AST R_s = 0.80, ABA-LDH R_s = 0.80 and ABA-AST R_s = 0.80). Similarly, at rec 2, some strong correlations were found both positive (SJ-CK R_s = 0.80 and SJ-ALT R_s = 0.80) and negative (ABA-CK R_s = −0.80 and SJ-ALT R_s = −0.80) among some biomarkers parameters and jump tests. Finally, at rec9, only ABA test showed a strong correlation (ABA-CK, R_s = −0.80) (see Table 5).

Table 5. Spearman's rank correlation coefficient (R_s) and probability (p) pre and post-race.

Bosco Jumps	CK Post-pre		LDH Post-pre		AST Post-pre		ALT Post-pre	
	(R_s)	p	(R_s)	p	(R_s)	p	(R_s)	p
SJ pre-post	0.4	0.6	0.80 **	0.2	0.80 **	0.2	0.4	0.6
CMJ pre-post	0.2	0.8	0.4	0.6	0.4	0.6	0.2	0.8
ABA pre-post	0.4	0.6	0.80 **	0.2	0.80 **	0.2	0.4	0.6
SJ pre-rec2	0.80 **	0.2	0.2	0.8	0.4	0.6	0.80 **	0.2
CMJ pre-rec2	−0.60	0.4	0.4	0.6	0	1	−0.60	0.4
ABA pre-rec2	−0.80 **	0.2	0.2	0.8	0.2	0.8	−0.80 **	0.2
SJ pre-rec9	−0.31	0.69	−0.31	0.69	−0.63 *	0.36	−0.64 *	0.36
CMJ pre-rec9	0.4	0.6	0.2	0.8	0.4	0.6	0.4	0.6
ABA pre-rec9	−0.80 **	0.2	−0.40	0.6	−0.20	0.8	−0.20	0.8

(R_s) * moderate correlation; ** strong correlation; p, p-value.

After the study of the training parameters with blood biomarkers and jumps test (pre-post), strong relations were found for LDH-Exp, $R_s = 0.94$ and LDH-Slo $R_s = -0.80$. Additional negative correlations were also found for CK-Exp, $R_s = -0.31$ and ALT-Exp, $R_s = -0.31$) (see Table 6).

Table 6. Spearman's rank correlation coefficient (Rs) and probability (p).

	Weekly Training Load (Hours/Week)		Accumulated Elevation (Meters)		Experience (Years)	
	(R_s)	p	(R_s)	p	(R_s)	p
SJ pre-post	−0.31	0.68	−0.40	0.6	0.63 *	0.36
CMJ pre-post	−0.63 *	0.36	−0.20	0.8	0.31	0.68
ABA pre-post	0.31	0.68	−0.40	0.6	0.63	0.36
CK post-pre	0.63 *	0.36	0.60 *	0.4	−0.31	0.68
LDH post-pre	−0.31	0.68	−0.80 **	0.2	0.95 ***	0.05
AST post-pre	0.31	0.68	0	1	0.31	0.68
ALT post-pre	0.63 *	0.36	0.60*	0.4	−0.31	0.68

(R_s) * moderate correlation, ** strong correlation; *** very strong correlation; p, p-value.

4. Discussion

The main purposes of this study were to analyze possible biochemical alterations related to muscle damage by determining their likely relationship with the loss of the contractile capacity of extensor muscles of the thigh and to evaluate the relationship between training parameters, biomarkers alterations and strength loss on lower limbs. To the best of the authors' knowledge, this has been the first study to compare muscle contraction capacity both in a resting and fatigued situation in such an extreme multi-stage ultra-trail race. Among these studies, another research study previously conducted has already included ultra-trail races with a wide range of durations (from 330 to 1600 km) [34–37], but no one has evaluated jointly the length and negatively and positively accumulated elevation included. Additionally, despite its design as a case study, this study shows strong correlations between training and biomarkers parameters; consequently, the main conclusions reported offer valuable information for runners, coaches and medical staff that help understand internal behaviors of the muscle and could be considered as a training response variable.

4.1. Increases in Blood Biomarkers

The results obtained in this study coincide with previous research that also shows an elevation in biomarkers of inflammation and muscle damage (CK, LDH, AST and ALT) after the completion of an ultra-trail race [6,19,21] and during the recovery period [4]. CK and LDH have been related to the extreme duration of these races [38,39]; consequently, it could be the cause of fatigue and muscle damage directly or indirectly [26,40]. In this line, Skenderi et al. established the elevation of both values by comparatively studying two groups of runners over two different distances, obtaining higher levels of CK and LDH in the group that covered the longest race [8]. Martínez et al., in a comparative study, analyzed CK and LDH in two different ultra-trail races of 65 km vs. 107 km, and significant elevation in both biomarkers was observed in the two study groups. However, the group of runners of the shortest race obtained higher increases in both values in addition to not returning to the baseline levels after 24h of recovery [21]. This fact seems to highlight the idea that the duration of the test could not only be the main determining factor in the release of CK and LDH [17].

Due to the lack of consensus, researchers have proposed two additional factors to study the kinetics of both biomarkers. On the one hand, one factor includes running speed, where high levels of CK and LDH have been observed in studies including shorter races such as marathon and half marathon [16,41,42]. On the other hand, the number of stages of the race prevents runners from suffering higher increases in CK and LDH. Multi-stage races may ease the recovery between sections of the event by diminishing the release of muscle damage biomarkers [4,16,43]. This disparity in conclusions has generated other biomarkers such as AST and ALT, which are also taken into account for the possible diagnosis of ER. We are aware that it seems that the elevation in these values is somewhat transient and does not have functional repercussions for the liver except for isolated cases [38]. In our study, extremely high values are described even during recovery periods (rec9; AST = 103% and ALT = 226%).

Therefore, we can conclude that apart from the speed and duration of the test, there are more factors involved in generating alterations in muscle damage biomarkers. Some authors determine temperature, both cold and hot, as a mechanism for increases in AST and ALT [38], but this is still a hypothesis with numerous doubts since authors such as Žákovská et al. [44] observed no differences in the values of ALT pre and post-race in a study with extremely cold temperatures (−1 °C to 1 °C).

4.2. Neuromuscular Fatigue

For the parameters of neuromuscular function, significant reductions in the height of the jumps have been reported in the scientific literature [7,21,27,44]. In this sense, both Martínez Navarro et al. and Balducci et al. reported decreases in flight height in the use of isolated tests of the Bosco jump protocol, both for SJ (pre = 24.4 ± 4.1 vs. post = 18.4 ± 42.2) [42] and for CMJ (pre = 30 ± 0.6 vs. post = 24 ± 0.5 cm) [43] respectively. It is also worth mentioning a more recent study about induced muscle fatigue that also describes losses in SJ, ABA and CMJ in pre and post-race situations. [7]. In the present study, we found that the flight height of SJ, CMJ and ABA decreased in the post-race and also during the recovery period (see Table 4). These findings would reinforce the theory that the duration of exercise, the type of contractile capacity and the muscle group involved in the effort, are responsible for a decrease in muscle contractile capacity by induced peripheral fatigue [45]. The decrease in flight height of the CMJ would show how elastic muscle elements require a longer recovery time compared to the muscle contractile capacity assessed from SJ. Therefore, we could intuit that the performance of very long-term running exercises during several stages causes higher deterioration in elastic contractile function [44]. The lower losses in strength capacity found in the ABK could be understandable by requiring coordinative involvement of the arms in the performance of the jump.

If we take into consideration that the reduction in contractile capacity depends to a large extent, on the muscle group involved in the movement and the trunk part is not implicit in the race, we could consider that this is the reason for why the loss in flight height in this jump is lower compared to the test performed without using the arms (SJ and CMJ). Finally, we can note that there was a great variety in the decrease in height jumps among runners involved in this study. This fact is referred to by Bregstrom et al. [46]. According to them, this variability in neuromuscular responses to the extreme effort is based on the characterization of neuromuscular fatigue subject-by-subject basis differences.

4.3. Biomarkers and Strength Loses

The present study describes a strong correlation between the loss of the contractile capacity of the musculature involved in the vertical jump and the increase in several biomarkers. This relationship was present at post and in the recovery period (rec2 and rec9). (See Table 5). Post values showed a strong correlation between SJ and LDH and AST, but not in the remaining variables analyzed (CK and ALT). Our results differ from previous research in which CK production is related to a decrease in flight height in all jumps of Bosco test [1,30]. Be reinforcing these findings, the negative elevation of the race studied and consequently more eccentric load increased the association between strength loses and rising muscle damage biomarkers [31,33]. On this matter, Hody et al. tried to predict the loss of muscle strength by using the serum value of CK by associating the loss in flight height with levels of CK produced [47].

Despite this evidence concerning the production of CK and the loss in flight height in a post situation, this is a conclusion that could not be fully contrasted by not taking into account the possible differences between runners (characterization of genetic subject-by-subject basis) [48] and, above all, by not considering the level of training of the study sample analyzed [7,21,49]. These considerations, together with the fact that our analysis test was a multi-stage race and the fact that our study sample was composed of subjects with similar levels of training and physical condition, mean that it could be the consequence of not obtaining relationships in the rest of the biomarkers analyzed for the losses in the capacity of fair contractile after the end of the event. Concerning data obtained in the recovery period (rec2 and rec9) we can observe a return to baseline levels or very close to them in the different tests used to measure muscle fatigue and in almost all biomarkers analyzed. An elevation in rec2 was observed only for those related to liver damage compared to the baseline value (AST rec2 = +87.00% and ALT rec2 = +115.94%), but in rec9 these values were still increasing (AST rec9 = +103.00% and ALT rec9 = +226.08%).

These described kinetics responds to the conclusions established in previous studies that determine that biomarkers related to liver damage require more than one week to return to their baseline state without the implication of a situation of liver damage [10,47]. Other parameters that established strong positive correlations were CK and ALT for the SJ test, a relationship that would determine that the higher losses of SJ would entail a higher increase in CK and ALT. Conversely, we found negative values in the association between CK and LT and CMJ but with a lower statistical correlation (Rs = 0.6). A relationship would indicate that a higher increase in CK would be associated with lower differences in the release of this biomarker at rec2. Considering the values obtained at rec9, no value showed strong positive associations except for ABA and CK. Such associations would probe that the rising levels of CK found at rec9 are related to a lower loss in flight height. The results described in our study would be in line with the data reported by the group of Landart et al., who also related higher decreases in CMJ compared to ABA [50]. Similar results were found by Balducci et al., who also found opposite results when comparing descents in height when using different Bosco jumping protocol tests (CMJ vs. SJ and ABA) [42]. This recovery time may allow runners to recover from the effort and return to pre-race baseline values with respect to both the contractile capacity and muscle

damage biomarkers analyzed, a situation that would cause this negative statistical relationship.

4.4. Training Parameters

The data obtained in this study about the possible relationship between the characteristics of some training parameters and their possible relationship with the loss in muscle strength production did not report any notable effects for the jumping tests used. We must emphasize that the current scientific literature is today contradictory in providing information about this possible relationship. For example, Eston et al. determined that performing eccentric work within the preparatory phase resulted in a decrease in mean strength losses from SJ [51]. However, another study carried out by Muanjai et al. did not find this relationship [52].On the contrary, an opposite effect was demonstrated by relating eccentric work with higher loss in contractile capacity and increased deficit in the coordination excitation contraction mechanism. Moreover, similar results were reported by the working group of Giandolini et al., who associated higher losses in strength production and higher muscle pain in the group that performed eccentric work in the preparation phase [9]. To the authors' knowledge, only one more study has tried to analyze the possible relationship of some parameters of ultra-trail training and its possible influence on the loss of strength [45]. Pradas et al., by separating the study sample between expert and non-expert runners (experts = 5.80 ± 2.52 and non-experts = 4.60 ± 1.26 years of experience) determined that the most expert subjects had lower losses in flying height in a post-race situation in SJ and CMJ [7]. Data that have not been corroborated by our study that have analyzed a sample with great homogeneity that did not allow establishing groups based on the experience of the runner (SD = ±1.26). Finally, the possible influence between training parameters and their relationship with the release of markers related to muscle damage is a little studied topic. Despite finding a strong correlation between the experience of the runner and the production of LDH, there is no work in the scientific literature that can corroborate this finding and we can only intuit that despite the relationship found it is the extreme volume that the studied test has that is the real reason for this elevation [39,40].

4.5. Limitations

It must be considered that the sample size (n = 4) and only male gender used in this study could be a limitation that had an impact on the results obtained. The selection of this study design is due to the uniqueness and extreme duration of this extreme multistage ultra-trail challenge. As far as we know the length and the positive and negative elevation of the race here analyzed makes the results unique and meaningfully relevant for coaches and researchers [13,36,53]. Future studies should explore the relationship between muscle damage blood biomarkers and neuromuscular function after completing an ultra-trail race by considering the characteristics of the race (length and elevation) as well as central fatigue to explain possible decreases in the jump test. Additionally, some training parameters should be investigated in future investigations to clarify their role in preventing acute effects on neuromuscular fatigue.

5. Conclusions

Ultra-trail competitions cause increases in muscle damage blood biomarkers (CK, LDH, AST and ALT) and lower-limb strength losses (SJ, CMJ and ABA) after finishing the race. These alterations returned to baseline after a recovery of 9 days, with the exception of liver damage biomarkers which remained higher. The duration and the great accumulated elevation associated with these races are largely responsible for the effects described. However, the influence of each of them individually is still contradictory. The results found in this study for the kinetics of muscle damage blood biomarkers and the response in the lower-limb strength of the runner can help both the comprehension of metabolism

implicit in this type of multi-stage ultra-trail events and the preparation of the race and its possible influence on the performance of the runner.

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Data Availability Statement: Information about the case report is available at http://gr11en11.org/ (accessed on 26 October 2021).

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3.4 Artículo 4

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MDPI

Case Report

Bone Turnover Alterations after Completing a Multistage Ultra-Trail: A Case Study

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Abstract: A series of case studies aimed to assess bone and stress fractures in a 768-km ultra-trail race for 11 days. Four nonprofessional male athletes completed the event without diagnosing any stress fracture. Bone turnover markers (osteocalcin (OC), serum C-terminal cross-linking telopeptide of type I collagen (CTX), bone-specific alkaline phosphatase (BALP), and serum turnover calcium (Ca^{2+})) were assessed before (pre) and after the race (post) and on days two and nine during the recovery period (rec2 and rec9), respectively. Results showed: post-pre-OC = −45.78%, BALP = −61.74%, CTX = +37.28% and Ca^{2+} = −3.60%. At rec2 and rec9, the four parameters did not return to their pre-run levels: OC, −48.31%; BALP, −61.66%; CTX, +11.93% and Ca^{2+}, −3.38%; and OC = −25.12%, BALP = −54.65%, CTX = +93.41% and Ca^{2+} = +3.15%), respectively. Our results indicated that the ultra-trail race induced several changes in bone turnover markers, uncoupling of bone metabolism, increased bone resorption: OC and BALP and suppressed bone formation: CTX and Ca^{2+}. Bone turnover markers can help determine the response of bone to extreme effort and might also help predict the risk of stress fractures.

Keywords: ultra-endurance; bone mass density; bone remodelling markers; bone formation; bone resorption

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1. Introduction

Regular physical activity has been recognized to have health benefits in general and specifically in the musculoskeletal system, increasing muscle strength and bone mineral density (BMD) [1]. Regular physical activity prevents multiple bone diseases, such as osteopenia or osteoporosis by increasing BMD [2]. Regardless of the importance of exercise in maintaining bone health, there is still no consensus in the scientific literature regarding the volume and intensity of effort required to prevent bone damage [3]. If exercise does not exert a minimum load on bone tissue, it will not increase BMD, as has been proven in studies carried out on swimmers [4]. Benefits in BMD depend on the type of exercise undertaken; weight-bearing exercise increases BMD, particularly at load-bearing sites, independent of muscular activity alone [5]. In contrast, when exercise overloads bone tissue because of excessive force, it may result in stress fractures and weaken BMD [6].

Among all the weight-bearing endurance sports, ultra-trail races include the longest (e.g., any distance in excess of the standard marathon distance 42.195 km or at least 6 h of duration) [7] and a great amount of negative and positive accumulated elevation, which increase the mechanical stress and consequently overload the microstructure of bone tissue (especially lower limbs) [8]. In addition, multi-stage ultra-trail competitions are usually held in extreme environments lasting several days and, as a consequence, athletes must carry their own provisions, resulting in additional weight and increasing the stress on bone tissue and reducing BMD [9]. Despite all these characteristics, the number of stress fractures found in competitions is relatively low [10,11]. Hoffman et al., in a descriptive study

including 1212 ultra-runners, who completed an ultra-trail race, only found 0.3% of stress hip fractures and 1.9% of stress fractures involving tibia or fibula [12]. However, Scheer et al. found a higher incidence when assessing the stress fractures in a 12-month following study (10.3%) [13]. Apart from the length of the race, these runners have to complete a high volume of training prior to the competition in order to acquire the physiological and biomechanical adaptations required to face this extreme effort. Average training loads are between 66-83 km/week in adults and around 57 km/week in youth athletes [14]. This great amount of training may result in overtraining syndrome and subsequently in BMD loss increasing the likelihood of suffering stress fractures [15]. Thus, predictive markers that reflect stress of bone are needed to prevent stress fractures and help runners and coaches plan their training routines effectively [16].

BMD is usually assessed by using Dual-energy X-ray absorptiometry (DEXA) at the femur and, in the current criteria, OP is defined as a BMD T-score of −2.5 or lower at any one location or presenting a previous fragility fracture [4]. However, BMD analysis is not always conclusive as a predictive factor of stress fractures in the sports field [3]. Due to this fact, many researchers have raised the importance of bone turnover (BT) by analysing bone turnover markers (BTMs), as an essential factor when assessing bone microarchitecture and, therefore, the state of bone tissue [15,17]. BT consists of two dynamic processes: bone formation (anabolism) regulated by osteoblasts (responsible for bone matrix synthesis) and resorption (catabolism) mediated by osteoclasts (responsible for the secretion of proteolytic enzymes which digest bone matrix) [18]. BTMs are biochemical products measured usually in blood or urine that reflect the metabolic activity of bone, but which themselves have no function in controlling skeletal metabolism [19]. They are traditionally categorized as markers of bone formation or bone resorption. It has been shown that during the practice of endurance exercise there is an increase in markers related to bone resorption (serum C-terminal cross-linking telopeptide of type I collagen (CTX)) [20,21] and a decrease in those of formation (osteocalcin (OC), alkaline phosphatase (AP) and serum procollagen type I N propeptide (s-PINP)) causing a net loss of bone [22–24]. The analysis of BTMs offer some advantages over DXA alone: they allow the assessment of bone metabolic activity at a specific time, there are a high number of selective markers and the techniques are easily applicable and minimally invasive [25]. The analysis of BTMs, therefore, allows the monitoring of the likelihood of suffering stress fractures during the practice of physical activity by using selective biomarkers, apart from BMD [26]. The relationship between stress fractures and BT seems predisposed by an acceleration in the destruction of bone tissue that precedes the remodelling phase, which may cause a weakening of the tissue during this period and, therefore, increased probability of suffering stress fractures [27].

The aim of this case study was to assess the alterations suffered by four runners after completing a 768 km extreme ultra-trail race on bone turnover markers. We hypothesized that bone resorption markers (CTX and Ca^{2+}) would increase and bone formation markers (CTX and BALP) would decrease. The alteration suffered in BTM may persist for days after the activity; a fact that may result in an increase in the suffering of stress fractures.

2. Case Report

Four non-professional healthy ultra-runners (38.08 ± 4.11 years) accepted participation in this case report after being invited by email. The four subjects included were males with broad experience (5 ± 1.26 years), well trained (11.61 ± 2.22 h·week^{-1}) and had accumulated large amounts of elevation both positive and negative in the preparatory period (116,615 ± 37,462 m). It took them 154 h 43 min (SD ± 23 min) (equivalent to 51% of VO_{2max}) to complete the 11 stages. They ran at an average speed of 5.11 ± 0.46 km h^{-1} and an average pace of 11 min 46 s (SD ± 3 min 4 s). No runner suffered any stress fracture. All participants were non-smokers and were not receiving medical, pharmacological, or dietary treatment.

Body composition measurements included: height, weight, skin folds and body mass index. All subjects were measured 2 h prior to the start of the race. Height measurement

was made to the nearest 0.1 cm using a wall-mounted stadiometer (Seca 220, Seca, Hamburg, Germany), body weight was measured barefoot to the nearest 0.01 kg on calibrated electronic digital scales (Seca 769, Seca, Hamburg, Germany), skin folds used a compass accurate to ±0.2 mm (Seca 212, Seca, Hamburg, Germany) and a tape with an accuracy of ±1 mm was employed. Six skin folds were taken: abdominal, suprailiac, subscapular, tricipital, thigh and leg and perimeters; arms and legs were in a relaxed 90° position. The equations of Yushaz were used to calculate the percentage of fat [28] and the equation according to Lee to determine the percentage of muscle [29].

A cardiopulmonary test assessed the following physiological outcomes: maximum oxygen consumption (VO_{2max}), heart rate maximum (HR_{max}) and maximal aerobic speed (MAS). The laboratory test was performed on a treadmill (Pulsar, h/p/cosmos®, Nussdorf, Germany). The test was run on a 1% slope and the start speed was set to 8 km h^{-1}, which increased 1 km·h^{-1} every minute. To warm up the subjects ran for 5 min on the treadmill operating at a speed of 6 km·h^{-1}. Respired gases were collected with an Oxycon Proanalyzer (Erich Jaeger GmbH, Hoechberg, Germany). The gas analysis system was calibrated according to ambient temperature and humidity, air flow and VO_2 and VCO_2 concentrations. A pulsometer was used to evaluate the maximal heart rate (Vantage M, Polar, Finland). The participants' pre-race characteristics are listed in Table 1.

Table 1. Pre-race individual characteristics of the population included (n = 4).

Parameters	Subject 1	Subject 2	Subject 3	Subject 4
Age (years)	33	37	41	42
VO_{2max} (mL/kg/min^{-1})	58.28	70.6	67.1	50.71
HR_{max} (beats·min^{-1})	194	186	194	176
Maximal aerobic speed (km·h^{-1})	18	17	16.7	16
Height (cm)	180.7	176.1	172.3	173.9
Weight (kg)	79.1	64.9	60.8	77.3
BMI	24.2	20.9	20.5	25.6
Fat mass (%)	8.82	6.88	8.70	8.14
Muscle mass (%)	43.4	47.38	57.63	38.55
Experience (years)	6	6	4	7
Distance covered (h·$week^{-1}$)	11	11	15	11
Annual slope accumulated (m)	140,655	120,404	156,000	70,000

BMI, body mass index; HRmax, heart rate maximum; VO_{2max}: maximum oxygen consumption.

The GR-11 route joins the Mediterranean and Atlantic coasts along the Pyrenees, covering 786 km in 11 stages. The average stage/day consisted of 71.49 km (SD ± 8.2), the average positive elevation was 4260.45 ± 1063.26, and the average negative elevation was 4258.63 ± 989.13. The race had a warm temperature, with values ranging from 13.1 to 17.6 °C, and the humidity was (60.1–70.9%). In-race hydration was provided ad libitum. The characteristics of the ultra-trail race are listed in Table 2. This table has been previously published [30]. This case report is part of a series of case studies aimed at studying the effects on runners' health after completing this unique ultra-trail challenge called "GR-11"; accordingly, the characteristics of the ultra-trail (i.e., duration, positive and negative elevation) are the same in both case studies.

Table 2. Characteristics of the extreme ultra-trail [30].

Stages	Distance (km)	Elevation (m+)	Elevation (m−)
1	78.5	3136	3024
2	72.3	3886	3458
3	72	4655	4044
4	68.1	5660	4581
5	72.6	5411	6336

Table 2. *Cont.*

Stages	Distance (km)	Elevation (m+)	Elevation (m−)
6	76.1	5344	4788
7	63.7	5492	5163
8	66	3641	4576
9	66.1	3361	3841
10	66.5	2958	2934
11	83	3321	4100
Total	784.9	46,865	46,845
Md	71.35	4260.45	4258.63
Sd	±6.00	±1063.26	±989.13

Twenty milliliters of venous blood (antecubital vein) were withdrawn from each participant pre- and post-race, rec2, and rec9 evaluations (90 min before and 10 min after finishing the race, two days and nine days in the morning). Blood samples were collected in two 5-mL Vacutainer tubes (Beliver Industrial State, Plymouth PL6 7BP, UK) without anticoagulant for serum isolation and in two 5-mL tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Once collected, the blood samples were coagulated for 25–30 min at room temperature and then centrifuged at 2500 rpm for 10 min to remove the clots. Serum samples were aliquoted into Eppendorf tubes (Eppendorf AG, Hamburg, Germany), washed with diluted nitric acid, and stored at −80 °C until biochemical analysis. To facilitate the interpretation of the data, the change in analytical parameters was measured as follows: post-race, 2, 9 days less pre-race respectively. Statistical analyses were carried out using the Statistical Package for The Social Sciences software (IBM SPSS Statistics for Windows, version 26.0, 64 bits Edition, IBM Corp., Armonk, NY, USA). Descriptive analysis was carried out on all variables, and average, median and standard deviations were calculated. Normal distribution of the variables was verified by using Kolmogorov-Smirnov and Shapiro-Wilk tests, but normality criteria were not met because of the low number of subjects. *p*-value was calculated, but due to low number of subjects included and the design of the study as a series of case studies, its value was not considered for final analysis. The BTM changes analyzed are listed in Table 3. Range values were expressed for OC, ALP, CTX and Ca^{2+} according to age, sex, and race [31]. All bone formation markers included (OC and ALP) decreased their values when comparing pre- and post-exercise. Conversely, all the bone resorption markers decreased after race completion. During the recovery period, OC and ALP values remained above the basal line, even at rec9 (OC = −45.78% and BALP = − 54.65%). In contrast, the CTX values increased slightly at rec2 (+11.93%) but soared at rec9, with values close to +100%. (CTX = +93.41%). Serum calcium levels decreased slightly when comparing pre- vs. post-and pre- vs. rec2. However, the rec9 values exceeded the pre-race levels (Ca^{2+} = +3.15%) (See Table 3). The chronological sequence of BTMs is fully shown in four different figures included in Figure 1.

Table 3. Blood parameters before (baseline) and after race (post-exercise day 2 and post-exercise day 9).

Parameter Blood (Reference Values)	Before-Race	Post-Race		
	Pre (Baseline) Value	Post (Post-Exercise) Value (% Difference)	Day 2 (rec2) Value (% Difference)	Day 9 (rec9) Value (% Difference)
OC (ng/mL) (13.98–41.99)	22.20 ± 7.41	11.15 ± 3.14 ↓ (−45.78)	10.30 ± 2.29 ↓ (−48.31)	15.14 ± 5.73 ↓ (−25.12)
BALP (ug/L) (6–30)	23.03 ± 4.68	8.64 ± 1.63 ↓ (−61.74)	8.50 ± 2.37 ↓ (−61.66)	10.29 ± 2.30 ↓ (−54.65)

Table 3. *Cont.*

Parameter Blood (Reference Values)	Before-Race	Post-Race		
	Pre (Baseline) Value	Post (Post-Exercise) Value (% Difference)	Day 2 (rec2) Value (% Difference)	Day 9 (rec9) Value (% Difference)
CTX (µg/L) (0.23–0.94)	0.24 ± 0.02	0.32 ± 0.09 ↑ (+37.28)	0.26 ± 0.12 ↑ (+11.93)	0.46 ± 0.14 ↑ (+93.41)
Ca^{2+} (mg/L) (8.70–10.40)	9.35 ± 0.33	9.22 ± 0.32 ↓ (−3.60)	9.03 ± 0.34 ↓ (−3.38)	9.64 ± 0.12 ↑ (+3.15)

Data are expressed as absolute values and as ± percentages from baseline values. OC, osteocalcin; BALP, alkaline phosphatase; CTX, C-terminal cross-linking telopeptide of type I collagen; Ca^{2+}, calcium.

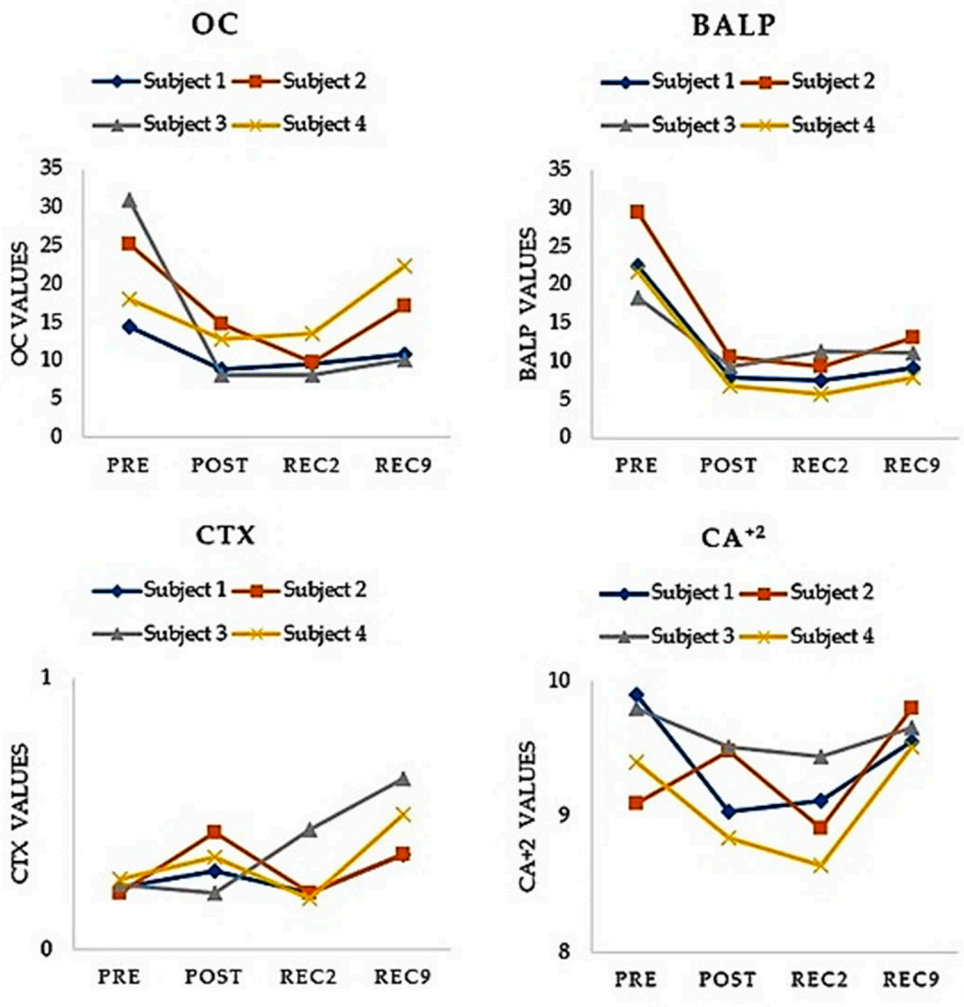

Figure 1. Chronological sequence of serum variables of bone metabolism of each of the 4 subjects. No statistically significant differences were found when comparing pre vs. post, rec2 and rec9 ($p > 0.05$).

3. Discussion

The objective of this case report was to assess the alterations suffered on BTMs after completing a multi-stage ultra-trail and in the recovery period. The main finding of our study was suppression in bone formation and an increase in the bone resorption process, not only after completing the race but also in the recovery period (2 and 9 days after), respectively. To the best of the authors' knowledge, this has been the first study to assess BTMs in such an extreme multi-stage ultra-trail after finishing the race and even nine days after in the recovery period. Only three previous studies have studied BTMs in ultra-endurance races so far, but the duration (from 245 to 308 km) and the elevation of

these events were not as extreme as in the race included in this study [23,24,32]. To better understand the discussion, the text contains different points listed below.

3.1. Bone Formation Biomarkers

The results included in our study showed that bone formation markers reduced their values after completing an ultra-trail race competition and two and nine days after finishing it (See Table 3). These results fall in line with those previously reported by other authors [23,24,32]. However, these previous studies did not include such a long race nor assessed BTMs after nine days after completing the event. Despite the increase of new BTMs in recent years (e.g., Procollagen type I N-terminal propeptide (PINP) and procollagen type I C-terminal propeptide (PICP) [21], OC and BALP are still reliable markers of bone formation. Many of the most prestigious institutions related to bone health, including The National Health Allegiance (NBHA) [33], the International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), support the use of OC and BALP as clinical biomarkers for OP [19,34].

OC is a non-collagen protein synthesized exclusively by the osteoblasts and plays a pivotal role in osteogenesis [34]. Several studies have analyzed OC as the main BTM of bone resorption in ultramarathons [20,23,24,35]. Three out of the four found decreases in OC levels [20,23,24] but only one of them found no differences after finishing the race [32]. OC decrease during strenuous exercise has been explained by an increase in parathyroid hormone and cortisol [24]. The action of these hormones suppresses the activity of the osteoblasts or reduces osteoblast release as a consequence. Malm et al. found a four-fold increase in cortisol levels after completing a marathon [35]. In the same line, Knectle et al. found rises in cortisol and catecholamines and decreases in growth hormone, which shows the complexity of the alterations suffered by the hypothalamic-pituitary axis in these efforts [36]. In the recovery period, our study showed a relevant decrease of OC levels, even nine days after, which implies bone formation function remained partially suppressed days after completing the ultra-trail race. Other studies have also evaluated the activity of OC and have found similar results. Nizet et al. in a study that evaluated a marathon race, observed a decline in OC levels after the race (from 4.9 to 3.9 g/liter, −20%) and three days later. BALP is a homodimer anchored to the membrane of osteoblasts and matrix vesicles [37]. Although its exact function is not completely clear, the presence of alkaline phosphatase on the cell membrane is required for bone mineralization [38].

The results found in our study support the idea that repeated weight-bearing exercise may result in a suppression of the activity of BALP. However, BALP is mainly affected by hormonal levels altering its release. Malm et al., in a comparative study, only found significant decreases of BALP in the female group [39]. In this sense, the validity of BALP as a conclusive bone formation biomarker seems lower than OC or PINP [33]. Our study found higher decreases of BALP in the three measurements (−61.74%, −61.66% and −54.51%), despite the subjects of our study only being of male gender. This fact may be due to the excessive distance and the extraordinary elevation of the ultra-trail race in our study in comparison with previous research.

3.2. Bone Resorption Biomarkers

CTX is the result of osteoclastic bone resorption, and it is a type I breakdown product [38]. CTX has been proposed as the gold standard for assessing the bone resorption process [33]. Our study showed relevant decreases in CTX values after finishing the race and in the recovery period. Similarly, previous studies on marathons [40] and on ultra-trail races [20] have shown CTX increases ranging from +8% to +19%. The values found in our study were higher, especially at rec9 (+93.41%). The duration of the effort has been proposed as the main factor responsible for the increase of CTX [20]. According to many authors, [22,41] prolonged mechanical usage increases microdamage. It seems reasonable that higher values of CTX were found in our study because of the extreme duration of a 768 km run.

Ca^{2+} values slightly varied in the three measurements of our study (−3.60%, −3.38 and +3.15%). Similar increases were reported by Nila et al. [42]. After the race Ca^{2+} increases from 9.2 ± 0.1 (mean ± SE) to 9.8 ± 0.1 mg/dL ($p < 0.01$). Another study that evaluated the alterations of Ca^{2+} in marathon runners (eleven men and seven women) found no increases in Ca^{2+} after the end of the race. The activity of Ca^{2+} has also been associated with duration and intensity of effort, apart from the adrenergic activation post-exercise [36]. The multi-stage here studied (768 km and 11 stages) exceeds the duration and the elevation of the races analyzed in previous research, so the higher increases in Ca^{2+} values at post and rec2 are mainly justified by these specific characteristics.

3.3. Stress Fractures and BTMs

The four subjects included in our study suffered no stress injury in the course of the ultra-trail race despite the BTM alterations found in our study. The incidence of stress fractures that the scientific literature has reported in ultra-endurance sports and ultra-trail, in particular, is relevant, oscillating their values from 0.3% in femur or hip to 1.2% tibia or fibula [12]. High rates of bone remodelling have been associated with an increase in suffering of stress fractures, regardless of BMD loss measured by DEXA or ultrasound [38]. Tian et al., in a systematic review. found positive relationships between CTX and risk fractures (1.20, 95%CI, 1.05–1.37). The ACSM has shown that the most influential factors for stress fractures are exercise mode, intensity and duration. Accordingly, stress fractures occur as a result of excessive training activity due to repetitive mechanical loading [16]. It is because of these microfractures that bone resorption activity increases. Vasikaran et al., in a systematic review, found several studies that associated BTMS changes and subsequent fractures [19].

Traditionally, the studies investigating hip fractures have usually focused on women, due to hormonal reasons behind the development of OP and, consequent higher incidence of hip fractures. Nevertheless, studies including men have also found BTM changes prior to suffering a stress fracture [16]. Studies that have analyzed the relationship between BMD loss and the likelihood of stress fracture in running activities have found significant associations [16,27,36].

Bennell et al., in a 12-month prospective study, found no differences in OC values between athletes who suffered a stress fracture and did not ($p = 0.010$) [15]. On the contrary, Sayaka et al., in another study including young athletes, found an incidence of stress fractures higher than in other similar studies (11.4% of 316 athletes), but there was no statistical difference in BTMs [16]. We can conclude that the value of BTM alterations as a tool for predicting stress fractures is contradictory. This discrepancy in the results obtained is due to the variety of BTMs analyzed, as well as the different characteristics in the population included and differences in running activities studied. The characteristics of the ultra-trail races (i.e., duration and elevation) would require further investigation considering these characteristics.

3.4. Limitations

It must be considered that the sample size ($n = 4$) and the only male gender used in this study could be a limitation that had an impact on the results obtained. The main reason to justify the design of this study was due to the uniqueness and extreme conditions of the race (e.g., duration, number of stages and positive and negative elevation accumulated). As our study shows, there are several alterations on BTMs after finishing an ultra-trail race and at least nine days after in the recovery period. However, further investigation is still required in order to clarify the mechanisms involved in BT and its relation with BMD and/or BC loss, and, ultimately, the etiology of stress fractures in these efforts. More epidemiological studies, including analyses of BTMS and DXA or ultra sound measurements, are needed to better elucidate the mechanisms involved in BMD.

4. Conclusions

According to this study, during an ultra-trail race it appears that bone resorption is increased and, conversely, bone formation is suppressed, resulting in a transient uncoupling of BT. The levels of all BTMs analyzed remained altered when compared with pre-run levels, especially in CTX and OC, even nine days later. This study showed that a 768-km multi-stage ultra-trail induces changes in the OC/Ca^{2+}/BALP/CTX interaction, which may result in an increase in the likelihood of stress fractures as a consequence of damage to bone tissue. The special preparation that these athletes had to carry out to face the race implies a great amount of training volume (e.g., kilometers accumulated, n° sessions·week^{-1}) prior to the race, so the study of which BTMs reflect bone damage may help in preventing runners suffering from BMD loss and in avoidance of stress fractures. Considering the results of this case report, runners and coaches should analyze alterations in BTMs, not only immediately after the race but also in the recovery period. The analysis of the BTMs here presented offers valuable information about load in bone tissue during the training process and may help runners reduce the likelihood of suffering stress fractures. The training process of these races requires a structured program of scientific monitoring in physiological, biomechanical and performance areas. According to these findings, BTMs should be measured as part of the preparation routine for ultra-races to prevent ultra-runners suffering bone turnover alterations.

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Data Availability Statement: Information about the case report is available at http://gr11en11.org/ (accessed on 26 October 2021).

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4. Discusión

A continuación, se presenta la discusión de los principales resultados hallados, y publicados en cuatro artículos científicos, uno de revisión sistemática y tres estudios de caso, considerando el objetivo e hipótesis general y los objetivos e hipótesis específicos planteados en esta Tesis Doctoral por compendio de publicaciones (apartados 1.1 y 1.1.2). Con el fin de facilitar la discusión en referencia a los diferentes objetivos específicos, la discusión se ha dividido en diferentes apartados. En primer lugar, se detallan efectos generales sobre el organismo provocados tras realizar una UT; posteriormente, se detalla la incidencia y alteraciones en biomarcadores relativos al AKI (4.2); en un tercer apartado se reflejan los aspectos relativos al EAH (4.3); en el cuarto apartado se describen los resultados hallados en relación con ER y las pérdidas asociadas de fuerza en miembros inferiores (4.4). Finalmente, y para concluir en el apartado (4.5) se valora el daño en el tejido óseo.

4.1 Efectos negativos en la salud de las carreras de ultra trail

Como se ha comentado anteriormente, las UT han experimentado un considerable aumento de popularidad (Spittler & Oberle, 2019). Este incremento es en parte debido a la incorporación y creciente participación de mujeres en estas pruebas, representando un 20% del total de participantes, y el incremento de corredores más jóvenes (Ehrensperger et al., 2013). El perfil tradicional de un corredor de UT era un varón de 45 años con una amplia experiencia en carreras de distancias menores. La experiencia previa (p.ej. números de carreras completadas y mejores tiempos), ha demostrado ser uno de los factores más importantes a la hora de predecir el rendimiento en este tipo de competiciones, tanto en hombres como en mujeres (Nikolaidis & Knechtle, 2020). Sin embargo, esta experiencia no ha demostrado ser un factor protector frente a la aparición de alteraciones en biomarcadores, que indican un proceso patológico en ciertos órganos y sistemas como el corazón (Predel, 2014), el hígado (Lecina et al., 2021), el riñón (Hodgson et al., 2017), el sistema músculo esquelético (Belli et al., 2018) o el sistema endocrino (Kupchak et al., 2014).

El impacto real de estas alteraciones en la salud del corredor es aún hoy en día motivo de discusión científica, siendo para algunos autores simples adaptaciones transitorias y reversibles fruto de la capacidad adaptativa del organismo, mientras que en algunas ocasiones, estas modificaciones en los biomarcadores traspasan los limites fisiológicos y se convierten en patologías agudas que pueden afectar seriamente la salud de los corredores (Runacres et al., 2021).

La diversidad de perfiles fisiológicos y morfológicos existente en las UT, a raíz del auge de participantes de diferentes edades, experiencia y sexo, obliga a estudiar de una forma más exhaustiva estas alteraciones, detallando los efectos que provocan en diferentes parámetros, con el fin de reducir los riesgos agudos asociados a la participación en este tipo de carreras, y prever posibles efectos deletéreos a largo plazo. Los artículos relacionados en la Tesis Doctoral incluyen, no sólo los efectos agudos visibles tras acabar la prueba, sino también el efecto crónico a los dos y nueve días de haber finalizado la prueba, con el fin de dilucidar si los efectos descritos se limitaban a la propia finalización de la misma o implicaban efectos más duraderos.

A continuación, se discuten los efectos hallados en las variables estudiadas relacionadas con biomarcadores renales, óseos, musculares y de desequilibrio de electrolitos.

4.2 Alteraciones en la función renal

La presencia de alteraciones en la función renal en deportes de ultra resistencia ha sido estudiada en revisiones sistemáticas previas, sin embargo, ninguna de ellas centró su estudio en el desarrollo de AKI en una UT, ni analizó la incidencia conjunta de AKI y EAH (Hodgson et al., 2017; Rojas-Valverde et al., 2020). El estudio del deterioro agudo de la función renal fue analizado en tres de los cuatro artículos incluidos en esta Tesis Doctoral, a través de dos estudios de caso (Lecina et al., 2021; Lecina et al., 2022) y una revisión sistemática (Lecina et al., 2022). La primera publicación es una revisión sistemática en la que se analizaron la incidencia de AKI y EAH en una UT con distancias mayores de 42,195 km y desniveles superiores a 500 metros, tanto de manera aislada como simultánea.

Dicha revisión es la primera publicación conocida en donde se estudian de manera conjunta ambas condiciones médicas, analizando su incidencia y gravedad de acuerdo al tipo de carrera (distancia y presencia o no de más de una etapa). Los estudios se clasificaron según la longitud y las etapas de la carrera en cuatro categorías: de alcance medio (42 a 69 km), largo (70 a 99 km), extra (> 100 km) y multietapa (si incluían varias etapas). El número total de casos de AKI encontrados fue de 468 con una incidencia del 42,04% (*n* = 1.113). Estos resultados son similares a los hallados en otras revisiones sistemáticas en donde se analizaba la incidencia de AKI en deportes de ultra resistencia (Hodgson et al., 2017; Rojas-Valverde et al., 2020). A pesar de la gran muestra analizada, tan solo un sujeto alcanzó un estadio de fallo, aunque no precisó de trasplante renal ni diálisis (Cuthill et al., 2009). Teniendo en consideración estos hallazgos se planteó la realización de un estudio de caso. En esta investigación se analizaron los valores de SCR (pre vs. post = 0,88 vs. 1,13 mg/Dl (+28,41%)). Los datos obtenidos fueron similares a los descritos en otros estudios en los que se analizaba la función renal en pruebas de UT, hallándose incrementos en el parámetro SCR aunque sin sufrir AKI (Khodaee et al., 2021), al utilizar las herramientas diagnósticas (AKIN, RIFLE o KDIGO) (Lopes & Jorge, 2013).

4.2.1 Biomarcadores y criterios diagnósticos asociados

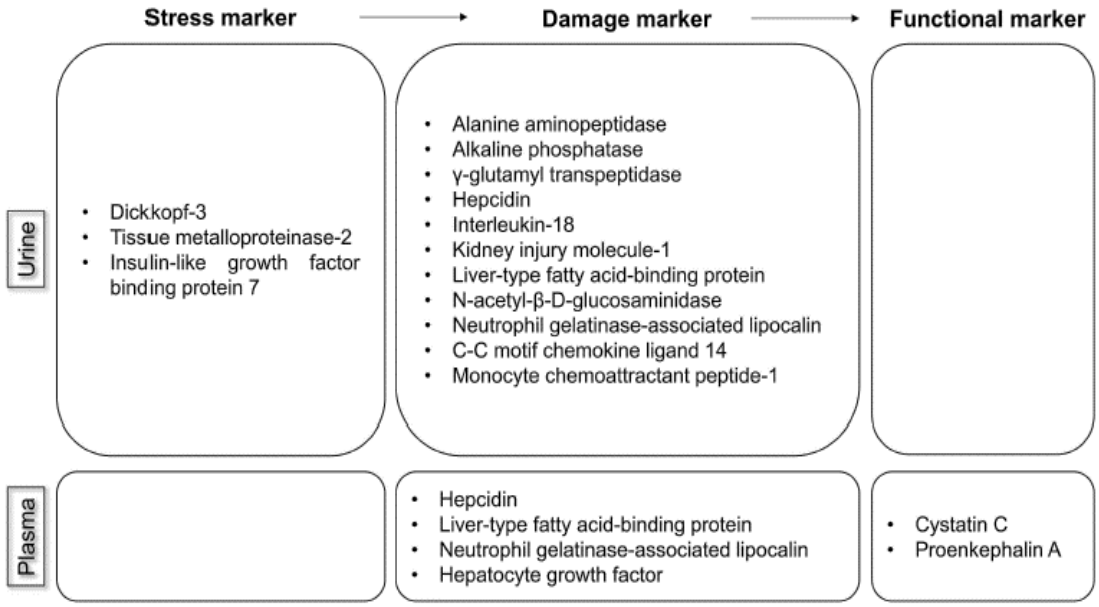
El diagnóstico de AKI sigue a día de hoy bajo discusión entre las sociedades médicas de nefrología (Makris & Spanou, 2016). Las principales herramientas diagnósticas utilizadas en las tres clasificaciones son: la medida de SCR, la determinación de EFG y la producción de orina (Lopes et al., 2016). La principal diferencia entre las tres son los porcentajes de variación de los parámetros SCR y EFG que se precisan para alcanzar cada estadio de gravedad (Barry & James, 2016).

Esta inexactitud en el diagnóstico de AKI en el ámbito hospitalario es todavía mayor en el ámbito deportivo y, en especial, en las UT (Little et al., 2019). Debido a la discrepancia de resultados obtenidos al diagnosticar AKI, mediante los marcadores disponibles en la actualidad, varios autores han planteado diferentes hipótesis que podrían explicar los posibles motivos de esta dificultad en su diagnóstico. La más aceptada de todas ellas se basa en que la fuerte ER que

sufren los deportistas no estaría contemplada en las fórmulas de EFG diseñadas, enfocadas mayormente al ámbito clínico y hospitalario (Poussel et al., 2020). Otro factor que podría afectar la validez de EFG es que al ser una fórmula calculada mediante el SCR, precisa de la estabilización de este biomarcador, acontecimiento que no ocurre durante las carreras de ultratrail hasta pasadas 16 horas desde la finalización de la prueba (Belli et al., 2018). Contrariamente, la elevación de CK, que es el marcador estándar de ER, sucede muy rápidamente, habiéndose encontrado valores por encima de 40.000 U/L a las pocas horas del comienzo de la prueba (Chlíbková et al., 2015; Knechtle & Rosemann, 2015). Sin embargo, los incrementos de CK no se correlacionan con incrementos lineales de SCR o EFG, por lo que no se plantea el uso de CK como diagnóstico de AKI en lugar de SCR o EFG. Lo que sí resulta muy evidente es que en estas carreras, en algún momento del transcurso de la prueba, los deportistas sufrirán una elevación del biomarcador SCR (Lipman et al., 2016; Martínez-Navarro et al., 2020).

A pesar de todos los esfuerzos realizados por la investigación científica, todavía no está claro hasta qué punto estas alteraciones en el biomarcador SCR son meras alteraciones transitorias compensatorias del sistema renal o, por el contrario, constituyen una entidad patológica real que pueda suponer un riesgo para los corredores. En este sentido, los estudios que han demostrado variaciones en el parámetro SCR al finalizar una prueba de UT, la repercusión real que los aumentos de SCR implican en el daño renal, y si éstos constituyen una entidad patológica o por el contrario son un simple signo del síndrome general de adaptación del organismo en su etapa de estrés agudo, continúa sin estar totalmente establecida (Hodgson et al., 2017; Lecina et al., 2022). En la actualidad se están desarrollando nuevos marcadores que sean capaces de diagnosticar la presencia de AKI en sus fases iniciales, pero su uso está de momento limitado al ámbito clínico y no han sido utilizados aún en ambientes ajenos al del uso médico (Mercado et al., 2019). Estos marcadores serían de tres tipos: de estrés, de daño renal y marcadores funcionales, y se obtendrían tanto de plasma como de orina (Figura 4).

Figura 4. Principales biomarcadores de AKI. (Mercado et al., 2019).



4.3 La hiponatremia post ejercicio en las carreras de ultra trail

EAH, se define como una concentración de sodio sérica por debajo de lo normal (< 135 mmol/L), durante o hasta 24 horas después de la actividad física (Spasovski et al., 2014). Ha sido clasificada en tres grados de severidad en función del descenso de [Na^+]: leve (130-135 mmol/L), moderada (125-129 mmol/L) y severa (< 125 mmol/L) (Hew-Butler et al., 2015). El mayor factor etiológico para la hiponatremia es un aumento en la ingesta de líquidos durante la carrera, provocando un descenso en la concentración de [Na^+] y, por tanto, un aumento en el peso corporal (Chlíbková et al., 2016). Sin embargo, la hiponatremia también puede ser euvólica (no se produce una alteración en el peso corporal pre carrera vs. post carrera), y representa el 60% de todos los casos en pacientes hospitalizados, o hipovolémica cuando se asocia con un bajo volumen plasmático (Sahay & Sahay, 2014). La EAH presenta una variedad de síntomas y resulta compleja y poco específica (Rosner & Kirven, 2007), en donde se incluyen síntomas como: náuseas, mareos, aumento de presión intracraneal, pérdida de consciencia y, en casos muy severos, incluso muerte (Shephard, 2011).

4.3.1 Incidencia de EAH en las pruebas de ultra trail

En nuestra revisión sistemática los casos totales de EAH que se hallaron fueron de un 6,16% (*n* = 195), sobre una muestra total de 2.778 corredores, siendo estos valores inferiores a los encontrados en estudios similares (Cuthill et al., 2009; Hoffman et al., 2018). Cabe destacar que ningún corredor sufrió EAH severa y tan solo 19 (1,13%) padecieron EAH moderada.

Este dato fue respaldado por nuestro estudio de caso (Lecina et al., 2021), en donde al corredor, a pesar de la duración de la prueba, no sólo no le descendieron sus valores basales de [Na^+], sino que aumentaron al comparar pre vs. post (136 vs. 140 mmol/L), sufriendo una hipernatremia. La hipernatremia ha sido tradicionalmente relacionada con el incremento de peso y el aumento de suplementos de sodio en las bebidas (Lipman et al., 2021).

En cuanto a las categorías de pruebas que sufrieron mayor incidencia de EAH, las carreras de categoría extra alcanzaron un 12,19%, seguidas de las largas con un 8,69%, las multi con valores del 7,73%, y finalmente las medias en donde se hallaron un 2,69% de casos. Parece evidente pensar que en la aparición del EAH, a diferencia de AKI, existen más factores a tener en cuenta que en el desarrollo de EAH. Diferentes investigaciones han demostrado una relación directa entre EAH y factores como la velocidad de carrera lenta (Rust, 2012), el sexo femenino (Hew-Butler et al., 2015), un bajo peso corporal (Lipman et al., 2021), un excesivo consumo de líquidos antes de comenzar la carrera, protocolos de hidratación erróneos (Hoffman et al., 2019) y una temperatura extrema o exceso de humedad (Costa et al., 2013; Žákovská et al., 2017).

En este sentido, la duración del esfuerzo ha sido considerada como otro de los factores predisponentes de poder sufrir episodios de EAH (Hew-Butler et al., 2015; Noakes et al., 1990; Rosner & Kirven, 2007). De forma paralela, diferentes investigaciones han valorado si la duración de las carreras aumenta la frecuencia y gravedad de los episodios de EAH. En pruebas de media distancia se han hallado resultados de casos de EAH leves (Hoppel et al., 2019; Page et al., 2007), por ningún caso de EAH moderada (Chlíbková et al., 2019; Hew-Butler et al., 2008; Knechtle et al., 2012). En cuanto a las UT largas, existen resultados contradictorios, en un

estudio se han diagnosticado cuatro casos de EAH (Boulter et al., 2011), todos ellos leves, mientras que por el contrario, en otra investigación de índole similar realizada sobre una carrera con la misma distancia no se registró ningún caso de EAH (Jouffroy et al., 2019). En pruebas de categoría extra se hallaron una cantidad mayor de casos de EAH. En uno de los estudios de mayor relevancia, por el importante número de participantes incluidos, se hallaron 88 casos de EAH leve y 13 moderados, de un total de 669 sujetos (Hoffman et al., 2013). Resultados similares fueron descritos por Winger et al. (2013), encontrando 12 casos de EAH en una muestra total de 207 participantes. En esta misma categoría, otros tres estudios también informaron de la aparición de casos de EAH, pero con una incidencia menor, con 7, 8 y 4 casos, respectivamente (Cejka et al., 2012; Cuthill et al., 2009; Shephard, 2012). En sentido contrario, se encuentran los resultados de Bracher et al. (2012), donde ningún participante sufrió un episodio de EAH. En cuanto a las pruebas de varias etapas, varios autores hallaron un alto número de casos de EAH, en concreto 7 y 8 casos de un total de 120 y 74 participantes respectivamente, aunque todos ellos de categoría leve (Costa et al., 2013; Knechtle et al., 2011).

4.3.2 Factores que predisponen a sufrir EAH

De entre todos los factores que predisponen a la aparición de EAH, los más importantes son el aumento de peso corporal debido a la sobrehidratación, y la supresión no osmótica de la secreción de la hormona vasopresina (Chlíbková et al., 2016; Hoffman et al., 2018; Lipman et al., 2021). Sin embargo, en la actualidad no existe un consenso en torno a la cantidad de líquido que se debería ingerir durante la prueba, ni en consecuencia el porcentaje de pérdida de peso corporal (BDWL) que deberían soportar los corredores durante la carrera. Ni siquiera en las guías más relevantes, en relación a la hidratación en carreras de ultratrail (Hew-Butler et al., 2015; Hoffman et al., 2019), cuantifican estas variables. En la actualidad coexisten tres grandes corrientes relativas a las estrategias de hidratación en carrera: *ad thirst, ad libitum* y planificadas (Meyer et al., 2012). Sin embargo, el porcentaje exacto de BDWL que los corredores pueden perder durante la carrera, así como los métodos y medidas de composición corporal para ser medidos, continúan sin estar totalmente establecidos en las carreras de ultratrail (Hoffman et al.,

2018). Cejka et al. (2012), propusieron una medida alternativa de la sobrehidratación a BDWL, basada en el aumento del volumen de los brazos y piernas de los corredores, demostrando que el aumento de volumen ingerido tenía un efecto proporcional en el incremento del volumen de los pies de los corredores (r = 0,54, p < 0,0001), e inversamente relacionado con la disminución de [Na^+] (r = -0,28, p = 0,0142), y por consiguiente de EAH. Sin embargo, este método no se ha estandarizado y en investigaciones actuales continúa utilizándose el BDWL.

Si bien, muchas guías aconsejan un 2% de BDWL (Costa et al., 2013; Hew-Butler et al., 2015; Hoffman et al., 2019), muchos de los estudios recogidos en esta revisión hallaron episodios de EAH con dichos valores de BDWL o incluso mayores. Esto podría ser debido a la efectividad de las vías metabólicas de estos corredores, que mediante la adaptación al entrenamiento logran, a partir de la oxidación de sustratos energéticos, obtener más agua a través de estas reacciones químicas. Como resultado, estos sujetos producen mayores cantidades de agua endógena (Hoffman et al., 2018). A falta de otra medida más fiable, el BDWL es la más usada en las investigaciones. Diferentes estudios han encontrado pérdidas de peso inferiores al 2% en corredores sin episodios de EAH (Boulter et al., 2011; C. Cejka et al., 2012; Chlíbková et al., 2019; Costa et al., 2013; Hoffman et al., 2013; Knechtle et al., 2011; Martínez-Navarro et al., 2020; Scotney & Reid, 2015). Por el contrario, un estudio informó de ganancias medias de peso previas y posteriores a la carrera en cinco corredores que sufrieron episodios de EAH, aunque todos ellos leves (Page et al., 2007).

Las condiciones ambientales, al igual que las ganancias de peso, han sido estudiadas como factor de riesgo asociado a la aparición de episodios de EAH en carreras por montaña de larga duración, especialmente cuando las temperaturas son extremadamente frías o cálidas (Kaufman et al., 2014; Lee et al., 2011; Shephard, 2011). Chlibkoval et al. (2019), en un estudio observacional realizado en temperaturas extremas por frío intenso (-7,96 a -20ºC), aunque con un desnivel acumulado de solo 764 m, no encontraron ningún caso de EAH, a pesar que los participantes apenas perdieron peso corporal (-0,2 kg).

Estos datos contradicen a la mayoría de investigaciones en donde se defiende que, pérdidas de peso de al menos el 2% del peso corporal durante la carrera, son necesarias para evitar sufrir EAH (Hew-Butler et al., 2015). En esta misma línea, Costa et al. (2013), en una investigación realizada en una UT de 225 km por etapas, con temperaturas extremas de calor (32º a 40ºC), en la que participaron 74 corredores, encontraron ocho casos de EAH, todos ellos de categoría de EAH mild, con BDWL de -1,2%.

4.4 Rabdomiólisis post esfuerzo

El objetivo específico C de este estudio analizó las posibles alteraciones bioquímicas relacionadas con el daño muscular, determinando su probable relación con la pérdida de la capacidad contráctil de los músculos extensores de las piernas, y evaluar la relación entre los parámetros de entrenamiento, las alteraciones de los biomarcadores y la pérdida de fuerza en los miembros inferiores.

En estos momentos, parece ser que este estudio ha sido el primero en donde se compara la capacidad de contracción muscular, tanto en una situación de reposo como de fatiga, en una UT de varias etapas tan extremas. Existen diferentes estudios en donde se han analizado ER en UT de categoría extra (de 330 a 1.600 km) (Fallon et al., 1999; Knechtle et al., 2008; Pesic et al., 2019; Saugy et al., 2013), pero estas pruebas no incluyeron desniveles positivos y negativos tan elevados como en nuestro estudio. Adicionalmente, y a pesar de su diseño como caso de estudio, este trabajo muestra fuertes correlaciones entre los parámetros de entrenamiento y biomarcadores, por lo que, en consecuencia, las principales conclusiones reportadas pueden ofrecer información valiosa para corredores, entrenadores y personal médico, que ayude a comprender el comportamiento interno del músculo y que podría considerarse como una variable de respuesta de entrenamiento.

4.4.1 Aumento de los biomarcadores sanguíneos de daño muscular

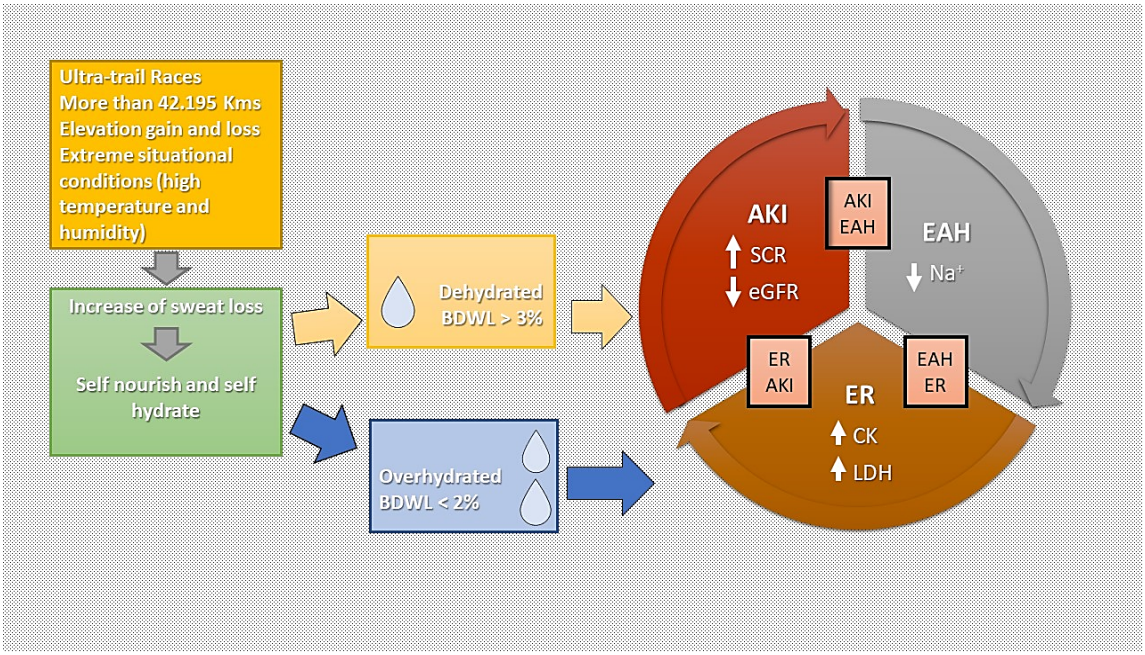
Los resultados obtenidos en este estudio coinciden con investigaciones previas que también muestran una elevación en los biomarcadores de inflamación y daño muscular (CK, LDH, AST y ALT), justo después de la finalización de una UT (Chlíbková et al., 2015; Kao et al., 2015; Martínez-Navarro et al., 2020), y después de un período de recuperación (Lecina et al., 2021). Parámetros como la CK y la LDH se han relacionado con la duración extrema de este tipo de carreras (Knechtle & Nikolaidis, 2018; V. Scheer et al., 2020), que según algunos estudios podrían ser la principal causa de fatiga y daño muscular, ya sea de manera directa o indirecta (Millet et al., 2002; Noakes & Carter, 1976). En esta línea, Skenderi et al. (2006), establecieron la elevación de ambos valores estudiando comparativamente dos grupos de corredores en dos distancias diferentes, obteniendo mayores niveles de CK y LDH en el grupo que cubrió la UT más larga. Otro estudio que reforzó esta hipótesis es el realizado por Fallon et al. (1999), en una prueba de 1.600 km, aunque en pista de atletismo y sin desnivel, y en donde hallaron incrementos significativos de CK y AST, aunque éstos no lo fueron significativos para los marcadores ALT y LDH. Considerando todos estos resultados se puede apreciar que existe una importante diferencia de criterio en las conclusiones encontradas en la literatura científica.

De manera específica, Martínez-Navarro et al. (2020), analizaron los incrementos de CK y LDH en UT de 65 km vs. una de 107 km, y observaron una elevación significativa en ambos biomarcadores en los dos grupos de estudio. Sin embargo, fueron en esta ocasión los corredores de la distancia más corta los que obtuvieron mayores incrementos en ambos valores, además de no regresar a los niveles basales tras 24 horas de recuperación.

Este hallazgo parece resaltar la idea de que no sólo podría ser la duración de la prueba el principal factor determinante en la liberación de CK y LDH (Koch et al., 2014).

Como consecuencia de estas discrepancias, en relación con las conclusiones presentadas por diferentes autores, se ha especulado con la importancia de tener en consideración dos factores más a la hora de estudiar la cinética de ambos biomarcadores. Por un lado, la velocidad de carrera, a partir de la cual se han registrado altos niveles de CK y LDH en estudios de prueba con una duración más corta, y donde la velocidad de carrera fue mucho mayor (maratón y media maratón), por el otro lado la distancia que siempre ha de ser superior a la prueba de maratón (Kyrolainen et al., 2000; Lippi et al., 2008; Rojas-Valverde et al., 2020). De forma paralela se sitúan las carreras multietapa, donde la recuperación entre las diferentes etapas de la carrera podría provocar una disminución en la liberación de ambos biomarcadores (Besson et al., 2020; Lecina et al., 2021; Rojas-Valverde et al., 2020). Esta discrepancia en las conclusiones ha generado que otros biomarcadores, como AST y ALT, también se tengan en cuenta para el posible diagnóstico de ER post. Estos elementos invitan a pensar que la elevación de estos valores es transitoria y sin repercusiones funcionales para el hígado, salvo en casos aislados (Knechtle & Nikolaidis, 2018). En nuestro estudio se describen valores extremadamente altos, incluso en los períodos de recuperación (rec9; AST = 103% y ALT = 226%). Estas diferencias conducen a un análisis en donde, además de la velocidad y duración de la carrera, existen otros elementos que todavía no se han tenido en cuenta como objeto de estudio y que podrían ser relevantes. Algunos autores determinan la temperatura, tanto fría como caliente, como un mecanismo influyente para los aumentos de AST y ALT (Knechtle & Nikolaidis, 2018), aunque es una hipótesis aún que presenta numerosas dudas. Autores como Žákovská et al. (2017), en un estudio realizado con temperaturas extremadamente frías (-1ºC a 1ºC), señalan que no hay diferencias en los valores de ALT pre y post carrera. Estos resultados muestran la posible interrelación fisiopatológica encontrada en nuestra revisión sistemática que relaciona las tres patologías AKI, EAH y ER (Figura 5).

Figura 5. Mecanismos fisiopatológicos de AKI, EAH y ER en las carreras de ultra trail.



4.4.2 Fatiga neuromuscular

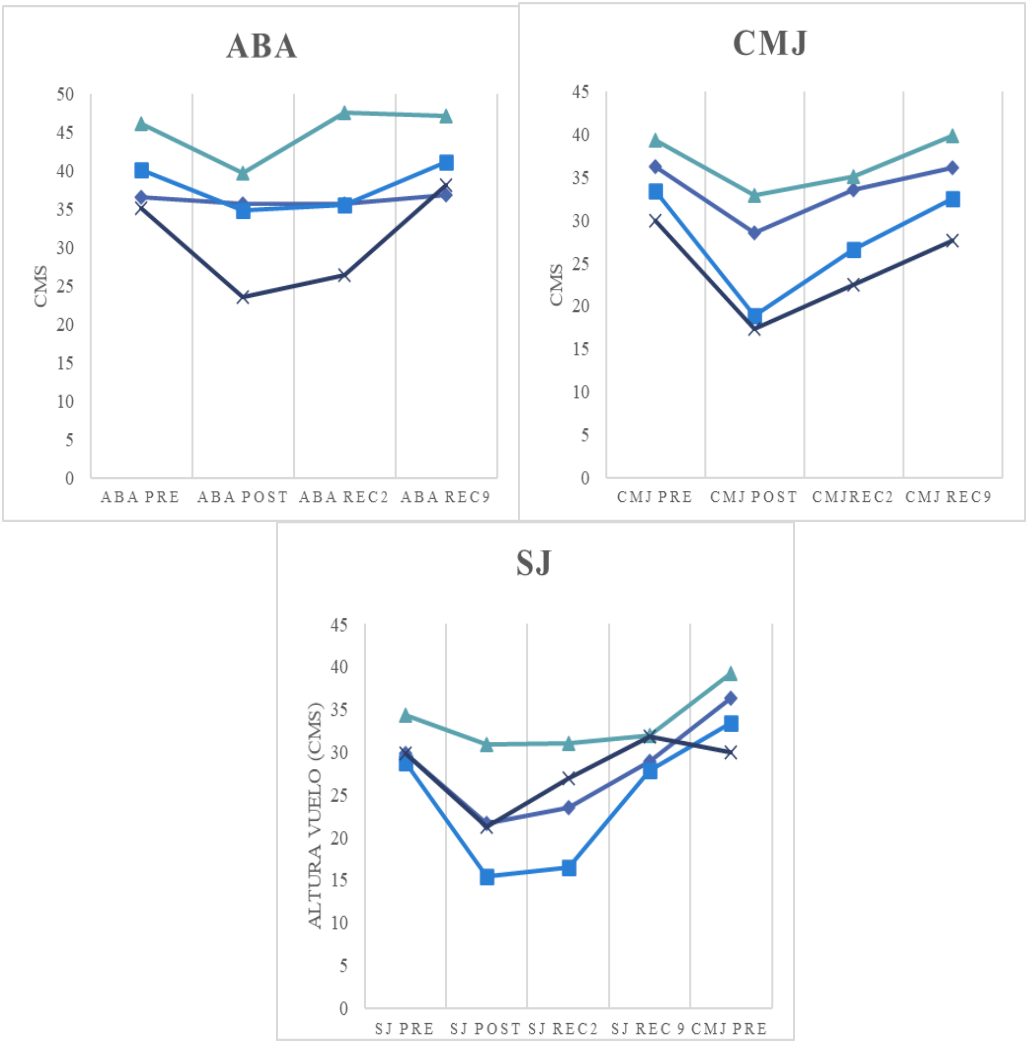
El estudio del efecto en la fatiga neuromuscular fue evaluado en el artículo *"768-km Multi-Stage Ultra-Trail Case Study-Muscle Damage, Biochemical Alterations and Strength Loss on Lower Limbs"* (Lecina et al., 2021). La fatiga muscular posee un doble componente: central y periférica. En esta Tesis Doctoral se evaluaron las pérdidas de fuerza muscular medida mediante tres saltos de la batería del test de Bosco (SJ, CMJ y ABA). Los corredores sufrieron pérdidas en la altura de vuelo al comparar los valores previos de la carrera respecto a los posteriores (SJ = −28%, CMJ = −36% y ABA = −21%), que se recuperaron gradualmente durante rec2 (SJ = −11%, CMJ = −15% y ABA = −8%), volviendo a sus resultados basales en rec9 (SJ = −1%, CMJ = −2% y ABA = +3%.).

Estos datos se encuentran en consonancia con los resultados reportados por otros estudios similares, aunque a diferencia del presente estudio de caso, en ellos se incluían carreras de menor duración y de una única etapa.

Martínez Navarro et al. (2020), en un estudio comparativo de dos UT (65 y 107 kms), hallaron diferencias significativas en los valores de vuelo pre-post en SJ (107 kms pre = 24,4±4,1 vs. post = 18,4±42,2; F = 7,40; p <0,01; η2 partial = 0,14). En este sentido, Balducci et al. (2017), mostraron descensos en altura de vuelo de CMJ pre-post significativos. Landart et al. (2020), en una maratón de montaña (42 km), también demostraron descensos en la altura de vuelo en ABA pre-post, aunque las diferencias no fueron significativas, hecho que reforzaría la hipótesis de que duraciones mayores de la carrera provocarían descensos mayores de los test de fuerza muscular en las extremidades inferiores. En nuestro estudio, los descensos mayores fueron hallados en el salto CMJ. De los tres saltos incluidos en el protocolo, el CMJ es el movimiento que posee una mayor participación del componente elástico muscular excéntrico (González et al., 2008). El componente excéntrico muscular parece ser el más afectado por el esfuerzo excéntrico, al afrontar descensos pronunciados implícitos en el desnivel negativo de los recorridos de este tipo de competiciones (Rojas-Valverde et al., 2019). En cuanto al test de ABA, se pone de manifiesto que las pérdidas fueron menores, posiblemente debido a la participación de los miembros superiores en la ejecución del movimiento y la producción final del salto (Sáez de Villarreal, 2004).

Los valores hallados (Figura 6), refuerzan la hipótesis que la duración y los desniveles asociados a este tipo de pruebas son los principales causantes de las disminuciones de fuerza contráctil en los músculos extensores de los miembros inferiores (Vuorimaa et al., 2006).

Figura 6. Variaciones de SJ, CMJ y ABA durante la prueba de cada uno de los sujetos.



4.4.3 Asociación entre biomarcadores y pérdidas de fuerza contráctil

El estudio *"768-km Multi-Stage Ultra-Trail Case Study-Muscle Damage, Biochemical Alterations and Strength Loss on Lower Limbs"* (Lecina et al., 2021), evaluó la asociación entre los incrementos en los marcadores sanguíneos de daño muscular en post, rec2 y rec9. A pesar de que no se hallaron asociaciones estadísticamente significativas (p <0,05), probablemente relacionado con el tamaño muestral del estudio (n = 4), sí se encontraron asociaciones fuertes e incluso muy fuertes (R_s <0.80), tanto en post como en rec2 y rec9. En cuanto a los valores obtenidos en post, se observa una fuerte correlación entre SJ y los biomarcadores LDH y AST, aunque no se aprecian para el resto de las variables analizadas (CK y ALT). Estas asociaciones difieren de otras investigaciones donde CK ha sido el biomarcador de daño muscular más

relacionado con las pérdidas de capacidad contráctil de los músculos extensores de las piernas (Bäcker et al., 2020). Hody et al. (2013), en un estudio que valoraba la relación entre un ejercicio excéntrico y los aumentos de CK, demostraron una asociación significativa. Kupchak et al. (2014), en la carrera "*The Western States Endurance Run*" de 161 km, encontraron incrementos de CK hasta de siete veces, pero este estudio no evaluó las pérdidas de fuerza en las extremidades inferiores sino en miembros superiores.

Tras analizar los datos aportados por nuestro estudio de caso, y el resto de los estudios descritos, se pone en evidencia que el uso de marcadores de daño muscular como evaluación de las pérdidas de fuerza periférica, puede ser útil para prevenir la aparición de ER, aunque se precisarían más investigaciones que aporten más datos y que permitan el desarrollo de esta línea de investigación.

4.4.4 Relación entre pérdidas de fuerza y parámetros del entrenamiento

En este trabajo se investigó la posible asociación entre la pérdida de altura de los saltos realizados al finalizar la prueba y diferentes parámetros del entrenamiento: experiencia en competición en UT (EXP), volumen de horas de entrenamiento semanales (VOL) y la acumulación total de metros entrenados en desnivel (SLO) (Lecina et al., 2021). No se apreciaron asociaciones estadísticamente significativas ($p < 0,05$), probablemente debido al reducido tamaño muestral ($n = 4$). A partir de un análisis estadístico no paramétrico más acorde al tipo de muestra analizada, sí se hallaron asociaciones fuertes y muy fuertes con las variables de LDH pre-post respecto a SLO y EXP. Estos resultados abren la vía de nuevas estrategias a la hora de plantear el entrenamiento de los corredores, aplicando cargas específicas del trabajo de fuerza para reducir el daño muscular durante la competición, así como optimizar la recuperación tras la prueba. La literatura científica se muestra contradictoria a la hora de evaluar si las adaptaciones de un trabajo excéntrico orientado hacia el desarrollo de la fuerza, puede tener efectos positivos o negativos en estas pruebas de ultra resistencia. En este sentido, trabajos como los de Eston et al. (1996), determinaron que la realización de trabajo específico de fuerza

con énfasis excéntrico en la fase preparatoria, produjeron una disminución de las pérdidas medias de fuerza a partir de la evaluación del rendimiento en el SJ. Resultados similares fueron descritos por Hinks et al (2021), demostrando en un estudio comparativo que el grupo que realizó un ejercicio excéntrico previo tenía un efecto atenuante en las pérdidas de fuerza. Sin embargo, estos resultados no tenían como objetivo los músculos extensores del tren inferior sino sobre la musculatura del bíceps braquial. Resultados opuestos fueron hallados por otros estudios como los de Muanjai et al. (2020), hallando un efecto contrario al relacionar el trabajo excéntrico con una mayor pérdida de capacidad contráctil, y un mayor déficit en el mecanismo de coordinación-excitación-contracción. Resultados similares fueron hallados por Royer et al. (2021), asociando mayores pérdidas en la producción de fuerza y manifestación de dolor muscular al grupo que realizó trabajo excéntrico en la fase de preparación.

En cuanto al parámetro EXP, nuestro estudio ha demostrado que los sujetos con mayor EXP no obtuvieron menores pérdidas de fuerza, ni menores incrementos en los marcadores de daño muscular. Investigaciones como las realizada por Pradas et al. (2021), en donde se distribuía la muestra del estudio entre corredores expertos y no expertos (expertos = 5,80 ± 2,52 y no expertos = 4,60 ± 1,26 años de experiencia), determinaron que los sujetos más expertos obtenían menores pérdidas en altura de vuelo, en una medición inmediatamente posterior a la carrera mediante las pruebas de SJ y CMJ.

Por último, la posible influencia entre los parámetros de entrenamiento y su relación con la liberación de marcadores, relacionados con el daño muscular en las UT, es un tema poco estudiado. A pesar de encontrar una fuerte correlación entre la experiencia del corredor y la producción de LDH en nuestro estudio (Lecina et al., 2021), no se ha encontrado ningún trabajo en la literatura científica que pueda corroborar este hallazgo mediante un análisis estadístico con diferencias significativas. Sólo podemos intuir, que a pesar de la relación encontrada, es el volumen extremo de la prueba analizada el principal responsable de esta elevación (Knechtle & Nikolaidis, 2018; V. Scheer et al., 2020).

4.5 Alteraciones en el tejido óseo

El objetivo del presente estudio de caso "*Bone Turnover Alterations after Completing a Multistage Ultra-Trail: A Case Study"* (Castellar et al., 2022), fue el de evaluar la variación de los marcadores de daño óseo en post, rec2 y rec9. Se analizaron marcadores en suero de resorción (CTX y Ca^{2+}) y de formación ósea (OC y BALP). Tras completar la prueba, se evidenció un descenso de los marcadores de formación ósea al comparar con los valores basales (OC = -45,78% y BALP = -61,74%). Contrariamente, los valores de resorción ósea mostraron valores diferentes al finalizar la prueba (CTX = +37,28% y Ca^{2+} = -3,60%). Estos resultados son similares a los mostrados por otros estudios que analizaron la dinámica del BT en UT. En este sentido Kerschan et al. (2009), en una prueba de 246 km, demostraron descensos significativos en los valores de OC, así como incrementos significativos de CTX. Shin et al. (2012), en una carrera de ultra maratón de 308 km evaluaron la dinámica del marcador OC durante tres puntos kilométricos: a los 100 km, 200 km y al finalizar la prueba. Se evidenció un descenso progresivo de OC, aunque únicamente se hallaron descensos significativos a partir del kilómetro 100 y en el kilómetro 200. Contrariamente a lo esperado, al finalizar la prueba los valores de OC fueron superiores a los valores iniciales. Mouzopoulos et al., (2007), evaluaron en una prueba de 245 kilómetros las alteraciones de OC y BALP en post, a los 3 y 5 días después de completar la competición. OC mostró diferencias significativas al comparar pre vs. post (3,8±0.8 ug/L vs. 3,4±0.5 ug/L, $p < 0,05$), del mismo modo BALP mostró también diferencias significativas (66,5±8,2 ug/L vs. 61,5±7,7 ug/L, $p < 0,05$). Recientes investigaciones han demostrado que OC puede actuar como una hormona en su forma decarboxilatada, con funciones en el desarrollo cerebral, la fertilidad y sobre todo, en la homeostasis de la glucosa (Rubert & De la Piedra, 2021). Debido a esta interacción de OC con el resto de sistemas, se podría explicar la supresión de OC durante las UT por la activación simpática y el aumento de la segregación de cortisol y catecolaminas (Belli et al., 2018). Esta hipótesis, ha sido probada por Malm et al. (2004), al encontrar un aumento de OC tras una prueba de maratón. Del mismo modo, Nicolas et al. (2011), también hallaron incrementos de catecolaminas y cortisol en una UT. Se ha tratado de buscar otras alternativas para explicar los mecanismos fisiológicos para el

aumento de OC, pero no se han obtenido conclusiones definitivas. Larsen et al. (2020), intentaron probar una relación entre marcadores de inflamación sistémica y OC, sin embargo, no se pudo demostrar dicha relación. Durante el periodo de recuperación sólo un estudio evaluó el valor de OC (Mouzopoulos et al., 2007), y no se encontraron diferencias significativas (4,6±1,2 μg/L vs. 4.3±1.2 μg/L). En otras pruebas de resistencia sí se han demostrado incrementos de OC significativos a los 3 días después de finalizar la prueba (4,9 g/L vs. 3,9 g/L, -20%) (Malm et al., 1993). Además de OC, nuestro estudio evaluó la acción de BALP. BALP es un homodímero anclado a las membranas de los osteoblastos y vesículas de la matriz. Su presencia en la membrana celular es necesaria para la mineralización ósea (Nizet et al., 2020). La presente Tesis Doctoral ha mostrado descensos en BALP en los tres puntos de medición (-61,74%, -61,66% y -54,51%). Estos descensos han sido mayores a los encontrados en estudios similares (Dolan et al., 2020; Mouzopoulos et al., 2007). Este hecho, puede ser debido a la mayor duración (768 km vs. 308 km) y desnivel de nuestro estudio, con 47.500 metros de desnivel positivo, respecto a las investigaciones comparadas.

El BT se compone de dos procesos: la formación y la resorción. En cuanto a la resorción ósea, nuestro estudio analizó dos biomarcadores específicos: Ca^{2+} y CTX. Altas tasas de resorción ósea medidas en estos biomarcadores han sido asociadas a formas más severas de osteoporosis (Coronado et al., 2021), y una mayor probabilidad de sufrir SF (Civitelli et al., 2009). Esta Tesis Doctoral ha mostrado incrementos en CTX al comparar valores basales con post (+37,28%) en rec2 (+11,93%), así como un incremento sustancial en rec9 (+93,41%). Estos datos son similares a los reportados por otros estudios que estudiaron carreras de ultra maratón en post (Kerschan-Schindl et al., 2009), pero los valores alcanzados en rec9 son muy superiores y muestran una tasa muy elevada de destrucción ósea en ese momento de la recuperación. Por lo tanto, la duración continuada de los impactos submáximos parece tener una mayor relación con el incremento de CTX que impactos de intensidad mayor, aunque mucho más cortos.

Resultados similares fueron encontrados por Scott et al. (2010), en un estudio comparativo en una prueba realizada sobre tapiz rodante durante una hora, donde no se hallaron

diferencias significativas entre los grupos que corrieron a diferentes intensidades a partir de su $VO_{2Máx}$. Otros deportes de ultra resistencia como el triatlón, han demostrado también incrementos menores de CTX al finalizar la prueba. (Wu et al. (2019), en una prueba de ultra triatlón (226 kilómetros), analizaron igualmente el CTX al finalizar la prueba, y no encontraron diferencias significativas. A la luz de estos hallazgos puede deducirse que las UT parecen aumentar de forma sustancial los valores de CTX. Este hecho podría ser debido a una mayor duración de estas pruebas, añadido a los desniveles acusados que presentan.

El segundo marcador de resorción ósea que fue evaluado en este estudio fue Ca^{2+}. Contrariamente a lo esperado, presentó descensos cercanos a los valores basales (-3,60%, -3,38% y +3,15%). Estos datos son similares a los mostrados por Spanidis et al. (2017), en una ultra maratón de montaña de 103 kilómetros y tres etapas, donde se hallaron incrementos de Ca^{2+} asociados a incrementos de marcadores oxidativos (glutatión y catalasa), y de estrés (catecolaminas y cortisol). Estas alteraciones se mantuvieron durante 24 y hasta las 72 horas posteriores. Vora et al. (1983), en una prueba de realizada en laboratorio sobre tapiz rodante, también hallaron variaciones de Ca^{2+} tras finalizar la prueba, registrando un aumento de los valores de Ca^{2+} de 9,2±0,1 mg/Dl a 9,8 ± 0,1 mg/Dl ($p < 0.01$). El aumento de Ca^{2+} ha sido asociado en todos estos estudios con la activación de las hormonas adrenales y el sistema simpático.

La relación entre las alteraciones de los marcadores de BT y la pérdida de BMD, ha sido ampliamente discutida en el ámbito clínico de la osteoporosis (OP). El diagnóstico de OP se realiza mediante el uso de absorciometría de rayos X de doble energía (DEXA) en el fémur. La OP se define como una T-score de BMD de -2,5 o menos, presentando una fractura de fragilidad previa a los criterios actuales (Romero Barco et al., 2012).

El uso de DEXA en UT por etapas es difícilmente viable, considerándose un tipo de análisis que no muestra cambios en periodos de tiempo tan pequeños. En cambio, BTM ofrecen un análisis dinámico y global del esqueleto, permitiendo la monitorización de los deportistas en periodos de competición o entrenamiento (Romero Barco et al., 2012).

Esta es la razón por la que el uso de BTM ha sido propuesto como una alternativa al DEXA, y parece especialmente útil para evaluar el estado óseo en pruebas deportivas. Este uso diagnóstico de BTM como factor predictivo, ha sido apoyado por diferentes estudios que demostraron el tipo de alteraciones en BTM previas a la aparición de fracturas por estrés (Civitelli et al., 2009; Tian et al., 2019). Sin embargo, qué biomarcadores y con qué valores aumentarían el riesgo de sufrir SF continúa sin estar plenamente establecido en la literatura científica, por lo que son necesarios nuevos estudios que evalúen el uso de BTM en las pruebas de ultra resistencia (Nose-Ogura et al., 2020). Johansson et al. (2014), en una revisión sistemática con sujetos de mediana edad, no deportistas y de ambos sexos, solo encontraron asociaciones entre CTX y SF en cadera. Igualmente, Tian et al. (2019), en otra revisión sistemática también hallaron una asociación entre CTX y riesgo de sufrir facturas por estrés, aunque igualmente en sujetos no entrenados y no en deportistas de pruebas de ultra resistencia. Vasikaran et al. (2011), analizaron en otra revisión sistemática la relación existente entre BTM y las SF en pacientes con osteoporosis diagnosticada, hallando igualmente una relación entre CTX y el riesgo de sufrir SF. Estas evidencias muestran que aumentos en los parámetros de resorción ósea CTX, al menos en sujetos no deportistas, parecen predecir de manera más eficaz la aparición de SF. En pruebas atléticas, dos estudios evaluaron la asociación entre BTM y SF, sin embargo, ninguno encontró una asociación significativa (Bennell et al., 2020; Nose-Ogura et al., 2020).

A pesar de que no se haya podido demostrar la relación entre las alteraciones BTM y las SF en pruebas de UT, la incidencia hallada de estas lesiones ha sido significativa, y por tanto, un motivo de preocupación sanitario (Scheer & Murray, 2011). Revisiones sistemáticas centradas en el estudio de SF en el ámbito deportivo han hallado incidencias muy variables en la cabeza del fémur y en la tibia (0,9% - 3,3% T-score) (Almekinders & Engle, 2019), en función de la especialidad deportiva y otros factores individuales como sexo, dieta y edad principalmente (Coronado et al., 2021). Duckam et al. (2015), en un estudio prospectivo de 12 meses realizado en corredoras de 800 metros lisos hallaron un 3,3% de SF.

Kahanov et al. (2015), en una revisión de las SF en los corredores de pruebas de ultra resistencia, mostraron que la incidencia de este tipo de patología osciló entre un 0,7% - 20%, siendo el 80% de las fracturas en las extremidades inferiores. Scheer et al. (2021), en una scoping review en UT, investigaron la incidencia de lesiones en el aparato músculo esquelético. Del total de lesiones analizadas, las fracturas de fémur supusieron un 0,3%, respecto al 1,2% de las de tibia o peroné.

Por lo tanto, y considerando los datos arrojados por los diferentes estudios científicos que han relacionado las fracturas por estrés y las alteraciones en BTM, se estableció en el presente trabajo una hipótesis derivada del objetivo D que, en nuestro estudio de caso, es la que valoraría las alteraciones en BTM y la posible aparición de fracturas por estrés. A pesar de las altas variaciones sufridas en los valores de resorción ósea, los cuatro sujetos completaron los 768 kilómetros en los que consistía la prueba, sin fracturas por estrés en ningún punto anatómico. Todo ello a pesar de haber sufrido alteraciones severas, principalmente en CTX, que permanecieron incluso hasta en rec9.

5. Conclusiones

Los cuatro estudios incluidos en esta Tesis Doctoral por compendio analizaron las alteraciones en biomarcadores de hiponatremia, daño muscular, óseo y renal en cuatro corredores varones sanos no profesionales, en una UT por etapas de 768 kilómetros y realizada durante 11 días consecutivos. Los resultados obtenidos en los cuatro artículos nos permiten responder al objetivo general y afirmar que, la realización de una UT de estas características provoca alteraciones en dichos parámetros, no solo en post sino también en rec2 y rec9. El análisis de estos resultados nos permite aceptar la hipótesis general de estudio y afirmar que estas alteraciones son transitorias y no provocan condiciones médicas severas en sujetos sanos (AKI, BT, EAH y ER). Para una mejor comprensión de estas conclusiones, el texto ha sido dividido en diferentes apartados de acuerdo con los objetivos e hipótesis específicos de esta Tesis Doctoral.

5.1 Objetivo e hipótesis A y B. Incidencia de AKI y EAH en pruebas de ultra trail

La incidencia de AKI y EAH correspondientes a los objetivos específicos A y B, fueron analizados en el artículo de revisión sistemática *"Acute Kidney Injury and Hyponatremia in Ultra-Trail Racing: A Systematic Review"*

- La duración e intensidad de las UT actúan como factores etiológicos de AKI, con tasas de incidencia mayores en las UT categoría extra (>100 kilómetros) o en las UT de categoría media (< 69 kilómetros).
- Las alteraciones en los marcadores actuales, SCR y EFG, no implican un daño real en fases iniciales de AKI, y, por tanto, estos marcadores mostrarían tan solo estados transitorios compensatorios de la función homeostática renal, que se revierten en la mayoría de los casos de forma natural con rehidratación y reposo.
- EAH refleja tasas mucho más bajas que AKI a pesar de que ha sido ampliamente estudiado y relacionado con la participación en este tipo de carreras a causa de la sobrehidratación y el consecuente aumento de BDWL.

- EAH, a diferencia de AKI, muestra una relación lineal con la distancia de la prueba, obteniéndose las mayores tasas de EAH en las UT extremas, y las menores en las UT medias. En este sentido, EAH sí que cuenta con unos criterios diagnósticos uniformes establecidos y por ello, a pesar de una sintomatología confusa, es más sencilla de diagnosticar en este tipo de pruebas.
- La aparición conjunta de AKI y EAH es mayor de lo esperado. La intensidad y la duración son los factores etiológicos de la presencia conjunta de ambas patologías, obteniéndose mayores tasas de AKI+EAH según incrementa la duración de la prueba. ER junto con la sobrehidratación parecen ser los mecanismos fisiopatológicos que subyace la aparición conjunta de ambas patologías.
- A pesar de la baja incidencia de casos graves de AKI y en menor medida aún de EAH hallados en esta revisión sistemática, la gravedad y el riesgo que pueden suponer estas patologías para la salud de los corredores debería obligar a los participantes, organizadores y personal médico a considerar su evaluación y rápido tratamiento en las UT.
- Estos resultados nos permiten responder a nuestras hipótesis A y B y aceptar que la participación en una UT no provoca incidencias altas de AKI ni EAH en sujetos sanos previamente entrenados y con suficiente experiencia en las UT en cuanto a la severidad de AKI solo un caso fue de grado 3 mientras que EAH ninguno de los sujetos alcanzo el grado más alto de severidad.

5.2 Objetivos e hipótesis específicos A, B y C. AKI, EAH y ER

El estudio de caso *"Extreme Ultra-Trail Race Induces Muscular Damage, Risk for Acute Kidney Injury and Hyponatremia: A Case Report."* evaluó los objetivos específicos A, B y C. Este trabajo fue diseñado en base a los resultados obtenidos en la revisión sistemática, en donde se destaca el papel de la duración de la prueba y la velocidad a la que corrían los atletas como factor fundamental etiológico de la aparición de AKI y EAH. Se evaluó la presencia de

AKI, EAH y ER, mediante un estudio de caso en la prueba GR11 en 11, que cumplía la premisa de una duración superior a las pruebas de categoría extra (> 100 km).

- En cuanto a AKI se mostraron alteraciones relevantes en la variación de SCR pre vs. post como en rec2 y rec9, estos aumentos de SCR no llegaron a alcanzar el diagnóstico de AKI en ninguno de los cuatro sujetos ni en post ni rec2 y tampoco en rec9 y se concluye que estas alteraciones de los parámetros SCR y EFG no implican una patología renal aguda en estas carreras.

- El parámetro [Na^+] apenas sufrió alteraciones ni en post ni rec2 ni rec9 con lo que no se diagnosticó ningún caso de EAH lo que nos permite afirmar que a pesar de la duración de la UT incluida en nuestro estudio ningún sujeto sufrió EAH.

- Se diagnosticó un episodio de ER que cursó de forma asintomática, pero con aumentos cinco veces el valor basal de CK. LDH aumentó también, aunque de forma más ligera lo que demuestra la importancia de CK como marcador más sensible para el diagnóstico de ER.

- Los valores de daño hepático (AST y ALT), también aumentaron multiplicando sus valores basales por dos, y se mantuvieron e incluso aumentaron hasta seis veces incluso en rec9.

- Adicionalmente, se evaluaron también marcadores de serie roja y serie blanca que demostraron variaciones mucho menores sin implicar ninguna condición médica relevante.

- No se registró MB en orina por lo que el diagnóstico de ER sólo se pudo realizar mediante análisis del incremento del biomarcador CK pre vs. post, este hecho demuestra la complejidad del diagnóstico de ER.

- Se demuestra la importancia de evaluar los parámetros sanguíneos de daño muscular al finalizar una UT y no solo confiar en los análisis de orina. La sintomatología de ER se basa en la clásica triada: mialgia, debilidad y MB que en esta ocasión no fue detectable y por lo tanto se precisó del análisis de CK para el diagnóstico de ER.

- Estos datos permiten aceptar las hipótesis de estudio especificas parcialmente, ya que, aunque el sujeto no alcanzó criterios diagnósticos de AKI ni EAH, sí que sufrió un episodio de ER en post, que cursó asintomático y se resolvió por sí mismo en rec2 y rec9.

5.3 Objetivo e hipótesis C. El daño muscular y las pérdidas de fuerza

El tercer artículo, denominado "*768-km Multi-Stage Ultra-Trail Case Study-Muscle Damage, Biochemical Alterations and Strength Loss on Lower Limbs.*" halló resultados similares en CK, LDH, AST y ALT.

- Se demostraron pérdidas de fuerza al finalizar la prueba en los tres saltos SJ, CMJ y ABA, volviendo a los valores pre en rec9. Las pérdidas fueron menores en ABA y mayores en CMJ.

- Se hallaron asociaciones fuertes ($R_s > 0,8$) sobre todo con el parámetro LDH y varios parámetros del entrenamiento lo que nos permite destacar el papel del entrenamiento previo a estas pruebas como factor preventivo de pérdidas de fuerza y de un aumento de daños de biomarcadores de daño muscular.

- Los parámetros del entrenamiento analizados VOL, SLO y EXP, podrían tener un papel importante a la hora de prevenir el aumento de marcadores de daño muscular especialmente con LDH. Estos parámetros parecen poder prevenir las pérdidas contráctiles en los músculos extensores de las piernas de los corredores no sólo en post si no en rec2 y rec9.

- Los resultados obtenidos podrían ayudar a establecer pautas de entrenamiento de la fuerza en estos corredores como medida preventiva del daño muscular en las UT.

- En cuanto a la hipótesis C también se aceptó que las pérdidas de fuerza fueron elevadas en post, de la misma forma que los incrementos en los marcadores de ER (CK, LDH, ALT y AST), sin embargo, todos marcadores se recuperaron en rec2 y rec9 al igual que las pérdidas de fuerza que retornaron a su nivel basal.

5.4 Objetivo e hipótesis D. Alteraciones en el proceso de remodelado óseo

El último de los artículos incluidos *"Bone Turnover Alterations after Completing a Multistage Ultra-Trail: A Case Study."* evaluó las alteraciones de BTM, siguiendo el objetivo específico D.

- Se registraron alteraciones severas en los marcadores de formación ósea OC y BALP, demostrándose una supresión de la actividad formadora de los osteoblastos en post que se mantuvo en rec2 e incluso en rec9. Contrariamente, los marcadores específicos de resorción ósea, sobre todo CTX, aumentaron su actividad en post y se mantuvieron por encima de valores basales hasta en rec9 demostrando que la actividad de CTX y Ca^{2+} continua incluso mucho después de completar la prueba.

- Los sujetos no sufrieron SF a pesar de las alteraciones en BTM, por lo que estas variaciones en dichos valores, no necesariamente implican un aumento de riesgo de fractura de estrés a corto plazo. Sin embargo, estas alteraciones pueden modificar a largo plazo la microestructura ósea y, en consecuencia, aumentar el riesgo de sufrir SF. Se precisarían estudios a largo plazo que evaluaran el uso de BTM como factor diagnóstico de SF en UT.

- El uso de BTM puede ser una alternativa complementaria al análisis DEXA ya que permite una fácil aplicación y ofrece información sobre el estado del tejido óseo de forma global y en el corto plazo.

- La hipótesis D quedó probada solo parcialmente, mostrando un desajuste negativo en los corredores entre los marcadores de formación ósea (OC y BALP) y los de resorción ósea (CTX y Ca^{2+}), este desbalance en BT, contrariamente a lo previsto en nuestra hipótesis D, no se normalizó en rec2 ni en rec9.

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7. Apéndices

7.1. Artículo 1

7.1.1 Carta de aceptación artículo 1

7.1.2 Temática e Índice de Impacto de la publicación

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7.2. Artículo 2

7.2.1 Carta de aceptación del artículo 2

7.2.2 Temática e Índice de Impacto de la publicación

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7.2.3 Justificación de la contribución del doctorando

El doctorando contribuyó a la elaboración de este artículo en las siguientes áreas: conceptualización y diseño del estudio, metodología del estudio de caso, recogida de datos de los corredores, recogida de muestras sanguíneas y de orina, análisis y estadística descriptiva de los resultados, redacción y revisión del manuscrito original y finalmente, traducción a la lengua inglesa.

7.3. Artículo 3

7.3.1 Carta de aceptación del artículo 3

7.3.2 Temática e Índice de Impacto de la publicación

International Journal of
Environmental Research and Public Health

an Open Access Journal by MDPI

Indexed in: PubMed | CITESCORE 3.4 SCOPUS | IMPACT FACTOR 3.390

CERTIFICATE OF ACCEPTANCE

Certificate of acceptance for the manuscript (**ijerph-1532193**) titled:

768-km multi-stage ultra-trail case study; muscle damage, biochemical alterations and strength loses on lower limb.

Authored by:

Miguel Lecina; Carlos Castellar; Francisco Pradas; Isaac López-Laval

has been accepted in *Int. J. Environ. Res. Public Health* (ISSN 1660-4601) on 10 January 2022

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CITESCORE 3.4 SCOPUS

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IMPACT FACTOR 3.390

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Basel, January 2022

7.3.3 Justificación de la contribución del doctorando

El doctorando contribuyó a la elaboración de este artículo en las siguientes áreas: conceptualización y diseño del estudio, protocolo de la valoración de la plataforma de fuerza, recogida de los saltos de los corredores, recogida de muestras sanguíneas y de orina, análisis y estadística descriptiva de los resultados, redacción y revisión del manuscrito original y finalmente, traducción a la lengua inglesa.

7.4. Artículo 4

7.4.1 Carta de aceptación del artículo 4

7.4.2 Temática e Índice de Impacto de la publicación

healthcare

an Open Access Journal by MDPI

Indexed in: PubMed | Indexed in: Scopus | IMPACT FACTOR 2.645

CERTIFICATE OF ACCEPTANCE

Certificate of acceptance for the manuscript (**healthcare-1649497**) titled:

Bone turnover alterations do not imply stress fractures after completing a 768-Kilometer Multistage Ultra-Trail: A Case Study.

Authored by:

Carlos Castellar; Miguel Lecina; Francisco Pradas

has been accepted in *Healthcare* (ISSN 2227-9032) on 23 April 2022

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Covered in: PubMed

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Aims and Scope

Healthcare (ISSN 2227-9032) is an international, peer-reviewed, open access journal (free for readers), which publishes original theoretical and empirical work in the interdisciplinary area of all aspects of medicine and health care research. This journal covers all topics related to all aspects of medicine and health care research. Healthcare is comprised of eighteen sections including the following:

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Basel, January 2022

7.4.3 Justificación de la contribución del doctorando

El doctorando contribuyó a la elaboración de este artículo en las siguientes áreas: selección de biomarcadores de remodelado óseo, estudio de la bibliografía relativa a daño óseo y ultra trail, recogida de muestras sanguíneas y de orina, análisis y estadística descriptiva de los resultados obtenidos, redacción y revisión del manuscrito original y finalmente, traducción a la lengua inglesa.