UNIVERSIDAD DE EXTREMADURA

TESIS DOCTORAL

INDICADORES DE CARGA INTERNA Y EXTERNA RELACIONADOS AL DAÑO AGUDO MUSCULAR Y RENAL EN CORREDORES DE TRAIL RUNNING

Daniel Rojas-Valverde

Programa de Doctorado en Ciencias del Deporte

2021

UNIVERSIDAD DE EXTREMADURA

DOCTORAL THESIS

INTERNAL AND EXTERNAL WORKLOAD INDICATORS OF ACUTE MUSCLE AND KIDNEY INJURY IN ENDURANCE TRAIL RUNNING

Daniel Rojas-Valverde

Sport Science PhD Degree

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Conformidad de los Directores

La conformidad de los directores de la tesis consta en el original en papel de esta Tesis Doctoral

Fdo: Guillermo Olcina Camacho Fdo: Rafael Timón Andrada

2021

UNIVERSIDAD DE EXTREMADURA

Dr. D. Guillermo Olcina Camacho, profesor del Área de Educación Física y Deportiva del departamento de Didáctica de la Expresión Musical, Plástica y Corporal de la Universidad de Extremadura.

CERTIFICA:

Que la Tesis Doctoral realizada por D**. Daniel Rojas Valverde**, con el título "**Indicadores de carga interna y externa relacionados al daño agudo muscular y renal en corredores de trail running**", bajo mi co-dirección, reúne los requisitos necesarios de calidad, originalidad y presentación para optar al grado de Doctor, y está en condiciones de ser sometida a valoración de la Comisión encargada de juzgarla.

Y para que conste a los efectos oportunos, firmo la presente en Cáceres, a 23 de marzo de 2021.

La conformidad del director de la tesis consta en el original en papel de esta Tesis Doctoral

Dr. D. Guillermo Olcina Camacho

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Dr. D. Rafael Timón Andrada, profesor del Área de Educación Física y Deportiva del departamento de Didáctica de la Expresión Musical, Plástica y Corporal de la Universidad de Extremadura.

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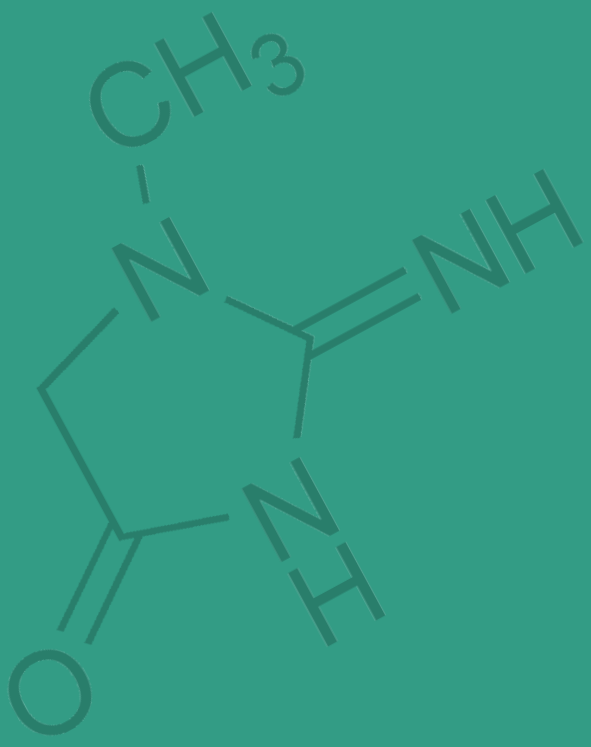
Y para que conste a los efectos oportunos, firmo la presente en Cáceres, a 18 de marzo de 2021.

La conformidad del co-director de la tesis consta en el original en papel de esta Tesis Doctoral

Dr. D. Rafael Timón Andrada

¨If we believe in human beings that we associate with
and apply the knowledge that we have and share,
we see and opportunity that people can realise their dreams,
boost their full potential of talents they are endowed with¨



ACKNOWLEDGEMENT

This thesis allowed me to develop my entrepreneurial spirit and represents an open window to boost my academic and research skills. Considering a thesis is a process with ups and downs, I would like to appreciate a multitude of people who gave their support, shared their knowledge, and provided invaluable assistance throughout this adventure.

First of all, I would like to recognize my parents MD. Sonia Valverde Arce and Eng. Marvin Rojas Valverde, who inherited a great capacity for adaptation, raised me in a scenario where conformity and comfort were two inadmissible conditions for a restless dreamer. My thesis is dedicated to them, who unconditionally gave me their support and taught me the hard work needed to reach my goals humbly.

Moreover, I would like to show my gratitude to my thesis director, Dr. Guillermo Olcina Camacho, my co-director Dr. Rafael Timón Andrada, for sharing their knowledge and directing my work with dedication and constructive guidance.

I am also very thankful to my colleagues from the Universidad Nacional de Costa Rica, Dr. Braulio Sánchez Ureña and MSc. Randall Gutiérrez Vargas for their encouraging support, tolerance and guidance. I am deeply grateful to my colleagues from the Universidad de Extremadura, Dr. Ismael Martínez Guardado and Dr. Sergio Ibáñez Godoy, and from Universidad de Murcia, Dr. José Pino Ortega; for their tireless help and support, and who were key elements for the development of this thesis. I am thankful to Dr. Jeffrey Mjaanes of the Northwestern University and Dr. Justin Kemp of the Australian Catholic University for their review of overall content and insights to improve the English expression. My gratitude to Dr. Sylvain Laborde from the Deutsche Sporthoschschule Köln for encouraging me to be a doctor. All my gratefulness to Dr. Nuno Batalha and Dr. Pablo Thomas Carús of the Universidade de Évora for their selfless support. Many thanks to all the participants who volunteered in our studies for their time, sweat, and sometimes pain.

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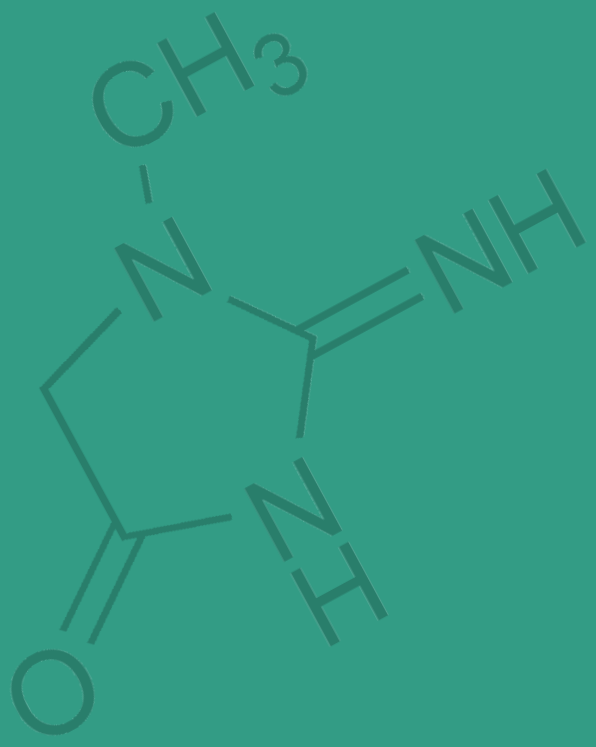

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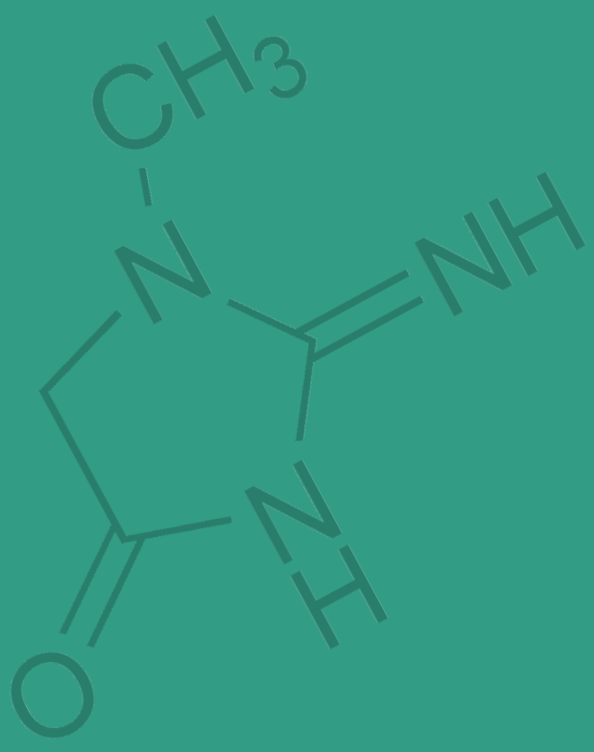


PUBLICATIONS AND DIVULGATIONS

Academic publications

According to the research topics, the studies were published in journals indexed in the Journal Citation Reports and Scimago Journal Rank in physiology, medicine, sport science, engineering, physical therapy and Public Health (see Table 1.).

Table 1. List of publications with authors, year, title, journal, impact factor, and quartile.

Thesis Aim	Article Type	Citation	Impact Factor	Quartile
A	Opinion	**Rojas-Valverde, D.**, Olcina, G., Gutiérrez-Vargas, R. & Crowe, J. (2019). Heat Strain, External Workload, and Chronic Kidney Disease in Tropical Settings: Are Endurance Athletes Exposed? *Frontiers in Physiology, 10*:1403. 10.3389/fphys.2019.01403	JCR: 3.367	Physiology Q2, Medical Physiology Q2
A	Systematic Review	**Rojas-Valverde, D.**, Sánchez-Ureña, B., Crowe, J., Timón, R. & Olcina, G., (2020). Exertional Rhabdomyolysis And Acute Kidney Injury in Endurance Sports: A Systematic Review. *European Journal of Sport Science, X* (X). 10.1080/17461391.2020.1746837	JCR: 2.781	Medicine Q1, Othopedics and Sport Medicine Q2, Physical Therapy, Sport Therapy and Rehabilitation Q1, Sport Science Q2
B	Original	**Rojas-Valverde, D.**, Pino-Ortega, J., Timón, R., Gutiérrez-Vargas, R., Sánchez-Ureña, B., Olcina, G. (2021). Agreement and reliability of magnetic, angular rate, and gravity (MARG) sensors to assess multiple body segment´s external loads during off-road running. *Journal of Sports Engineering and Technology*. *Ahead of Print*	JCR: 1.0	Engineering Q2, Sport Science Q4,
B	Original	**Rojas-Valverde, D.**, Sánchez-Ureña, B., Pino-Ortega, J., Gómez-Carmona, C.D., Gutiérrez-Vargas, R., Timón, R. & Olcina, G. (2019). External Workload Indicators of	JCR: 2.849	Health, Toxicology and mutagenesis

		Muscle and Kidney Mechanical Injury in Endurance Trail Running. *International Journal Environmental Research & Public Health, 16* (3909). doi:10.3390/ijerph16203909		Q2, Public Health, Environmental and Occupational Health Q2
C and D	Original	**Rojas-Valverde, D.,** Timón, R., Sánchez-Ureña, B., Pino-Ortega, J., Martínez-Guardado, I. & Olcina, G. (2020). Potential Use of Wearable Sensors to Assess Cumulative Kidney Trauma in Endurance Off-Road Running. *Journal of Functional Morphology and Kinesiology*; 5(4):93. doi:10.3390/jfmk5040093	SJR: 0.27	Anatomy Q3
C and D	Original	**Rojas-Valverde, D.**, Olcina, G., Sánchez-Ureña, B., Pino-Ortega, J., Martínez-Guardado, I. & Timón, R. (2020). Proteinuria and bilirubinuria as potential risk indicators of acute kidney injury during running in outpatient settings. *Medicina*, 56 (11). 10.3390/medicina56110562	JCR: 1.205	Medicine Q3

Conferences papers

The results of the studies developed were presented in two of the most important forums in the area of sport science and medicine (see Table 2.).

Table 2. List of conferences papers, year, authors and hosting conference.

Author	Title	Conference details
Rojas-Valverde, D., Sánchez-Ureña, B, Olcina, GJ., Pino-Ortega, J., Gutiérrez-Vargas, R., Timón, R. & Mjaanes, J.	Muscle damage and acute kidney injury in endurance mountain running.	67th ACSM Annual Meeting, 11th World Congress on Exercise is Medicine and World congress on Basic Science of Exercise in Regenerative Medicine, San Francisco, California, United States of America (26-30th May, 2020)
Rojas-Valverde, D., Olcina, G., Martínez-Guardado, I., Sánchez-Ureña, B., Timón R.	Proteinuria and bilirubinuria as indicators of acute kidney injury in endurance mountain runners.	25th European College of sport Science Annual Congress, Sevilla, Spain (28-30th October, 2020).

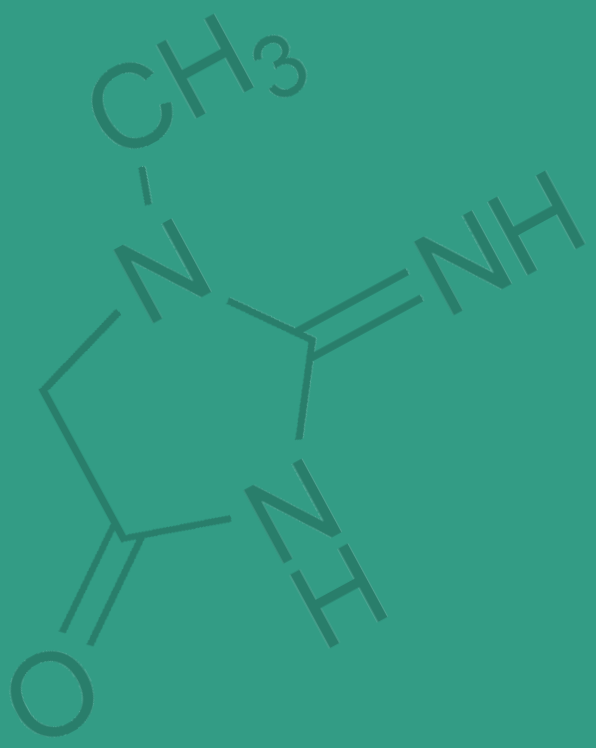
Research internships and traineeships

The internships and traineeships were developed in two different institute recognized by their research level and quality (see Table 3.).

Table 3. List of internship and traineeships with year, researchers in charge, institute and university.

Year	Researcher in Charge	Institute and University
2019 (1 month)	Dr. Sylvain Laborde	Abteilung Leistunspsychologie, Deutsche Sporthochschule Köln, Köln, Deutschland
2020-2021 (2 months)	Dr. Nuno Miguel Prazeres Batalha	Departamento de Desporto e Saúde, Universidade de Évora, Évora, Portugal

*Both certificates are attached in the chapter C of this document.



ABBREVIATIONS

AKI

acute kidney injury

eGFR

estimation of glomerular filtration rate

ER

exertional rhabdomyolysis

IMU

inertial measurement devices

L

lumbar

MARG

magnetic, angular rate and gravity

MB

myoglobine

MP

malleolus peroneus

PC

principal component

PCA

principal component analysis

sALB

serum albumin

sBUN

serum blood ureic nitrogen

sCK

serum creatin kinase

sCr

serum creatinine

sLDH

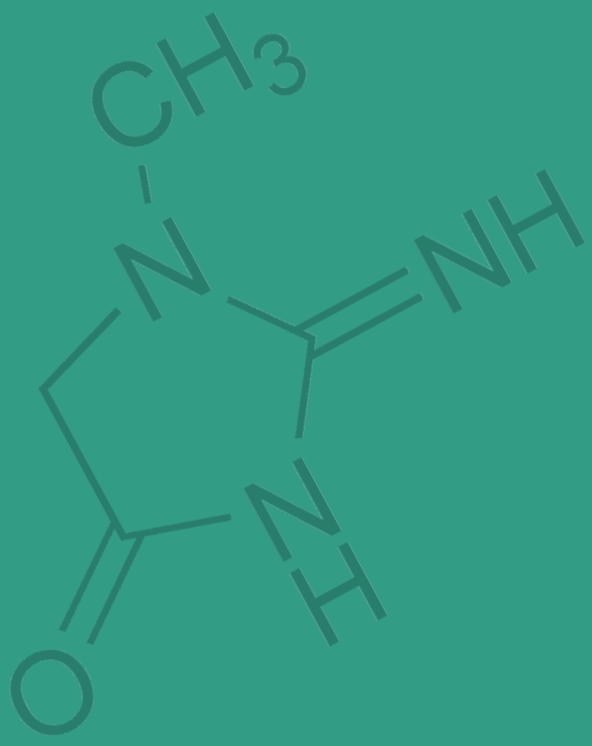
serum lactate dehydrogenase

T

thoracic

VL

vastus lateralis



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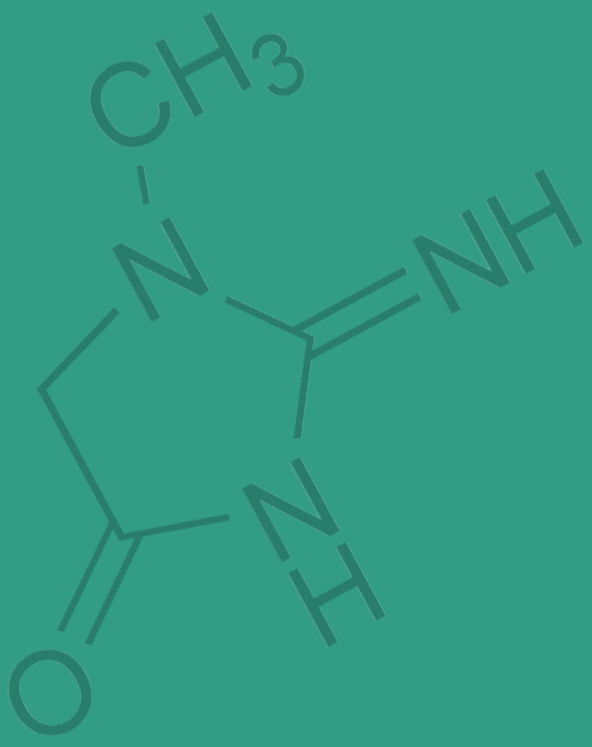

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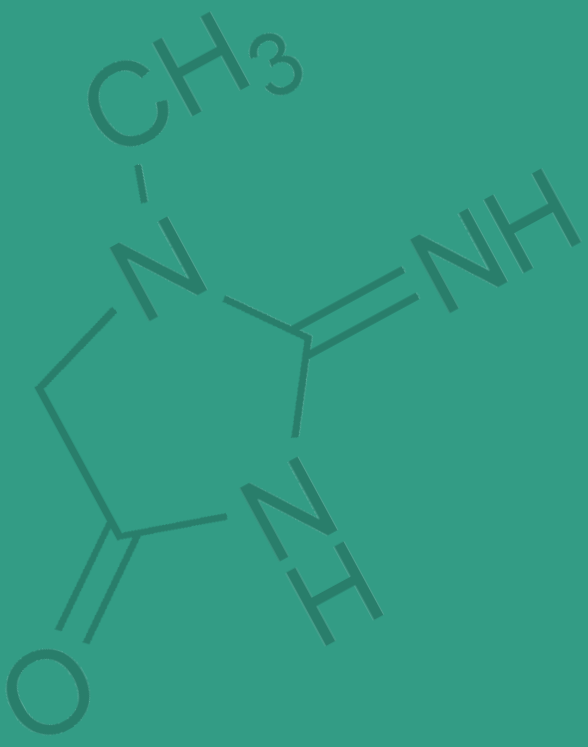


ABSTRACT

Introduction: The popularity of trail running and endurance sports has increased substantially in the last two decades. Research around trail running has focused mainly on acute injuries and medical problems considering physiological, biochemical, nutritional, aspects, and external loads. More recently, researchers have been concerned with investigating the short- and long-term effects of prolonged and strenuous exercise, given that trail running is one of the most physically and physiologically demanding sports, and can potentially lead to related chronic health problems. The link between these acute health problems and the development of subsequent chronic diseases is not clearly established. However, the relationship has been reported in certain populations (e.g., harvesters) that have similarities such as high physical load, exposure to adverse temperature conditions and severe dehydration. Considering the new technologies such as wearable devices, this thesis focuses on deepening the interrelation between internal and external load aspects related to acute muscle and kidney injury (AKI). This information will clarify the causality and potential consequences of muscle and kidney damage and how this could impact the long term.

Aims: The aims of this thesis were: a. delve into the findings on exertional rhabdomyolysis (ER) and AKI in endurance sports, emphasizing the diagnostic criteria used, the physical and environmental contextual conditions in which the ER and the AKI are developed. b. Analyze which external load indicators have the most influence on the biomarkers of muscle and kidney injury. c. Explore potential exposure to kidney disease in endurance athletes in countries with adverse hot and humid conditions. And d. Globally explore the variables of heat stress, dehydration and external workload as indicators of acute kidney injury in endurance running.

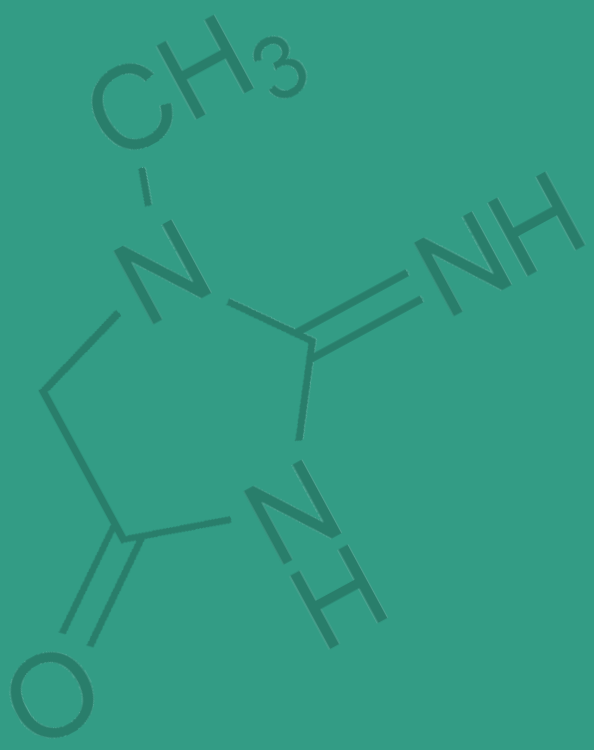
Methods: The four specific aims of this thesis were investigated through a systematic review, an exploration of the agreement and reliability of wearable devices for the quantification of renal and muscular load, and three cross-sectional observational studies on the effects of trail running on markers of muscular and renal injury at the level of external load, blood and urinary variables. For the systematic review, a digital search was carried out (PubMed [MEDLINE], Science Direct, [EMBASE] and Web of Science [WoS]) using the keywords “sport”, “exertional rhabdomyolysis”, “acute renal failure”, “acute kidney injury ". For the study of agreement and reliability of wearable devices (MARG sensors), 18 trail runners participated who ran two 12 km circuits using six MARG sensors in different parts of the body. The lineal relationship, the difference of means, the intraclass correlation and the bias of load variables were investigated. In the

cross-sectional observational studies, a total of 67 participants were measured who ran a distance of approximately 35km where they were evaluated before and after running variables such as: serum creatine kinase, serum creatinine, serum urea nitrogen, serum albumin, urinary variables such as protein, bilirubin, glucose, leukocytes, erythrocytes, urobilinogen, urine specific gravity and relative variables of external load such as player load, impacts, entropy.

Results: A total of 43 publications were extracted from the systematic review (sample = 813) and 345 (43.5%) individuals were diagnosed with ER (creatinine kinase> 5000 IU / L) and 130 (16.39%) concomitantly with AKI (creatinine ≥ 1.88 mg / dL). Of the total cases of ER + AKI, 96.92% were in endurance runners. In the agreement and reliability study, good agreement and a substantial almost perfect reliability of the external load variables were obtained. Observational studies suggest that: a. There are pre-post differences in variables such as creatine kinase, creatinine, ureic nitrogen, albumin and bilirubin and protein in the urine, which suggests a significant muscle and renal injury due to trail running. Additionally, these internal load results are related to external load variables such as player load and impacts. It is important to note that the position of the L_3-L_5 sensors explains the behavior of internal blood load variables.

Conclusions: It was concluded that trail running is one of the sports that causes the most significant muscle and kidney damage, and inconsistencies were found between the studies in the diagnostic criteria for ER and AKI, which represented difficulty in interpreting the data. It can be concluded that the MARG sensors offer significant reliability, good agreement when evaluating the external load of six different segments of the body during the trail running. External load assessment devices are useful for measuring cumulative renal trauma due to impacts. A new hypothesis of the effect of running this type of event with mechanical forces on the kidney is derived. In this sense, variables such as impacts, player load and entropy can predict muscle and kidney damage. Additionally, urine tests can be useful to identify potential AKI cases on an outpatient basis.

Keywords: acute kidney injury, exertional rhabdomyolysis, wearable devices, renal health, endurance.



RESUMEN

Introducción: La popularidad del trail running y los deportes de resistencia ha incrementado sustancialmente en las últimas dos décadas. Las investigaciones entorno al trail running se han enfocado principalmente en lesiones agudas y problemas médicos considerando aspectos fisiológicos, bioquímicos, nutricionales, desempeño y cargas externas. Más recientemente, sin embargo, los investigadores se han preocupado por indagar los efectos a corto y largo plazo del ejercicio prolongado y extenuante, considerando que el trail running es uno de los deportes con mayor demanda física y fisiológica y puede conducir potencialmente a problemas crónicos relacionados con la salud. El enlace entre estos problemas de salud agudos con futuros padecimientos crónicos no esta claramente establecido a pesar de que si se ha reportado en otras poblaciones (e.g., agricultores) que cuentan con similitudes como la alta carga física, exposición a condiciones adversas de temperatura y deshidratación severa. Considerando las nuevas tecnologías existentes como lo son los *wearables devices*, esta tesis se centra en profundizar sobre la interrelación entre aspectos de carga interna y externa relacionados con el daño muscular y renal agudos (AKI). Esta información servirá para esclarecer la causalidad y potenciales consecuencias del daño muscular y renal y cómo esto podría impactar a largo plazo.

Objetivos: Los objetivos de esta tesis fueron: a. Profundizar en los hallazgos sobre la rabdomiólisis por esfuerzo (ER) y el daño renal agudo (AKI) en los deportes de resistencia, haciendo hincapié en los criterios de diagnóstico utilizados, las condiciones contextuales físicas y ambientales en las que se informa la ER y la AKI. b. Analizar cuáles índices de carga externa tienen más influencia en los biomarcadores de lesiones musculares y renales. c. Explorar la exposición potencial a la enfermedad renal en atletas de resistencia en países con condiciones adversas de calor y humedad. y d. Explorar globalmente las variables de tensión de calor, deshidratación y carga de trabajo externa como indicadores de lesión renal aguda en la carrera de resistencia.

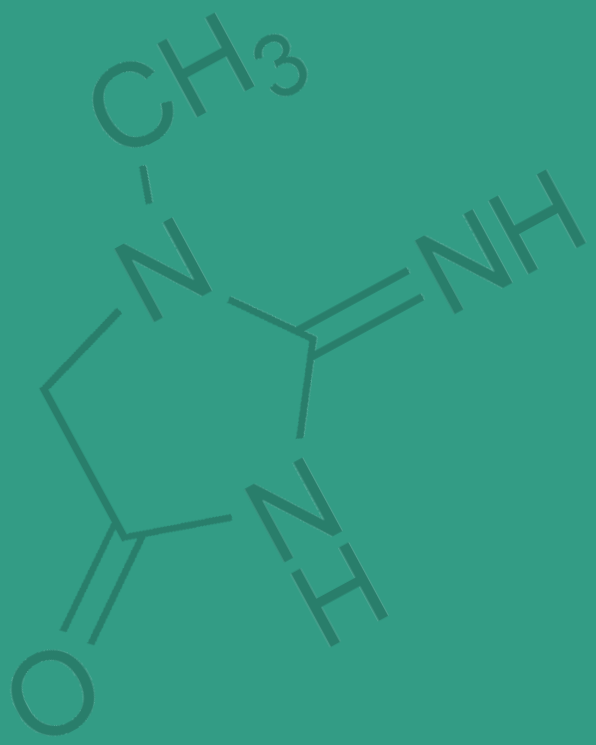
Método: Los cuatro objetivos específicos de esta tesis fueron investigados mediante una revisión sistemática, una exploración del acuerdo y confiabilidad de dispositivos portátiles para la cuantificación de carga renal y muscular, y tres estudios observacionales transversales sobre los efectos del trail running sobre marcadores de daño muscular y renal a nivel de carga externa, variables sanguíneas y urinarias. Para la revisión sistemática se realizó una búsqueda digital (PubMed [MEDLINE], Science Direct, [EMBASE] and Web of Science [WoS]) utilizando las palabras clave “sport”, “exertional rhabdomyolysis”, “acute renal failure”, “acute kidney injury”. Para el estudio

de acuerdo y confiabilidad de dispositivos vestibles (sensores MARG) participaron 18 trail runners quienes corrieron dos circuitos de 12km utilizando seis sensores MARG en diferentes partes del cuerpo. Se indagó la relación lineal, la diferencia de medias, la correlación intraclase y el bias de variables de carga. En los estudios observacionales transversales se midió a un total de 67 participantes quienes corrieron una distancia de aproximadamente 35km en donde se les evaluó antes y después variables como: creatinquinasa sérica, creatinina sérica, nitrógeno ureico sérico, albúmina sérica, variables urinarias como proteina, bilirrubina, glucosa, leucocitos, eritrocitos, urobilinógeno, gravedad específica de orina y variables relativas de carga externa como player load, impactos, entropía.

Resultados: Un total de 43 publicaciones se extrajeron de la revisión sistemática (muestra=813) y 345 (43.5%) individuos fueron diagnosticados de ER (creatinina quinasa>5000UI/L) y 130 (16.39%) en concomitancia con AKI (creatinina ≥1.88mg/dL). Del total de casos de ER + AKI, 96.92% fueron en corredores de resistencia. En el estudio de acuerdo y confiabilidad se obtuvo un buen acuerdo y una confiabilidad sustancial a casi perfecta de las variables de carga. Los estudios observacionales sugieren que: a. Existen diferencias pre-post en variables como creatinquinasa, creatinina, nitrógeno uréico, albúmina y bilirrubina y proteína en orina, lo que sugiere una carga muscular y renal significativa. Adicionalmente, estos resultados de carga interna son explicados y se relacionan con variables de carga externa como player load e impactos. Es importante rescatar que la posición de los sensores de L_3-L_5 explican el comportamiento de variables de carga interna sanguínea.

Conclusiones: Se concluyó que el trail running es uno de los deportes que causa mayor daño muscular y renal y se encontraron inconsistencias entre los estudios en los criterios de diagnóstico para ER y AKI, lo cual representó una dificultad en la interpretación de los datos. Se puede concluir que los sensores MARG ofrecen una confiabilidad significativa, buena concordancia al evaluar la carga externa de seis segmentos diferentes del cuerpo durante la carrera de trail running. Los dispositivos de carga externa son útiles para medir el trauma renal acumulativo por impactos, de ello se sugiere una nueva hipótesis del impacto de correr este tipo de eventos las fuerzas mecánicas sobre el riñón. En este sentido, variables como impactos, player load y entropía pueden predecir el daño muscular y renal. Adicionalmente, las pruebas de orina pueden ser útiles para identificar de manera ambulatoria potenciales casos de AKI.

Palabras Clave: daño renal agudo, rabdomiólisis por esfuerzo, dispositivos portátiles, salud renal, resistencia.



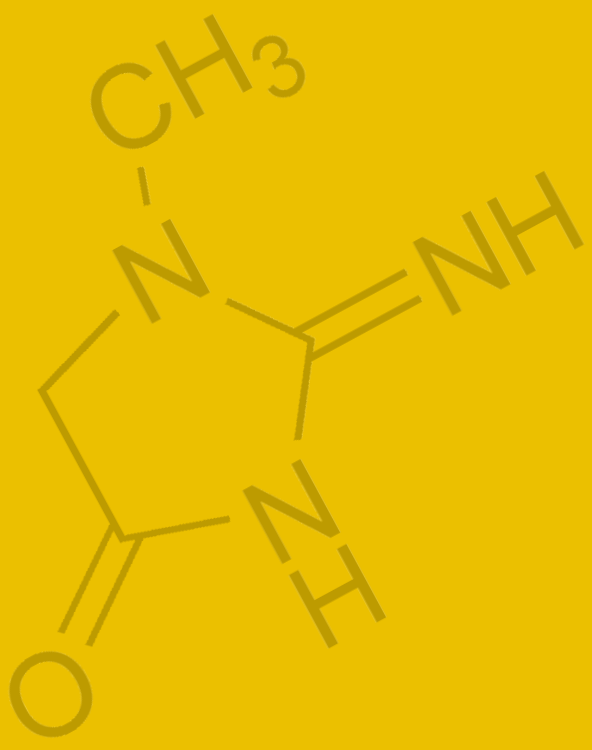
I. BACKGROUND

It is well established that regular physical activity offers multiple health benefits and can minimize the effect of age, prolonging not only the duration of life but its quality (Donnelly et al., 2016; Riebe et al., 2015). Despite this evidence, the acute and long-term implications of performing high volumes and intensities as endurance exercise is not entirely understood. This knowledge gap could be due to the need for more transversal and longitudinal studies in the implication on endurance sports' health.

By now, it is known that frequent endurance strenuous exercise can provoke pro-inflammatory conditions that may lead to some acute and chronic health problems as kidney issues (e.g., acute kidney injury, proteinuria, hematuria), cardiovascular disease (e.g., atrial fibrillation, myocardial remodeling, myocardial fibrosis), musculoskeletal problems (e.g., stress fractures, tendinopathies) or pulmonary issues (e.g., airway hyperresponsiveness) (Almekinders & Engle, 2019; Barros et al., 2017; Rojas-Valverde, Sánchez-Ureña, et al., 2020; Scheer, 2020; Tiller, 2019; Tiller et al., 2020).

Considering that youth and master-level participation in endurance running has increased, the development of strategies to monitor, control, and treat these types of health issues in such vulnerable populations is needed (Lepers & Stapley, 2016; Scheer, Di Gangi, Villiger, Nikolaidis, et al., 2020; Scheer, Di Gangi, Villiger, Rosemann, et al., 2020; Scheer & Hoffman, 2019; Zingg et al., 2013). Youth athletes may be at risk for issues due their skeletal immaturity and ongoing growth and development (Scheer, Sousa, Valero, Knechtle, et al., 2020), and master athletes could have pre-existing health conditions that may be impacted by prolonged running (Knechtle & Nikolaidis, 2018; Tso & Kim, 2020).

In this sense, the present thesis aim to review the available evidence around the acute muscle and kidney injury effects of trail running as the most physically and physiologically demanding sport. Additionally, the thesis will present some cross-sectional studies of the impact of trail running on blood, urine, and external workload indicators of muscle damage and kidney injury.



A. ENDURANCE

TRAIL RUNNING

Trail running stands out as the most famous modality within a group of Off-Road events, defined as events that take place on outdoor surfaces and in natural environments. These Off-Road events also include Fell-Running, Skyrunning, and Mountain Running (Scheer, Basset, Giovanelli, Vernillo, et al., 2020).

The International Trail Running Association (ITRA) defines trail running as pedestrian running events held in natural environments. These can be a mountain, forest, jungle, plains, desert, beach, among others. The lands can vary according to the geographical area; among them, it can found grass, sand, snow, earth or ballast, mud or mud, stone, among others (ITRA, 2017). According to the Spanish Federation of Mountain Sports and Climbing (FEDME) (2016), trail running races must have less than 10% contact with the pavement, cement, or asphalt. They must be carried out in less than 50% on terrain passable by motor vehicles. Other authors point out that contact with asphalt or paved terrain should not exceed 20-25% of the entire route (World Athletics, 2019).

i. Categories of trail running

The events can be of different modality according to the essential characteristics based on a general concept called Off-Road (Scheer, Basset, Giovanelli, Vernillo, et al., 2020):

Trail running: Any distance on terrain with less than 10-25% contact with asphalt or pavement (terrain passable by motor vehicles).

Mountain Running: Distances greater than 42.195km, natural environment with contact less than 10-25% with vertical elevation gains between 10-15%.

Skyrunning: With distances ≤ 99 km, separated into three categories: sky (20-49km, 1300m vertical ascent), ultra (50-90km; 3200 vertical climb) and vertical

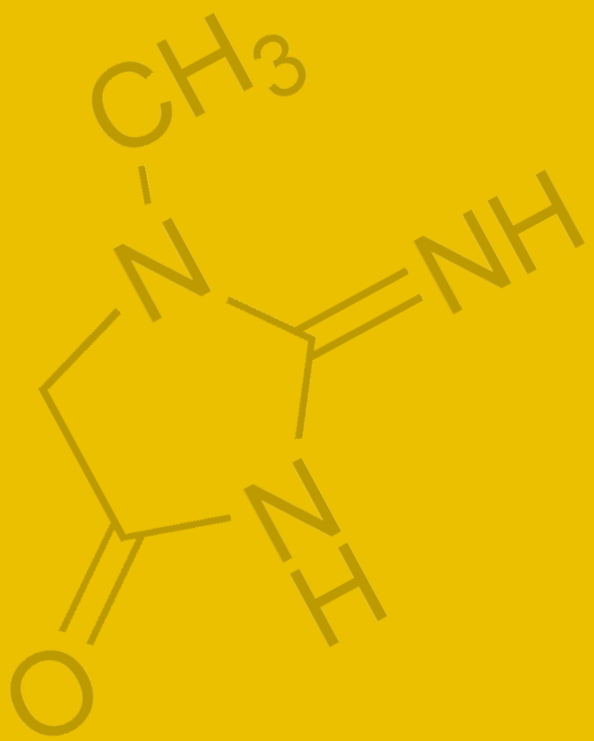
(maximum distance of 5km of climbing). They usually are carried out at heights greater than 2000 meters above sea level and with an average slope of 6%.

Orienteering: events by time approximately between 12-100min, with three categories of the distance between the short, medium and long. Made in natural terrain using navigation skills. There are short-distance urban modalities.

Obstacle course Racing: events where the competitor must overcome obstacles such as walls, water, carrying objects, jumping, etc. One of these sub modalities is called adventure races that last several days. There are no limits on the land used.

Cross country running: with distances between 4 and 10 km, mainly on grass circuits with recommended elevations of 10m / lap.

Ultramarathon running: defined by distance> 42,195 km, it can be a single-stage or multi-stage without terrain restriction.



B. MUSCLE FUNCTION

Throughout evolution, human beings have been forced to cover significant distances to nourishment and environments suitable for living. This is why running has been considered as a mode of locomotion that influences human evolution. Despite this, humans usually expend twice the energy while running, which is slower than most mammals of equal body mass (Bramble & Lieberman, 2004).

Exercise promotes several cardiovascular, neural and metabolic intra and extra-muscle responses (see Figure 1.).

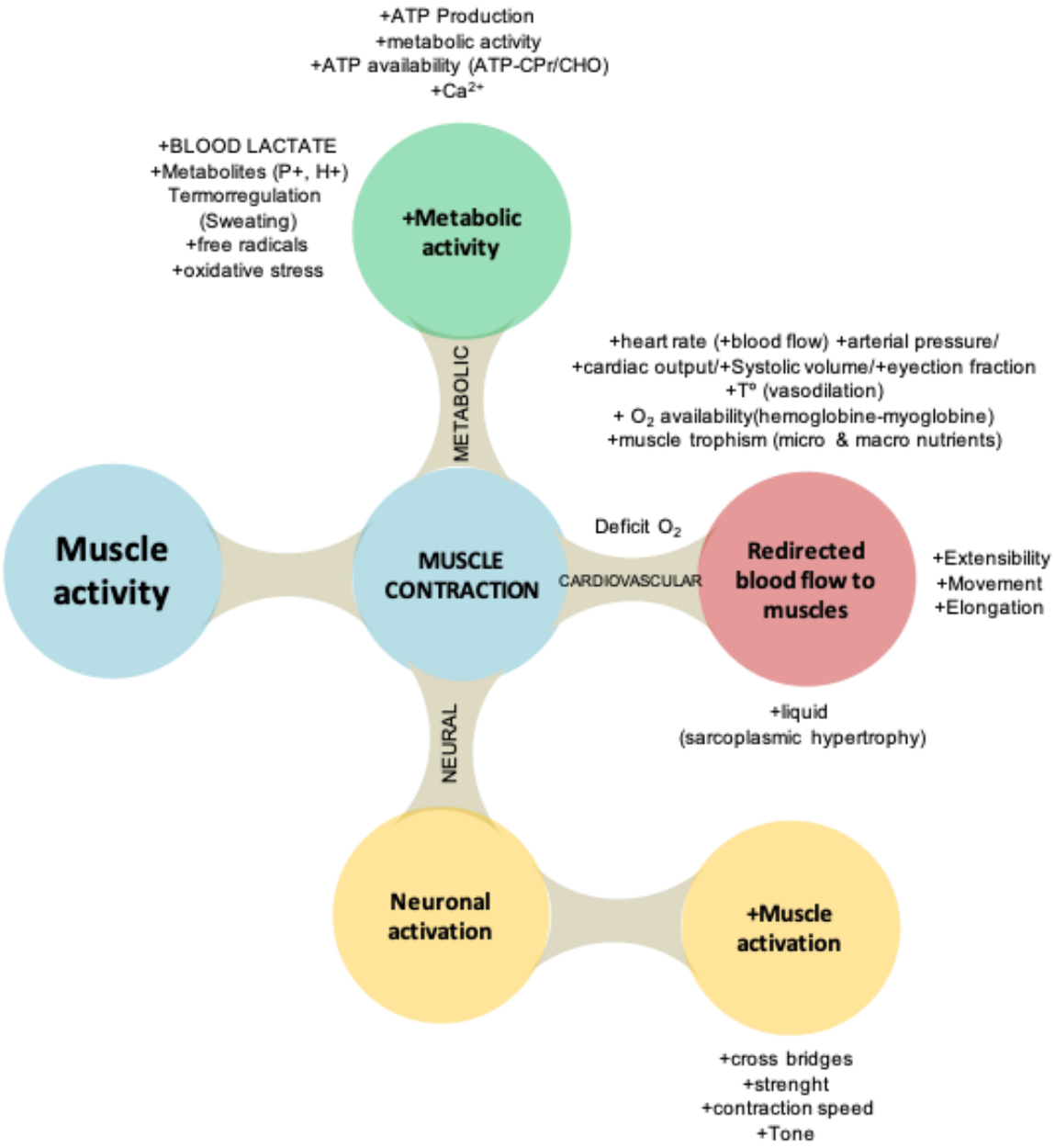


Figure 1. Muscle activation and neural, metabolic and cardiovascular responses.

i. Definition of muscle damage

Running exposes the musculoskeletal system to higher stress than walking, especially in each foot stroke, which produces a shock wave that passes up to the body from the foot through the spine to the head. This represents peak vertical forces that are 3-4 fold times greater when running than walking (Bramble & Lieberman, 2004).

Muscle damage is defined as a condition involving injury to the muscle tissue. It is provoked by two main factors, the mechanical strain and the metabolic overload during running (Assumpção et al., 2013). Metabolic causes could be but are not limited to fluid and electrolyte shifts from the bloodstream into the damaged muscle cells and vice versa. Skeletal muscle can regenerate almost entirely in response to minor fiber injuries such as strain. Still, after severe injuries or massive muscle damage, healing could be incomplete and could result in the formation of fibrotic tissue that affects muscle function (Laumonier & Menetrey, 2016).

Endurance exercise-related muscle damage usually is a result of high intensity and long-duration actions. Additionally, muscle damage and reduced muscle strength could lead to delayed onset muscle soreness and swelling. This is especially for muscle damage induced by an acute bout of repetitive, intense, and prolonged actions as downhill running (Assumpção et al., 2013).

Prolonged physical exercise as in endurance running induces a wide range of metabolic changes resulting in micro-injuries to the muscles and other tissues. This injury process provokes a rise in leukocytes' migration to the injury area and induces acute-phase inflammatory reactions (H. J. Kim et al., 2007). With this, trail and mountain running events include a significant amount of eccentric muscle contractions that may result in more exercise-induced muscle damage (Rojas-Valverde, Sánchez-Ureña, et al., 2020). Moreover, eccentric exercise has been shown to elevate serum levels of biomarkers as myoglobin and creatine phosphokinase (CK), suggesting protein leakage from skeletal muscle (Ramos-Campo et al., 2016).

In this sense, it should be considered that muscle damage has a fundamental role in exercise-related adaptations (see Figure 2). The remodeling of the damaged muscle fiber leads to hypertrophy and increases the strength necessary to perform better. When this muscle damage turns massive, as is usual during endurance running due to the high rate of eccentric muscle actions, potentially dangerous conditions could result (e.g., chronic pain, rhabdomyolysis).

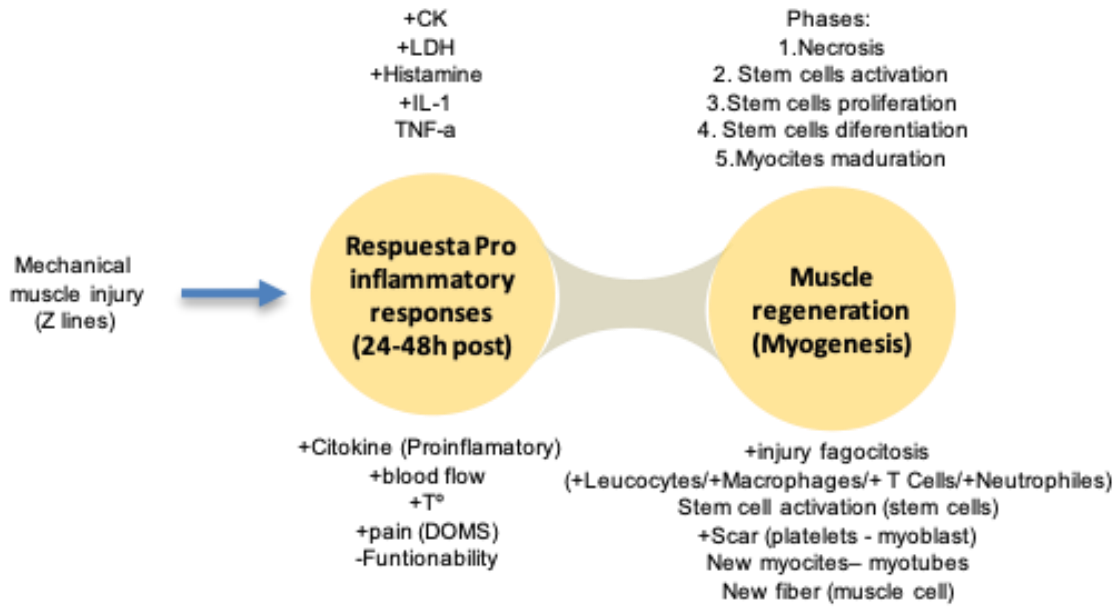


Figure 2. Muscle damage and regeneration process.

ii. Definition of rhabdomyolysis

Rhabdomyolysis is a condition provoked by the liberation of proteins into the bloodstream with various causes (Chlíbková et al., 2015). According to their etiology, the types of rhabdomyolysis could be categorized as; trauma, muscle hypoxia, genetic defects, infections, body temperature changes, metabolic or electrolytic disorders, idiopathic or physical effort (Bosch et al., 2009). Rhabdomyolysis could be caused by strenuous physical exercise, known as exertional rhabdomyolysis (Parmar et al., 2012). This condition is caused by massive damage of the skeletal muscle due to strenuous physical exertion leading to muscle disintegration. This commonly triggers the release of myoglobin and other cellular contents to the extracellular space and circulatory system (Kupchak et al., 2014b), such as electrolytes and sarcoplasmic proteins, including serum creatine kinase (sCK), aspartate transaminase (AST), aldolase, alanine transaminase (ALT) and serum lactate dehydrogenase (sLDH), myoglobine (MB) (Abbas et al., 2019; McVane et al., 2019).

CK is a by-product of muscle breakdown. When massive muscular necrosis occurs, exertional rhabdomyolysis could manifest weakness, myalgia, edema and macroscopic pigmentation with and without the presence of hematuria in the urine (Bruso et al., 2010a; DeFilippis et al., 2014). During ER, markers such as MB and sCK can increase 4 to 5 fold normal value; additionally, sLDH and AST can be two fold compared

to their normal reference values (Brancaccio et al., 2010). The most used biomarker to diagnose and identify ER is sCK, which increase until achieving the maximum peak 24–48 h after the endurance exercise and recovers the basal levels at a rate of 40% per day until 48–72 h later (Atias-Varon et al., 2017; D. Kim et al., 2015; Oh et al., 2015). sCK has replaced MB as the most preferred biomarker for ER diagnosis since MB is rapidly removed from the bloodstream (Hou et al., 2015). In this sense, ER is diagnosed in addition to clinical history and physical examination, when sCK values increase over five times the upper limit of normal reference values (~200 IU/L) or sCK exceeding 5000 IU/L. More recently, a diagnosis criterion was set at >1000 UI/L after a systematic review about rhabdomyolysis definition (Stahl et al., 2019) but it could not be applied to endurance exercise settings.

ER could cause severe organic conditions that could result in acute and chronic injuries, ranging from an increase in sarcoplasmic proteins at the circulatory level to health issues and medical emergencies (Asserraji et al., 2014; Rojas-Valverde, Sánchez-Ureña, et al., 2020). Among these health problems are but not limited to hyperkalemia, hypernatremia, lactic acidosis and hyperphosphatemia, kidney tubule damage and subsequent acute kidney dysfunction (McCullough et al., 2013a), muscle ischemia, cardiac arrhythmia and even death (Asserraji et al., 2014; Rojas-Valverde, Sánchez-Ureña, et al., 2020).

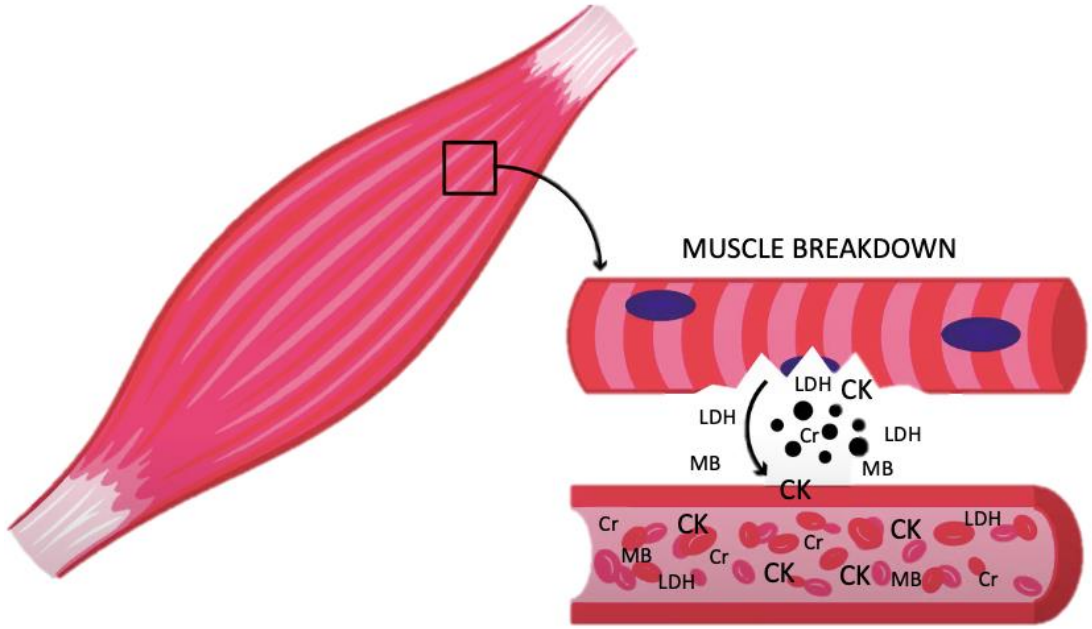


Figure 3. Muscle breakdown and rhabdomyolysis: the release of protein waste products to the bloodstream (CK, MB, Cr, LDH).

iii. Etiology of rhabdomyolysis

ER is the outcome of muscle damage induced by prolonged and intense exercise. This damage is represented in myocyte damage, usually in the Z lines and energy depletion at the cellular level (Hernández-Contreras et al., 2015; Stella & Shariff, 2012). When muscle damage occurs on massive scale due to intense and repetitive exercise, this in turn can result in further release of CK and MB into the bloodstream causing ER. CK molecule is released by urine only when 0.5–15 mg/dL of CK protein is exceeded in the bloodstream, which represents 100 g of muscle; these high biomarkers release lead to future damage to the tubules of the glomerulus and could cause subsequent AKI (DeFilippis et al., 2014; Shimizu et al., 2017).

The lower CK values in participants of mountain marathons tend to be lower than those of participants in road marathons, because road marathons are run on asphalt, a more rigid and compact surface on which repeated impacts could trigger more significant muscle fiber breakage compared to running in mountain marathons, where the surface is less stiff and more able to absorb shock (Rojas-Valverde, Sánchez-Ureña, et al., 2020). But when there are downhill slopes the speed and the eccentric muscle actions provoke a rise in muscle damage and future sCK levels (Rojas-Valverde, Sánchez-Ureña, et al., 2019, 2020; Vernillo et al., 2017).

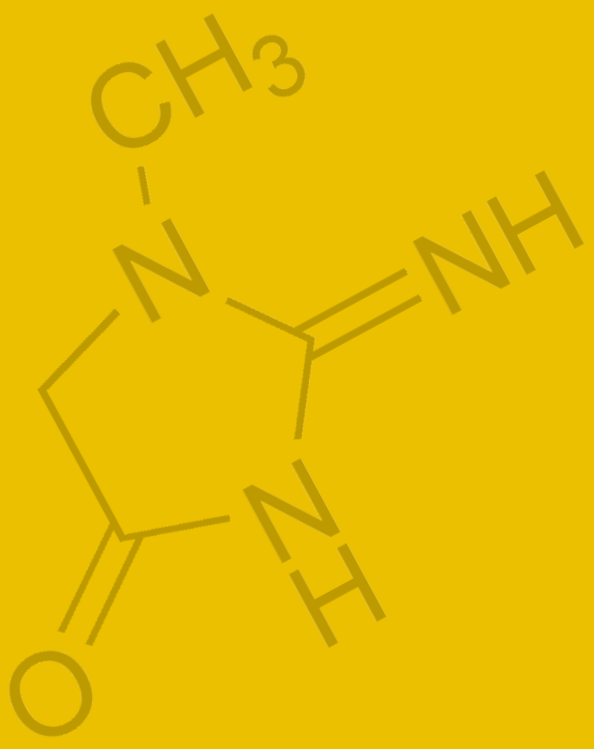
iv. Traditional biomarkers of muscle damage and rhabdomyolysis

Some of the traditional biomarkers to assess muscle damage and rhabdomyolysis are: sCK, ALT, AST, sLDH, MB. These lab analyses can be combined with medical imaging to aid medical decision-making. Although the extensive use of sCK as the preferred biomarker for ER (Lippi et al., 2018), some authors have exposed different unfavorable factors of sCK when assessing ER (e.g., CK activity strongly varies between human muscles) (Delanghe et al., 2019).

v. Novel biomarkers of muscle damage and rhabdomyolysis

Some novel biomarkers have been proposed to be assessed when identifying ER as fatty acid-binding proteins and carbonic anhydrase III, myosin light chain III and muscle micro RNAs. However, their practical and clinical effectiveness, reference values,

standardization, access in clinical laboratories and costs are still the major limitations of it use (Lippi et al., 2018).



C. RENAL FUNCTION

Kidneys play a critical role in maintaining physiological factors as osmotic pressure, electrolyte balance and pH. The electrolyte balance is a fundamental process necessary for exercise, and it is performed by excreting metabolic waste products and excreting and reabsorbing electrolytes and water (Suzuki, 2015).

i. Renal function during exercise

During exercise, to allow optimal mechanical muscle function and the requirement to dissipate heat, blood flow is redirected to the muscles allowing the proper delivery of nutrients, energy, and oxygen to the muscle tissues. To avoid a decrease in blood pressure, the body restricts the blood flow to some non-essential organs during exercise as the liver, gastrointestinal tract, and kidneys (75% reduction). As such, a decrease in renal function results, mainly due to the sympathetic nervous system's activity that provokes renal vasoconstriction, elevation in the antidiuretic hormone, renin, angiotensin II and aldosterone (Poortmans, 1984). Regularly, these cardiovascular and renal responses allow to maintain sodium levels and permits water conservation during exercise (Poortmans, 1984).

In endurance running, the exercise is prolonged and intense, which leads to a significant impact on internal and external load resulting in a tremendous effect on renal function. The association of this reduction in renal function and renal failure with endurance exercise is relatively low. In regular conditions, proper hydration and fluid intake before and throughout exercise can reduce kidney injury risk. In endurance running characterized by prolonged and strenuous exercise bouts, the body maintains systemic and renal homeostasis. Some internal and external conditions could magnify the potential for renal problems associated with endurance running as high heat and humidity, massive muscle damage due to increased external load, low rate of liquid restore, nonsteroidal anti-inflammatory drugs and pain drugs consumption, and some clinical and genetic background (de Souza et al., 2020; Kao et al., 2015a; Lipman et al., 2017; Page et al., 2007; Scheer, 2020).

ii. Definition of acute kidney injury

The definition of AKI has experienced a remarkable evolution during the last two decades, with more than 30 to 35 separate definitions found in the literature (Bellomo et

al., 2004; Kellum et al., 2002). Other authors defined AKI as an injury or damage accompanied in some cases by renal dysfunction over a relatively short period (less than 48h) with a relatively fast recovery (usually days) (Bellomo et al., 2012; Bosch et al., 2009; Gameiro et al., 2018; Rojas-Valverde, Sánchez-Ureña, et al., 2020). Some of the clinical signs include but are not limited to a decrease in glomerular filtration rate, disturbances in acid- and electrolyte-based balance, derangement of extracellular fluid volume, decreased urine output, and retention of nitrogenous waste products (Nissenson, 1998). Historically, there is no consensus on the definition or diagnosis criteria for AKI (Kellum et al., 2002), making it challenging to analyze and interpret studies data and differentiate when or not it is AKI (Rojas-Valverde, Sánchez-Ureña, et al., 2020). This could be due to the lack of consensus of when to consider a renal dysfunction or not, based on the fact that human kidneys have a significant glomerular function reserve, so a dysfunction may be only evident when more than 50% of the total renal mass is compromised (Ronco et al., 2012a).

This sudden kidney injury episode causes a build-up of waste products in the bloodstream, making it difficult for kidneys to maintain fluid homeostasis (Rojas-Valverde, Sánchez-Ureña, et al., 2020). AKI is defined as an abrupt or sudden decrease in kidney function over a relatively short period. This condition could be the result of a specific disease of the kidney (e.g., glomerulonephritis, interstitial nephritis) or extrarenal conditions (e.g., dehydration, muscle damage, heart failure, sepsis, obstruction) (Bellomo et al., 2004).

AKI is a relatively uncommon condition but an under-recognized problem in endurance sports (Rojas-Valverde, Sánchez-Ureña, et al., 2020). Concerning exercise, between 4-33% of the cases of exertional rhabdomyolysis could lead to or be accompanied by AKI (Chatzizisis et al., 2008).

iii. Etiology of acute kidney injury

The etiology of AKI has been traditionally understood as three different categories (Gameiro et al., 2018):

Pre-renal

Some clinical conditions can result in pre-renal kidney injury, including but not limited to: extracellular fluid losses secondary to burns, prolonged vasoconstriction, reduced cardiac output, reduction in renal perfusion pressure due to drugs (e.g., NSAID,

angiotensin-converting enzyme inhibitors). The above causes could lead to ischemic damage to the nephron and acute tubular necrosis. Some of the exercise-related reasons provoking pre-renal injury are dehydration, trauma, heart failure, strenuous exercise.

Renal

Damage to the nephrons and renal parenchyma usually characterize intrarenal failure, resulting in kidney diseases or acute tubular necrosis. One of the exercise-related causes leading to renal impairment is rhabdomyolysis due to massive muscle damage (mechanical and thermal).

Post-renal

Usually, post-renal failure is caused by obstruction of the urine flow (e.g., tumors, benign prostatic hypertrophy, kidney stones, and bladder neck obstruction). If not treated, post-renal failure could result in nephron damage and intrarenal failure. There is still no relationship between this kind of damage and exercise.

iv. Traditional markers of renal function and acute kidney injury

Several consensus definitions for AKI have been developed for use in the general population (Lopes & Jorge, 2013). Still, no specific considerations have been undertaken considering renal function variations during exercise (Rojas-Valverde, Sánchez-Ureña, et al., 2020).

Some diagnosis criteria and definitions have been considered considering the timing and magnitude of creatinine and urine output changes. These consensus definitions and diagnosis criteria include the Risk, Injury, Failure; Loss, End-Stage Renal Disease (RIFLE) (Bellomo et al., 2004), the Acute Kidney Injury Network (AKIN) criteria (Mehta et al., 2007), and the Kidney Disease: Improving Global Outcomes (KDIGO), modifications to AKIN (Kellum et al., 2012) (see Table 4.).

Table 4. Diagnosis criteria for acute kidney injury

Criteria	RIFLE	AKIN	KDIGO	Urine output
Risk (Stage 1)	↑sCr ≥ 1.5x baseline or ↓in GFR ≥25%	↑sCr ≥ 0.3mg/dL or a ↑1.5 to 1.9 fold from baseline	↑sCr ≥ 0.3mg/dL within 48h or ↑1.5 fold from baseline presumed occurred within prior seven days	<0.5 mL/kg/h for ≥6 h
Injury (Stage 2)	↑sCr ≥ 2.0x baseline or ↓in GFR ≥50%	↑2 to 2.9 fold from baseline		<0.5 mL/kg/h for ≥12 h
Failure (Stage 3)	↑sCr ≥ 3x baseline or ↓in GFR ≥75% or an absolute sCr ≥ 4mg/dL with an acute rise of at least 0.5mg/dL	↑≥3 fold from baseline or an absolute sCr ≥ 4mg/dL with an acute rise of at least 0.5mg/dL		<0.3 mL/kg/h for ≥24 h or anuria ≥12h

As mentioned, the variation in creatinine parameters considered in these three criteria makes it challenging to establish an incidence in the general population (Hsu et al., 2007). Also, considering the functional variations in creatinine during exercise due to renal vasoconstriction and muscle damage, these criteria have been strongly criticized (Colombini et al., 2012; Poussel et al., 2020; Rojas-Valverde, Sánchez-Ureña, et al., 2020). Although the creatinine sensitivity due to some contextual factors as food intake, age, race, gender, muscle mass, hydration status, drugs consumption, other novel biomarkers have been proposed (Beker et al., 2018; Beunders et al., 2017; McCullough et al., 2013a; Mingels et al., 2009; Watson et al., 2019).

v. Novel biomarkers of renal function and acute kidney injury

Considering the concerns about the AKI criteria based on creatinine, several new biomarkers have been proposed. The research community's attention stays focused on finding a novel AKI biomarker that identifies AKI in the early stages. Additionally, some ideal characteristics of an AKI biomarker have been proposed: a. easy, quick and cheap, and use readily available specimens (e.g., serum, urine), b. precise, reliable and use

standardized assay methods, c. highly sensitive to permit early detection of AKI, d. can detect the severity and trajectory of AKI, and d. specific, allowing discriminating between AKI´s subtypes (Bagshaw & Bellomo, 2007; Devarajan, 2007).

The proposed biomarkers are neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (Il-18), kidney injury molecule-1 (KIM-1), monocyte chemotactic peptide-1 (MCP-1), tissue inhibitor of metalloproteinase-2 (TIMP2), N-acetyl-β-D-glycosaminidase (NAG), insulin-like growth factor-binding protein-7 (IGFBP7), uromodulin, tumor necrosis factor-α (TNFα), cystatin-C (Cyst-C), and netrin-1(Bagshaw & Bellomo, 2007; Beker et al., 2018; Sandokji & Greenberg, 2020).

These novel biomarkers are very promising in the identification of early stages. There is a need for more studies to validate the sensitivity and specificity in clinical samples from large cohorts and multiple clinical situations, including during exercise. Considering the above, there are still no available reference values or definitions of various types and etiologies of AKI based on these biomarkers. Finally, some studies should be undertaken to resolve doubts regarding when each marker should be measured.

vi. Acute kidney injury in trail running and endurance sports

During prolonged and strenuous exercise bouts that define endurance running, some physical (external load) and physiological (internal load) conditions could impact renal function. The decrease in renal function associated with prolonged exercise is relatively low (Rojas-Valverde, Sánchez-Ureña, et al., 2020), and commonly optimal hydration before and throughout the endurance exercise can minimize the risk of kidney injury. Still, in endurance running, the body manages to maintain systemic and renal homeostasis. Internal and external load conditions could increase the potential prevalence for renal issues associated with endurance running as high heat and humidity, massive muscle damage due to high external load, low rate of liquid restoration, nonsteroidal anti-inflammatory drugs and pain drugs consumption, and some clinical and genetic background (de Souza et al., 2020; Kao et al., 2015a; Lipman et al., 2017; Page et al., 2007; Scheer, 2020).

These internal and external conditions could lead to serious renal issues related to renal function such as dehydration, hyponatremia, bilirubinuria, proteinuria, hematuria, hemoglobinuria, myoglobinuria, cylindruria, indicators of acute kidney injury (AKI)

(Belli et al., 2018a; Boulter et al., 2011a; Bruso et al., 2010a; Gerth et al., 2002; Khodaee et al., 2020; Rojas-Valverde, Olcina, et al., 2020; Wołyniec et al., 2019). These renal issues are generally minor and require relatively short periods to achieve full baseline recovery (1-10 days) (Kao et al., 2015b; Lipman et al., 2014b, 2016; Rojas-Valverde, Sánchez-Ureña, et al., 2020). In some cases, AKI is identified in combination with rhabdomyolysis, making it a more complicated condition (Rojas-Valverde, Sánchez-Ureña, et al., 2020). Due to AKI being usually asymptomatic, most cases are not detected in the field during and after endurance running events. This is why some methods (e.g., urine dipsticks) have been proposed to identify AKI in outpatient settings (Hoffman et al., 2013; Rojas-Valverde, Olcina, et al., 2020).

The release of some blood and urine biomarkers could imply a transitory functional or subclinical renal injury. Although there is extensive evidence of acute renal dysfunction during endurance running, the link between AKI and some long-term diseases has not been established in the exercise population (Hodgson et al., 2017; Rojas-Valverde, Sánchez-Ureña, et al., 2020). There is evidence suggesting that the cumulative or subsequent functional or structural AKI events could contribute to future chronic damage (Coca et al., 2012; Palant et al., 2016). In endurance running, this association is not clear and a better understanding of physiological cascade events is needed. Some risk factors could strengthen the AKI link to long-term renal issues as age, race, genetics, and other pathological conditions due to the potential maladaptive repair (Chawla et al., 2014) and progressive renal scarring (Ferenbach & Bonventre, 2016) due to cumulative AKI events.

Additionally, exploration should be undertaken into the influence situational, contextual, and individual factors have on AKI incidence and how this temporary loss of kidney function is related to possible long-term kidney problems (Rojas-Valverde, Sánchez-Ureña, et al., 2020). To do this, longitudinal studies are required to observe the behavior of kidney function through a season, multistage races, and, if necessary, investigate how to prevent and treat these acute and chronic kidney conditions promptly for the endurance runners' health. New technological developments such as wearable devices (Rojas-Valverde, Timón, et al., 2020; Wieringa et al., 2017), could allow researchers to monitor in real settings and remotely those conditions that could increase

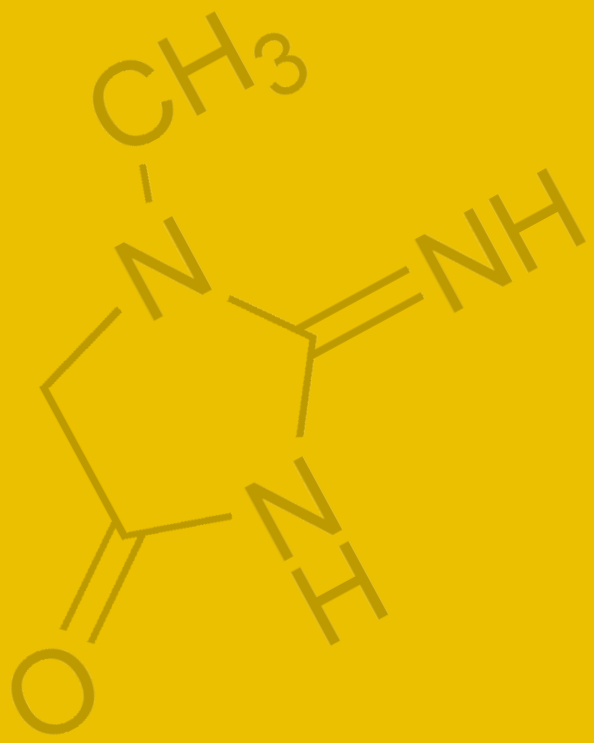
the incidence of acute or chronic kidney health problems (e.g., environment temperature and humidity, internal, muscle damage, water balance, sweating rate).

vii. Acute kidney injury leading to chronic kidney disease

For more than 50 years, specialists have classified diminished kidney function as two different syndromes, acute kidney failure (e.g., AKI) and chronic kidney failure (e.g., CKD). The interconnection of the two syndromes is not well established. Still, some hypotheses suggest that the acute tubular necrosis caused by pre-renal conditions could turn from functional and reversible damage (e.g., AKI) to structural and irreversible damage (e.g., CKD). The first link between both syndromes is based on similar risk factors (e.g., black race, diabetes mellitus, advanced age, repeated intense exercise bouts, heat strain, dehydration) (Chawla et al., 2014).

For patients who recovered renal function after an acute episode of kidney injury, the common belief was that there would not be long-term outcomes related to AKI (Chawla & Kimmel, 2012; Liaño et al., 2007), but some observational studies have shown a strong association between AKI and subsequent development of CKD (Chawla et al., 2011; Chawla & Kimmel, 2012; Ishani et al., 2009). These studies have shown that patients who suffered AKI and without any precondition of CKD often recover some degree of renal function but then the condition progress to CKD (Chawla et al., 2011; Chawla & Kimmel, 2012; Coca et al., 2012; Ishani et al., 2009). This is why it is proposed that AKI could lead to CKD regardless of the AKI cause (Chawla et al., 2014). Moreover, multiple AKI episodes could predict the development of CKD (Thakar et al., 2011).

Although the physiological mechanisms underlying renal dysfunction's progression are not entirely understood, studies in animals suggest two causal pathways, including maladaptive repair and disordered regeneration (Chawla & Kimmel, 2012; Venkatachalam et al., 2010). More in-depth analysis is needed to associate exercise-related AKI with nephron damage (structural) and no only functional as known until now, to consider the maladaptive and disordered regeneration hypothesis among athletes.



D. WEARABLE DEVICES AND EXTERNAL LOAD ASSESSMENT

The combination of internal load and external load through wearable devices is widely common. These wearable sensors are usually classified into three main categories: mechanical, physiological, and biochemical. Among the mechanical sensors, depending on the type of sensors incorporated in the same wearable device, some denominations are given. Some mechanical sensors (e.g., magnetic, angular rate and gravity [MARG] sensors, inertial measurement units [IMU]) use accelerometers to measure 3D movement, gyroscopes to evaluate rotation, and magnetometers to calculate relative position. Both MARG sensors and IMU usually combined other hardware as temperature sensors, Bluetooth, global navigation satellite systems (GNSS), altimeters to register, control, correct, communicate, and analyze registered data.

Wearable mechanical sensors usually calculate translational and rotational motion, applied forces, and magnetic fields to assess the athlete's external load (Dunn et al., 2018; Seshadri et al., 2019). Wearable mechanical sensors can acquire external load frequency, intensity, the magnitude of physical exercise. These sensors are widely used due to their high accuracy, low cost, small size, integration into existing sensor networks (Lai et al., 2013). These wearable mechanical sensors are used in sports and exercise sciences in multiple applications, such as assessing external load while running (de Dionisio et al., 2020; Gutiérrez-Vargas et al., 2020). For this reason, evaluating both internal and external workload variables simultaneously can provide complementary and highly relevant information for the planning and prescription of physical exercise (Eston et al., 1998). And potentially identify some health exercise-related issues (Li & Wen, 2020; Li et al., 2016; Rojas-Valverde, Timón, et al., 2020).

i. Interaction of internal and external load in trail running

Currently, it is essential to have information related to the integration of internal and external load variables. The interaction between internal and external load has been studied in sports. There is evidence of how several perceptual, physiological, and physical variables are related (Rossi et al., 2019). Some of the variables involved in this interaction between internal and external load in team sports are the rate of perceived exertion, total distance, TRIMP, high-speed relative distance, among others. Finally, internal and external load assessment has shown consistently positive associations with external load variables (Impellizzeri et al., 2019; McLaren et al., 2018; Rossi et al., 2019). Some authors have reinforced the importance of measuring both types of load due to their

complementary nature; although external load measurement has caught the attention of coaches and scientists, internal load represents the functional outcome of training, and therefore both should be monitored (Impellizzeri et al., 2019).

The optimal way to assess training and competing load is to use internal and external load together (Paulson et al., 2015). The monitoring of external load variables such as distance and speed-related accelerometry variables has been commonly used due to the development of technological devices such as inertial measurement units, global and local positioning systems, high video systems (McLaren et al., 2018). Combining these external load variables with other well-known internal load indicators may allow sports scientists and sport medicine stakeholders to develop and manage physical and physiological attributes in a more specific and optimal way, considering the activity demands and the responses provoked at the physiological level. In this sense, some studies have studied the interaction between internal and external load in the identification of AKI, considering that muscle damage, the forces involved in the running mechanics, and biomarkers could be strongly related (Rojas-Valverde, Timón, et al., 2020; Rojas-Valverde, Sánchez-Ureña, et al., 2019).

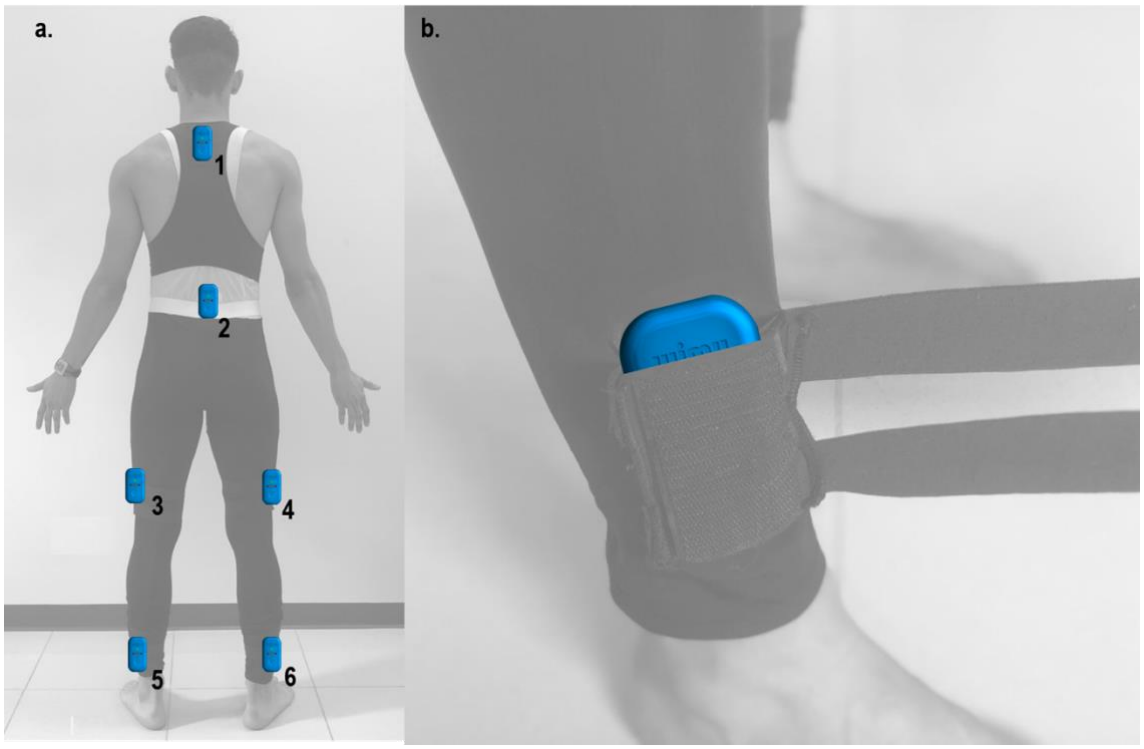
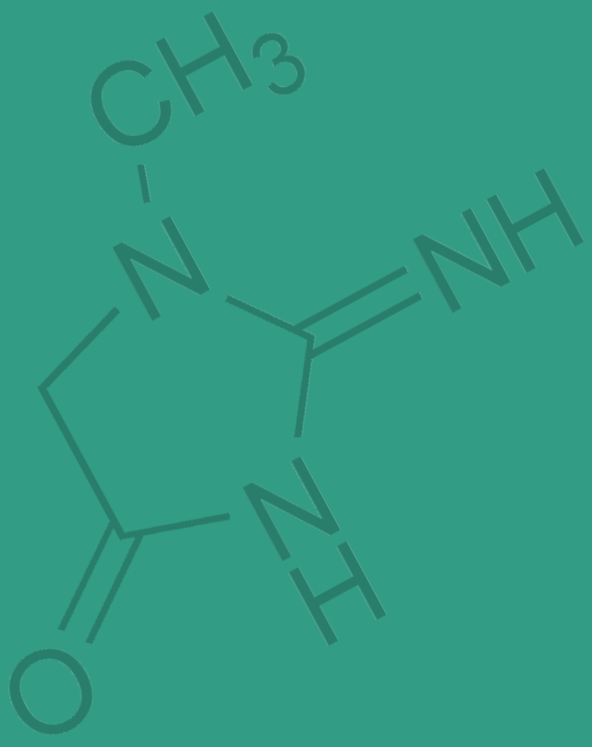


Figure 4. Example of MARG sensors position (a) and attachment (b) for body multi-segment external workload assessment (Rojas-Valverde, Sánchez-Ureña, et al., 2019).



II. AIMS

A. General aim:

Determine the effect of different internal and external factors on acute muscle and kidney damage in well-trained runners during mountain competition.

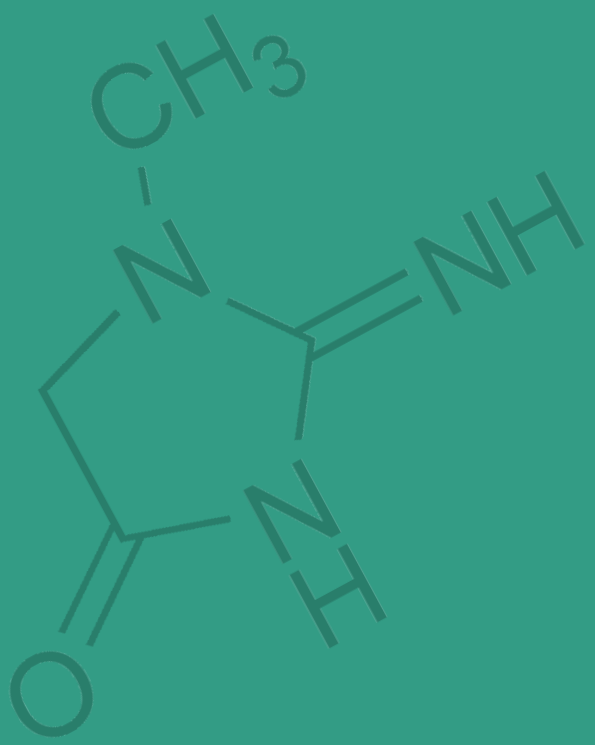
B. Specific aims:

a. Investigate findings on exertional rhabdomyolysis and acute kidney injury in endurance sports, emphasizing the diagnostic criteria used, and the physical and environmental contextual conditions in which ER and AKI occur.

b. Analyze which external load indicators have the greater association with biomarkers of muscle and kidney injury.

c. Explore potential exposure to kidney disease in endurance athletes in countries with adverse hot and humid conditions.

d. Globally explore the variables of heat stress, dehydration and external workload as indicators of acute kidney injury in endurance running.



III. METHODS

A. Designs

i. Specific aim A

To delve into the knowledge about ER and AKI in endurance sports and explore which are the most used and pertinent diagnostic criteria, a systematic review of was performed. This systematic review aimed to synthetize existing evidence and identify research needs regarding rhabdomyolysis and acute kidney injury in endurance sports, emphasizing the diagnostic criteria used as well as the physical and environmental factors and conditions in which ER+AKI is reported (Rojas-Valverde, Sánchez-Ureña, et al., 2020). This systematic review was developed following the Preferred Reporting Items for Systematics Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2015). Also risk of bias was considered and the internal quality of each study was assessed via the Office of Health Assessment and Translation (OHAT) Risk of Bias Rating Tool (OHAT, 2015).

Additionally, an opinion article was proposed with an overview of how endurance athletes could suffer from AKI and how it could result in long-term renal issues considering other population evidence that have some similarities with athletes as firefighters and sugarcane harvesters (e.g., high external load, heat strain, dehydration).

ii. Specific aim B

To analyze which external load indicators have the most influence on the biomarkers of muscle and kidney injury, two different studies were performed. The first one aimed to to analyze MARG sensors' test/retest agreement and reliability when assessing multiple segments' external loads during off-road running. This process was fundamental for the use of MARG sensors in trail running monitoring.

To explore the agreement and reliability of these sensors, participants ran two laps of a 12 km off-road circuit under a temperature of 25ºC and a relative humidity of 80%. Runners wore six different MARG sensors attached to a special neoprene suit six different body segments during both laps: (MP_{Left}) and right (MP_{Right}) malleolus peroneus, left (VL_{Left}) and right (VL_{Right}) vastus lateralis, lumbar (L_1-L_3) and thorax (T_2-T_4). (see figure 4 and 5).

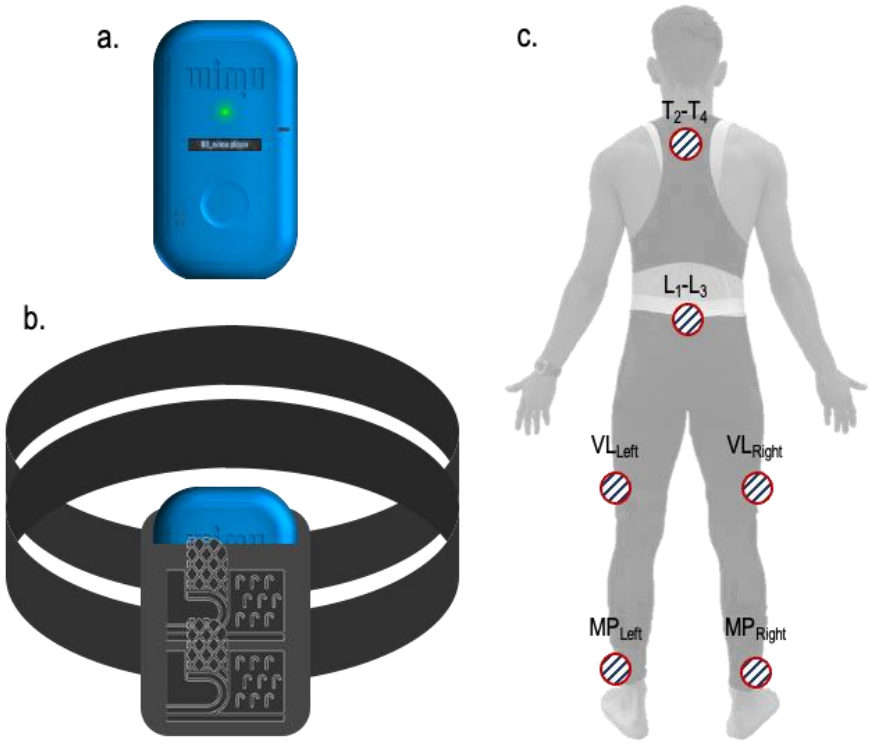


Figure 5. a. Magnetic, angular rate, and gravity (MARG) sensors, b. Adjustment Velcro straps system and, c. Special spandex bib-pants and MARG sensors positioning (Rojas-Valverde et al., 2021).

The second study aimed to explore which external workload indicators have more influence on the responses of muscle and kidney injury biomarkers in experienced endurance trail runners. To achieve this purpose, participants ran 3 × 11.76 km trail circuit (total distance: ~35.27 km, cumulative positive ascend: 1815 m [from 906 to 1178 m.a.s.l.]) under a temperature of 24.5°C and humidity 77.88%. And they were assessed -pre (serum test), during (physical external workload of six different regions, see Figure 5.), -post$_{0h}$ (serum test: sCr, sALB and sBUN) and -post$_{24h}$ (serum test) the trail running event (Rojas-Valverde, Sánchez-Ureña, et al., 2019) (see Figure 6.).

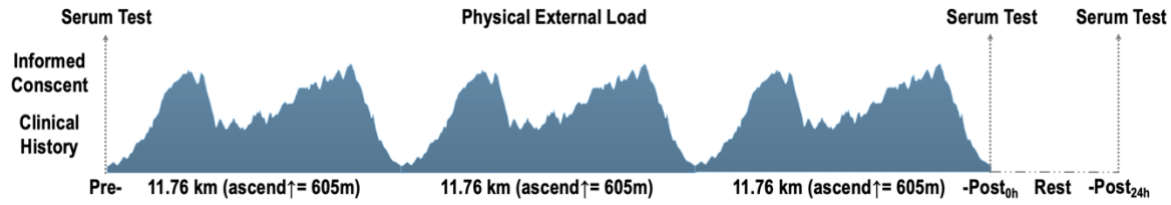


Figure 6. Schematic design of study variables with time measurement and trail altimetry (Rojas-Valverde, Sánchez-Ureña, et al., 2019).

iii. Specific aim C and D

To explore the potential exposure to kidney disease in endurance athletes and analyzed the heat stress, dehydration and external workload as indicators of acute kidney injury in endurance running, two studies were performed. The first one aimed to explore which urinary markers could indicate AKI during prolonged trail running in outpatient settings and the second one had the purpose to explore wearable sensors' potential use to assess cumulative mechanical kidney trauma during endurance off-road running.

In the first study, participants ran a 35 km event (cumulative positive ascend of 1815 m, altitude= 906 to 1178 m.a.s.l.) under a temperature of 25.52 ± 1.98 °C and humidity of 79.25 ± 7.45%. Serum and urine samples were collected ~15 min before and ~15 min after the race. In the second one participants ran the same distance wearing a MARG sensor attached to the lower back during all race and variables of time-related impacts were extracted (see Figure 7). Two blood samples were collected pre- and post-race to assess serum creatinine (sCr) and albumin (sALB).

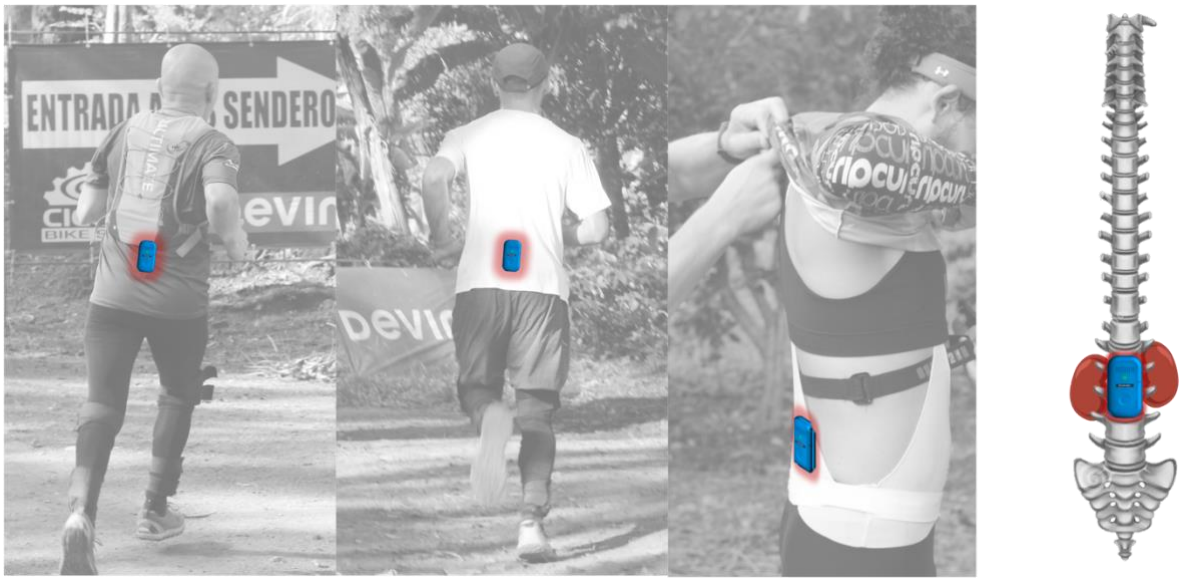


Figure 7. MARG sensor attachment at runner´s lower back (L1-L3) (Rojas-Valverde, Timón, et al., 2020).

B. Participants

For all studies participants were notified of the procedures and details of the study protocol. All potential risks and rights during the study were informed and agreed upon by both parties. All procedures were performed following biomedical guidelines based on the Helsinki Declaration. The study protocol was reviewed and approved by two Institutional Review Boards: Universidad Nacional, Costa Rica (Reg. Code 2019-P005) and Universidad de Extremadura, Spain (Reg. Code 139/2020).

All participants were experienced trail runners, heat-acclimatized (live and train near study location) and well-trained. Also, Participants who reported any muscular or metabolic diseases or recent (<6 months) physical injury of the lower limbs were excluded from the study.

i. Specific aim A

For the systematic review, web searches were performed in well-known databases in the areas of health, medicine, exercise and sport: PubMed (MEDLINE), Science Direct (EMBASE) and Web of Science (WoS). The combined keywords used as search descriptors were: ¨sport¨, ¨exertional rhabdomyolysis¨, ¨acute renal failure¨, ¨acute kidney injury¨.

ii. Specific aim B

In the agreement and reliability study a total of 18 off-road male runners (38.78 ± 10.38 years, 73.24 ± 12.6 kg, 172.17 ± 9.48 cm) took part. And in the second one a total of 20 male runners (age = 38.95 ± 9.99 years, weight = 71.94 ± 12.59 kg, height = 171.15 ± 9.52 cm) were involved in this study.

iii. Specific aim C and D

For the first study twenty-nine mountain runners (age 39 ± 9.1 years, weight 71.7 ± 10.8 kg, height 172.2 ± 8.3 cm) took part. And in the second one eighteen experienced mountain runners participated (age 38.78 ± 10.38 years, weight 73.24 ± 12.6 kg, height 172.17 ± 9.48 cm).

C. Instruments and procedures

i. Data selection, collection and extraction in the systematic review

The studies with the following inclusion criteria were selected: a. articles related on rhabdomyolysis or kidney injury with reports of at least two variables such as sCr, MB, sCK, Cyst-C and sLDH. The search was limited to articles published between 2009 and 2019. Due to the inconsistences around the S-CK and S-Cr levels for the diagnosis of ER and AKI respectively, reference values were selected as follow: exertional rhabdomyolysis was considered to be S-CK exceeding 1000-5000 U/L or 5 times the upper limit (Stahl et al., 2019) and AKI was considered to be when the AKIN or RIFLE criteria's for S-Cr were met (Lopes & Jorge, 2013). Baseline S-Cr was standardized in 1.25 mg/dL for rating proposes (Bellomo et al., 2012), therefore AKI was classified according to the following thresholds S-Cr ≥1.88 mg/dL (AKI-*risk*), ≥2.5 mg/dL (AKI-*injury*) and ≥4.5 mg/dL (AKI-*failure*) (Kellum, 2015).

The procedure followed in the systematic review is presented in the figure 8.

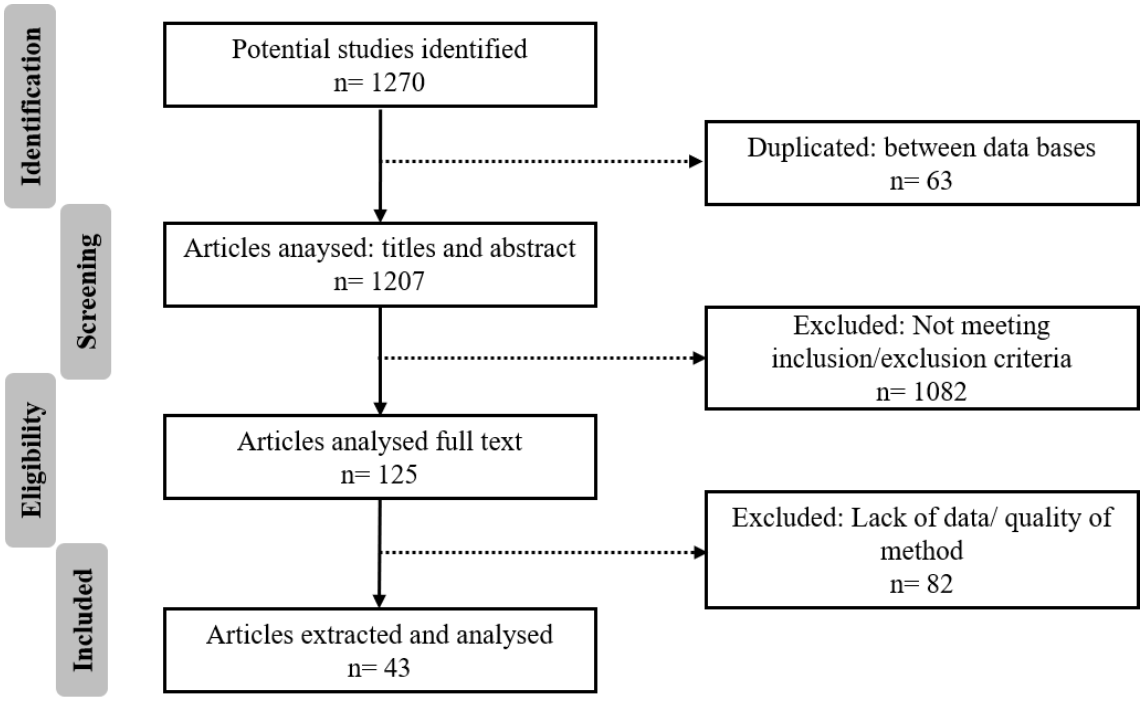


Figure 8. Data/articles selection process flowchart (Rojas-Valverde, Sánchez-Ureña, et al., 2020).

ii. Accelerometric-based external workload Indicators

All ABELIs (Gómez-Carmona, Pino-Ortega, et al., 2019) variables were registered and extracted Variables were assessed using MARG sensors (WIMU PRO^{TM}, RealTrack Systems, Almería, Spain) (see Figure 4 and 5.). The MARGs sensors integrate four 3-axis microelectromechanical system accelerometers (2x ± 16g, 1x ± 32g, and 1x ± 400g), gyroscope, and magnetometer. All MARGs calibration and settings were developed following published guidelines (Oliva-Lozano et al., 2020; Rico-González et al., 2020). Its reliability for lineal and curvilineal running external load assessment (Gómez-Carmona, Bastida-Castillo, et al., 2019) in different body parts (Gómez-Carmona et al., 2018) has been proven. Considering that the method (e.g., Velcro strap, adhesive tape, utility tape) and the exact point (e.g., MARG orientation and position) where the sensors are attached could cause measurement outcome differences, a standard protocol was performed following previous studies assessing external load variables (Fong & Chan, 2010; Riley et al., 2007; Rojas-Valverde, Sánchez-Ureña, et al., 2019). Consequently, the attachment was made using a special spandex dark-suit, equipped with six different pockets where the MARG sensors were introduced and secured using a particular Velcro strap system (see Figure 6). The six pockets were designed to be at T_2–T_4 (between scapulae), L_1–L_3 (in lumbar vertebrae), VL_{right} and VL_{Left} (5 cm cephalic from lateral femoral condyles), and MP_{Left} and MP_{Right} (3 cm cephalic from malleolus peroneus) (see Figure 6). These attachment spots were selected considering previous literature using body-worn sensors (Fong & Chan, 2010; Riley et al., 2007; Rojas-Valverde, Sánchez-Ureña, et al., 2019).

The IMU's were previously calibrated following protocols for this specific microsensor [39]. This IMU has been used for the assessment of neuromuscular running workload [6] and its reliability had been tested in it use, attached to different body places [39]. Total variables data extracted from IMU software were analyzed using a principal component analysis (PCA) in order to explain total variance [40].

Total variables analyzed were: Player Load per min (AU, PL/min), Player Load difference between segments (AU, PL_{Dif}), approximated entropy (ApEn, AU), maximum acceleration (m/s^{-1}, Acc_{max}), total accelerations (Acc, n/min), total decelerations (Dec, n/min), average acceleration (Acc_{avg}, m/s^2), average deceleration (Dec_{avg}, m/s^2), maximum speed ($Speed_{max}$, m/s), average speed ($Speed_{avg}$, m/s), metabolic power (MP, W/kg), high metabolic load distance (HMLD, m/min), explosive distance (>16 km/h)

($D_{>16\ km/h}$, m/min), maximum heart rate (HR_{max}, bpm), average heart rate (HR_{avg}, bpm), total impacts ($Impacts_{total}$, n/min), and total impacts at 1 g ranges from 0 to >30 g ($Impacts_{total}$, n/min).

Considering that MARG sensors reported more than 200 variables, the selection of the variables studied in each research were selected using a principal component analysis (PCA). A PCA was performed for each body segment to select the most representative variable when running in this specific sample. PCA is a widely used multivariate technique to manage big data resulting from different assessments in sports (Rico-González et al., 2020). This statistical model was selected to extract the most relevant variable that explains most of the variables' data set variance. The three ABELIs of each of the six body segments were introduced in the model.

A PCA for each body segment was performed following previously published protocols, meeting 21 out of 21 quality items (Rico-González et al., 2020). The variables were explored using a correlation matrix (factorability of *r*) to select the most representative variable (Tabachnick & Fidell, 2007); those correlations with $r<0.7$ between variables were considered for extraction (Hair et al., 1995). After excluding variables with variance=0, the variables were scaled and centered using *Z*-Scores if applied. PCA suitability was confirmed through Kaiser-Meyer-Olkin (*KMO*=0.62-0.75) and Bartlett Sphericity test significance ($p<0.05$) (Hair et al., 1995; Kaiser, 1960). Those eigenvalues >1 were considered for the extraction for each Principal Components (PC, factors). A VariMax orthogonal rotation method was used to identify high correlations between components to offer different information (D. J.-O. Kim & Mueller, 1978). Those PC loadings >0.6 (Liu et al., 2003) were considered for extraction, and when a cross-loading was found between PCs, only the highest factor loading was retained (Hair et al., 1995).

iii. Serum biomarkers

A five mL of blood was drawn from an antecubital vein directly into a blood collection sterile tube (BD Vacutainer®, New Jersey, USA) containing spray-coated silica particles activator and a gel polymer for serum separation. Samples were centrifuged at 2000 g relative centrifugal force (RCF) for 10 min using tube centrifuge (PLC-01, Gemmy Industrial Corp., Taipei, Taiwan). During sample collecting process, blood samples were stored on ice in a special cooler (45QW Elite, Pelican™, California, USA)

until they were stored in a freezer (−20 °C) the same sample extraction day. Sample processing was performed a day after the event under controlled and isolated room using an automatic biochemical analyzer (BS-200E, Mindray, China) by photometry method.

The variables extracted from analysis were serum creatinine (sCr, mg/dL), serum creatine kinase (sCK, IU/L), serum ureic nitrogen (sBUN, mg/dL), serum albumin (sALB, IU/L). Delta percentage of change was calculated for each variable between pre- and -post$_{0h}$ or -post$_{24h}$. Kidney functional loss and Acute Kidney Injury (AKI) was considered and classified following Acute Kidney Injury Network (AKIN), the Risk, Injury, Failure, Loss of kidney Function, and End-stage kidney disease (RIFLE) [36] and the Kidney Disease Improving Global Outcomes (KDIGO) [37] criteria as follow: AKI$_{risk}$ (sCr increase of 150% or acute increase or ≥0.3 mg/dL) and AKI$_{injury}$ (sCr increase of 200%). Additionally, exertional rhabdomyolysis (ER) was considered if sCK level exceeded 1000 UI/L [38].

iv. Urine markers and specific gravity

Urine samples were collected *in-situ* in a 30 mL polypropylene sterile urine sample container (Nipro Medical Corp., Osaka, Japan). Samples were analyzed using high sensitive and accurate dipsticks for urine screening (Combur$_{10}$Test M, Roche, Mannheim, Germany) during distance running [25]. Urine dipsticks were examined immediately after collection by two different microbiologists simultaneously using the manufacturer's color scale. In case of disagreement between observers, a consensus was obtained by the opinion of a third researcher. The following parameters were screened: leucocytes, erythrocytes, bilirubin, ketones, nitrites, protein, glucose, and urobilinogen. There were no reported urination problems or difficulties neither before nor after the race. Traces were considered as negatives, and those scores >1 were reported. Urine test interpretation an reporting was made as follow: >1 score was equivalent to leucocytes >10 cells/µL, erythrocytes >5 cells/µL, bilirubin >1, ketones >1, nitrites +, protein >30mg/dL, glucose >50mg/dL, and urobilinogen >1 mg/dL.

Urine specific gravity (USG) was assessed as a hydration status marker. USG was confirmed and double-checked with a digital valid (Wyness et al., 2016) handheld refractometer (Palm AbbeTM, Misco, OH, USA). It was classified following the hydration status ranges: well-hydrated <1.01, minimal dehydration 1.01-1.02, significant dehydration 1.02-1.03, and severe dehydration >1.03 (Casa et al., 2000). The

refractometer was cleaned with distilled water and calibrated previously. There were no reported urination problems or difficulties neither before nor after the race.

D. Statistical analysis

In all studies the results were presented as means and standard deviation. The Shapiro-Wilk test was used to confirm data normality, verifying the feasibility of using parametric inference statistics. The magnitude of the significance was assessed using Cohen's *d* effect size (Cohen, 1988) in all t-test performed, qualitatively rated as follows: < 0.2 *trivial*, 0.2-0.6 *small*, 0.6-1.2 *moderate*, 1.2-2.0 *large*, and 2.0-4.0 *very large* (Hopkins et al., 2009). In addition, ANOVA and MANOVA analysis were qualified with the omega squared (ω_p^2) to qualify the magnitude of the differences as follows: <0.01 trivial; >0.01 small; >0.06 moderate and >0.14 large (Cohen, 1988). Statistical differences were considered if $p<0.05$. Statistical analyses were developed using special software (v.24, Statistic Package Social Sciences, Chicago, IL, USA).

i. Specific aim A

For the systematic review, no specific statistical analysis was performed.

ii. Specific aim B

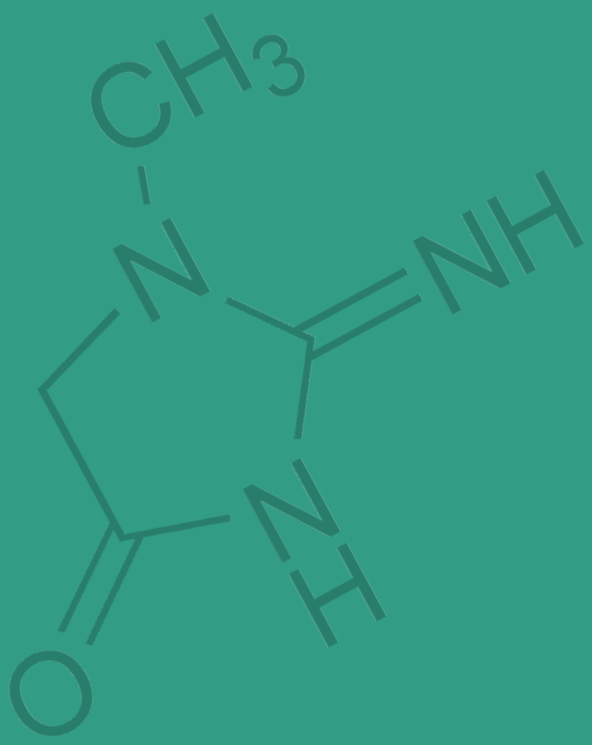
For the agreement and reliability study, over time, test consistency was assessed via reliability using intraclass correlation coefficient (*ICC*) with the respective 95% *IC*, which was confirmed via the linear correlation between measurements. *ICC* was interpreted following previous proposed ranks as: 0 *poor*, 0.01-0.20 *trivial*, 0.21-0.40 *regular*, 0.41-0.60 *moderate*, 0.61-0.80 *substantial* and 0.81-1 *almost perfect* (Kramer & Feinstein, 1981). A linear correlation was qualified as follows: 0.10-0.20 *poor*, 0.30-0.50 *fair*, 0.60-0.70 *moderate*, 0.80-0.90 *very strong*, 1 *perfect*. Bias and agreement between measurements were assessed using the Bland Altman Plotting method with respective 95% *IC*; it was complemented by mean differences analysis between measurements using *t*-tests.

For the second study, the data was reported using Change delta's percentage (Δ%) was reported between time measurement in each variable. After performing a PCA to select external workload indicators, and after PC's were extracted, multiple lineal regressions (R^2) were performed in order to analyze how the principal components found from each body segment explain both muscle and kidney injury markers change after the event

iii. Specific aim C and D

In the first study, participant´s data was compared using an independent t-test. Differences between AKI and No-AKI groups in blood biomarkers and USG were explored using a mixed analysis of variance, and Post-Hoc of Bonferroni was performed to look after specific differences. Also a McNemar non-parametric test was used to explore the possible change in proportion for the paired data of urinalysis. In those observed cases, the intersection frequency value was <5; the binomial test was performed. The data of urinalysis were paired by measurement moment using a 2 × 2 contingency table.

For the second study, a paired t-test was used to explore sCr and sALB changes between pre- and post-race data and the Change delta´s percentage (Δ%) was calculated as follows: ((sCr post-race – sCr pre-race)/ sCr pre-race)*100. Unpaired t-test was performed to explore potential differences in the number of impacts between those participants who met AKI diagnosis and those who did not. USG data were analyzed using a repeated measure t-test. Finally, a stepwise regression model (R^2) was applied to resulted factor scores obtained from impact´s PCA using the Δ% of sCr and sALB as the dependent variable. This statistical technique was applied to identify which PC could predict the Δ% of sCr, and Δ% of sALB.



IV. RESULTS

A. Specific aim A

Table 5. presents the systematization of the results obtained from the extracted studies in relation to ER and AKI.

i. Sample characteristics

A total of 43 studies were included in analysis, all of them met inclusion criteria around ER and AKI. A total of 813 cases were studied (mean age: 31.6 y). Women were involved in a total of 13 (30.2%) of the 43 researches. Following previous reported diagnosis criteria for ER and AKI, there were 318 (40.1%) cases that did not meet the established ER or AKI criteria for the purposes of this review, 345 (43.5%) cases presented ER after exercise, and 130 (16.4%) reported ER + AKI. Total of cases were distributed considering the activity type as follow: 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.

ii. Situational Conditions

Twenty six (60.47%) of total studies reported distance or total activity time, Twenty (46.51%) reported distance runned, cycled or swimmed. Seven (16.27%) ER+AKI studies reported that participants ran 2.5-177 km(mean 112.97 km), two studies (4.65%) explored multi-stages events. Two (4.65%) ER+AKI studies reported cumulative ramp (5.5km of total ascend).

iii. Environmental conditions

Seven (16.27%) researches informed about environmental conditions, seven different studies reported temperature (16.27%) and three reported relative humidity (6.98%). Three (6.98%) ER+AKI researches referenced environmental conditions (mean 23.9ºC, range 14-37ºC) while only one (2.33%) study informed about the relative humidity (mean 63%).

iv. ER-AKI diagnosis and biomarkers

Different biochemical and physiological parameters were reported including dehydration (eg. dark urine), muscle damage (eg. LDH, delayed onset muscle soreness [DOMS]), ER (eg. S-CK, S-MB) and AKI indicators (eg. S-Cr, S-Cyst C). In addition to these indicators some studies gave information about biochemical muscle damage and renal function parameters including : S-Cr (n= 32, 74.4%, 0.3-17.6 mg/dL), S-CK (n= 32, 74.4%, 108-494000 UI/L), MB (n= 12, 27.9%, 0.3-20000 ng/dL, S-LDH (n= 12, 27.9%, 335-11370 UI/L), S-Cyst C (n= 1, 2.3%, 0.7-0.8 mg/L). Other physiological and perceptual indicators were reported as: delayed onset muscle soreness (n= 16, 37.2%), urine color (n= 10, 23.3%), state of consciousness (n= 1, 2.3%). Some biomarkers were reported but not included in this review as: albumin, Fe, Ca, blood ureic nitrogen, alanine aminotransferase and others with no direct relevance of study in ER+AKI.

v. Return to Baseline

A total of nineteen (44.2%) studies on ER or ER+AKI reported intervention and number of hospitalization days (a mean of 5.9, from 1 to 17 days). A mean of 5.86 (from 1 to 10) days were necesarry for recovery in ER cases, and 6 days (from 1 to 17) for recovery in ER+AKI cases. One death was registered after 8 days of hospitalization.

Table 5. Sample characteristics, environmental conditions, diagnosis and studies outcomes of endurance events assessing AKI-related biomarkers (Rojas-Valverde, Sánchez-Ureña, et al., 2020).

#	Author/Year	Sample Characteristics	Situational Conditions (Activity, duration, distance)	Environmental Conditions	ER-AKI Diagnosis/Biomarkers	Diagnosis	Return to baseline
1	(DeFilippis et al., 2014)	Sedentary female and male (n= 2) (Age: 24 y)	Spinning class for first time	NR	Muscle soreness Dark urine S-CK (14960-161550 UI/L) S-Cr (0.8-3.5 mg/dL)	ER+AKI-*injury*	5 days hospitalization and compartment tight syndrome
2	(Boulter et al., 2011b)	Active male (n= 4) (Age: 35 ± 6 y)	89.3km, endurance running	14-24ºC, 63% relative humidity	Muscle soreness S-CK (5718-54231 UI/L) S-Cr (2.99-12.88 mg/dL)	ER+AKI-*failure*	Hospitalization and acute renal dialysis
3	(Bruso et al., 2010b)	Active males (n= 5) (Age: 36.7 ± 7.8 y)	161km, 5500m cumulative climb, endurance running	15-37ºC	Muscle soreness Dark urine S-Cr (1.1-4.9 mg/dL) S-CK (38218-95940 UI/L)	ER+AKI-*failure*	Hospitalization and hyponatremia
4	(McVane et al., 2019)	Sedentary female (n= 1) (Age: 24 y)	2 days walking in desert	NR	Muscle soreness S-CK (5892 UI/L) S-Cr (3.88 mg/dL)	ER+AKI-*injury*	7 days hospitalization
5	(Abbas et al., 2019)	Active male (n= 1) (Age: 31 y)	Marathon running	NR	Muscle soreness S-CK (131900 UI/L) S-Cr (7.97 mg/dL)	ER+AKI-*failure*	17 days hospitalization

6	(Hou et al., 2015)	Active males (n= 26)	100km Ultramarathon	NR	S-CK (4274 IU/L) MB (4462.4 ng/mL) S-Cr (17.64 mg/dL)	ER+AKI-*failure*	NR
7	(Hoffman & Weiss, 2016)	Active runners (n= 38)	161km, 5500m cumulative climb, Ultramarathon	NR	S-CK (120-200000 IU/L) S-Cr (0.4-2.2 mg/dL)	ER+AKI-*risk*	NR
8	(Lipman et al., 2014a)	Active runners (n= 30)	Ultramarathon (7 days-6 stages-177km)	NR	S-Cr (1-2.4 mg/dL)	ER+AKI-*risk*	NR
9	(Kao et al., 2015c)	Active males (n= 22) (age: 49y [22-60 y])	100k ultramarathon	24.9-28.7ºC	S-CK (112-4650 UI/L) S-Cr (0.87-1.77 mg/dL) MB (37-5897 ng/dL)	ER + AKI: risk	1 day hospitalization
10	(Shimizu et al., 2017)	Active male (n= 1) (18 y)	Professional Track running training (2500m)	NR	S-Cr (1.1-7.5 mg/L)	ER+AKI-*failure*	2 days hospitalization
11	(Rojas-Valverde, Sánchez-Ureña, et al., 2019)	Active males (n= 20) (age = 38.95 ± 9.99 years)	~35.27 km (cumulative-ascend = 1815 m)	thermal-index = 23.2 ± 1.8 °C	S-Cr (1.71 mg/dL) S-CK (680.87 UI/L)	ER+AKI-*failure*	NR
12	(Kim et al., 2015)	Sedentary female (n= 11) (age: 23.2±8.3 y)	Spinning class for first time	NR	Muscle soreness Dark urine S-LDH (1446-7350 UI/L) S-CK (>11000 UI/L) MB (8472->20000 ng/mL) S-Cr (0.6-0.9 mg/dL)	ER	Hospitalization 7.6 ± 1.9 days until S-CK dropped <5000

13	(Asserraji et al., 2014)	Experienced male (n= 11) (Age: 35 y)	6 hours marathon	41ºC and 64% relative humidity	Unconscious Muscle soreness Dark urine S-Cr (0.3-0.99 mg/dL) S-CK (22300-91596 IU/L) MB (>500 ng/mL)	ER	Patient died after 8 days hospitalization
14	(Oh et al., 2015)	Military personnel (28 males and 2 females) (n= 30) (age: 25.7 y)	Physical fitness training + ruck marches	NR	S-Cr (1.12 mg/dL) S-CK (61391 IU/L)	ER	Hospitalization 3.6 days
15	(Atias-Varon et al., 2017)	Males military personnel (n= 3) (Age: 18-19 y)	2 days military selection process: crawling + equipment carrying	NR	Muscle soreness Dark urine S-CK (45000-176599 UI/L) MB (1409-12555 ng/mL) S-LDH (2818-11370 UI/L)	ER	3-7 days Hospitalization
16	(Jeong et al., 2016)	Female college student (n= 1) (Age: 21 y)	2x 40 min spinning class	NR	Muscle soreness Dark urine S-CK (16370 UI/L) S-LDH (2310 UI/L) S-Cr (0.5-0.6 mg/dL)	ER	10 days hospitalization
17	(Tibana et al., 2018)	Active female (n= 1) (Age: 35 y)	Extreme 2 days conditioning program	NR	S-CK (1257-77590 UI/L) S-LDH (555-1835 UI/L)	ER	4 days hospitalization

					MB (1409-12555 ng/mL) S-Cr (0.9-1.22 mg/dL)		
18	(Chlíbková et al., 2015)	Active males and female (n= 5) (Age: 26-51 y)	24-100 km running	NR	S-CK (6381-26209 UI/L) S-Cr (↑9.9-33.4%)	ER	Hospitalization no required
19	(Ramme et al., 2016)	Sedentary Females (age: 24, 27 y) and male (age: 27 y) (n= 3)	Spinning class for first time	NR	S-CK (59651-152684 UI/L) S-Cr (0.6-0.9 mg/L) MB (1075 ng/mL)	ER	5-6 days hospitalization
20	(Hernández-Contreras et al., 2015)	Sedentary males and females (n= 11) (Age: 27.63 y)	Spinning class for first time	NR	S-CK (6888-494000 UI/L) S-Cr (0.53-1.57 mg/L) S-LDH (113-1611 IU/L)	ER	1-8 days hospitalization
21	(Stella & Shariff, 2012)	Sedentary male (n= 1) (Age: 32 y)	Endurance swimming	NR	Muscle soreness Dark urine S-CK (112400 UI/L) S-LDH (3566 UI/L)	ER	Recovery after one week
22	(Hoffman et al., 2012)	Active runners (27 females, 132 males) (n= 159) (Age: 42 ± 9.2 y)	161km, 5500m cumulative climb, Endurance running	4-33ºC	S-CK (1500-264300 UI/L)	ER	No intervention, no AKI reported
23	(Belli et al., 2018b)	Active males (n= 6)	135 miles Ultramarathon	NR	Muscle soreness	ER	NR

					S-CK (132-19157 UI/L) S-LDH (371-2026 UI/L) S-Cr (1-1.34 mg/dL)		
24	(Li et al., 2017)	Active runners (n= 26) (Age: 46. 9 ± 9 y)	100km ultramarathon	NR	S-CK (172-3-5530 IU/L)	ER	Hospitalization no required
25	(Kupchak et al., 2014a)	Active men (n= 12) (Age: 45. 9 y)	161km, 5500m cumulative climb, ultramarathon	NR	S-CK (114-20484 IU/L) MB (0.3- 118.5 ng/mL)	ER	Hospitalization no required
26	(Rubio-Arias et al., 2019)	Active males (n= 16)	54-110km, mountain Ultramarathon	NR	S-LDH (335-751 UI/L) S-Cr (1.06-1.5 mg/dL) S-CK (174-8976 IU/L) MB (14-1525 ng/mL)	ER	Hospitalization no required
27	(Halldorsson et al., 2016)	Patients (n= 54) (Age: 28 y)	Diverse long-term activities	NR	S-CK (24.132 IU/L)	ER	NR
28	(Tran et al., 2015)	Active female (n= 1) (Age: 23 y)	Different Conditioning activities	NR	Dark urine S-CK (156339 UI/L)	ER	1-4 day hospitalization
29	(Shin et al., 2016)	Active male runner (n= 1) (Age: 51.76 ± 6.88y)	marathon, 100km and 308km ultramarathon	NR	S-CK (108-4970 UI/L) S-Cr (1.03-1.27mg/dL) S-LDH (338-1002 UI/L)	ER	NA
30	(Dekeyser et al., 2009)	Active female (n= 1) (Age: 26 y)	Two session of cardio training and aerobic exercise	under 38ºC	Muscle soreness S-CK (20241 UI/L) S-LDH (900.6 UI/L)	ER	4 days hospitalization

31	(Parmar et al., 2012)	Active Male (n= 1) (Age: 38 y)	First time spinning class	NR	Muscle Soreness Dark Urine S-CK (149985 UI/L)	ER	4 days hospitalization
32	(Inklebarger et al., 2010)	Active female (n= 1) (Age: 63 y)	Indoor cycling	NR	Muscle soreness Dark urine MB (5330 ng/dL) S-CK (38120 IU/L)	ER	2 days hospitalization
33	(McDermott et al., 2018)	Active males(n= 40) (Age: 52 ± 9 y)	cycling event (5.7 ± 1.2 h)	33.2 ± 5.0 °C, 38.4 ± 10.7% relative humidity	S-Cr (0.82-0.88 mg/dL)	No ER or AKI reported	NA
34	(Bongers et al., 2018)	Active males (n= 35) (Age: 23 ± 3 y)	Cycling laboratory (incremental protocol 25watts/min)	NR	S-Cr (0.95 mg/dL)	No ER or AKI reported	NA
35	(Little et al., 2019)	Active males and females (n= 48) (Age: 39 ± 10 y)	Multi-stage ultramarathon (250km)	NR	S-Cr (0.99 mg/dL) only pre-race	No ER or AKI reported	NA
36	(Mccullough et al., 2011)	Active males and females (n= 25)	Marathon	NR	S-Cr (0.9-1.2 mg/dL) S-CK (186-1984 IU/L)	No ER or AKI reported	NA
37	(Omassoli et al., 2019)	Active males (n= 20) (Age: 25 ± 3 y)	Running (60 min heat run)	NR	S-Cr (0.96-1.6 mg/dL)	No ER or AKI reported	NA
38	(Mydlík et al., 2012)	Active male-female (n= 29)	Marathon and 16km running	NR	MB (0.85-5.2 ng/mL) S-Cr (0.99-1.27 mg/dL) S-CK (72-312 UI/L)	No ER or AKI	NA

39	(Cappuccilli et al., 2016)	Active males (n= 54) (Age: 51 ± 9.5 y)	Cycling (6 h)	NR	S-Cr (0.96-1.43 mg/dL)	No ER or AKI reported	NA
40	(Maynar et al., 2018)	Active and athletes (n= 47)	Exercise until exhaustion (3112-4429 m, run)	NR	S-Cr (1.61-1.72 mg/dL)	No ER or AKI reported	NA
41	(Colombini et al., 2012)	Professional male athletes (n= 9)	3 stages pro tour cycling	NR	S-Cr (0.61-0.63 mg/L) S-Cyst-C (0.7-0.8 mg/L)	No ER or AKI reported	NA
42	(Todd et al., 2016)	Sedentary female (n= 1) (Age: 17 y)	Firt time 5km	NR	Muscle soreness S-LDH (2310 UI/L) MB (34 ng/dL) S-CK (1327 IU/L)	No ER or AKI reported	NA
43	(Junglee et al., 2013)	Active males (n= 10) (Age: 20 ± 2 y)	Running Downhill (60 min, -10% gradient)	NR	S-Cr (0.9-1 mg/dL)	No ER or AKI reported	NA

Note: S-Cr: serum creatine, S-CK, serum creatine kinase, LDH: lactate dehydrogenase, MB: myoglobin, S-Cyst-C: serum cystatin-C, NR: no reported, NA: not apply.

B. Specific aim B

The variables selection (PCA) for the agreement and reliability testing for MARG sensors during trail running resulted that for each body segment was performed, three variables (PL_{RT}, impacts and entropy) were considered and grouped into two PCs in the whole circuit, accounting for a total of 76.2 to 92.78% of the data sets variance. In the 1st Lap of each body segment, the total variance was 81.15 to 97.85%, while in the 2nd Lap of each body segment, the total variance was 76.63 to 94.72%. This total variance was explained by the 1st PC by 44.08 to 68.67% and 46.78 to 70.64%, respectively, in the 1st and 2nd Lap. Considering these precise results, the variables selected to perform the agreement and reliability tests were PL_{RT} for all body segments and Impacts for L_1–L_3

The variables shows that MARG sensors obtained a substantial to almost perfect reliability in all six segments with good agreement between laps (see Table 6.).

Table 6. Agreement and reliability of selected accelerometry-based external load indicators in off-road running (D Rojas-Valverde et al., 2021).

ABELI Variables	n	*ICC (rating)*	95% *IC*	*r* (*rating*)	*p*-value	Bias	95% *IC*
PL_{RT} T_2–T_4 (n/min)	36	0.71 (*substantial*)	0.28; 0.89	0.73 (*very strong*)	0.01	-0.10	-0.17; 0
PL_{RT} L_1–L_3 (a.u./min)	36	0.84 (almost perfect)	0.45; 0.98	0.84 (*very strong*)	<0.01	-0.01	-0.03; 0
Impacts L_1–L_3 (n/min)	36	0.94 (almost perfect)	0.77; 0.99	0.95 (*very strong*)	<0.01	-5.57	-16.61; 5.33
PL_{RT} VL_{right} (a.u./min)	36	0.74 (*substantial*)	0.20; 0.91	0.75 (*very strong*)	0.01	-0.03	-0.07; 0
PL_{RT} VL_{Left} (a.u./min)	36	0.76 (*substantial*)	0.23; 0.96	0.78 (*very strong*)	0.01	-0.02	-0.06; 0
PL_{RT} MP_{Right} (a.u./min)	36	0.72 (*substantial*)	0.24; 0.93	0.76 (*very strong*)	0.01	-0.12	-0.20; 0
PL_{RT} MP_{Left} (a.u./min)	36	0.75 (*substantial*)	0.22; 0.94	0.80 (*very strong*)	0.01	-0.02	-0.06; 0

ABELI= accelerometry-based external load indicator; PL_{RT}= $PlayerLoad_{RT}$; L= lumbar; T= thorax; VL= vastus lateralis; MP= malleolus peroneus.

In the second study The body segments that explained the highest percentage of variance were MP of both legs and L_1–L_3, while the location that explained the lowest was VL_{left}. Additionally, PL, PL_{Dif} between segments, ApEn, different levels of impacts

and $impacts_{total}$/min were the most common variables that explained total variance of the workload (see Table 7.).

Table 7. External workload variables outcome and extracted principal components of each body segment spot (Rojas-Valverde, Sánchez-Ureña, et al., 2019).

		Outcome, M ± SD (95%CI)	PC1	PC2	PC3	PC4
T_2–T_4	Eigenvalue		3.198	1.352	1.324	1.101
	% variance		35.53	15.02	14.71	12.24
	% cumulative variance		35.53	20.55	65.26	77.5
	PL_{Dif} T_2–T_4–L_1–L_3 (AU)	274.17 ± 251.37 (−306 to 654.67)	−0.818			
	ApEn (AU)	0.43 ± 0.1 (0.26 to 0.64)	0.81			
	$Impacts_{total}$/min	314.77 ± 55.56 (201.29 to 417.54)		0.781		
	Acc_{max} (m/s^{-1})	4.41 ± 1.23 (3.19 to 7.21)		−0.624		
	PL/min (AU)	1.6 ± 0.57 (0.86 to 2.73)		0.766		
	$Speed_{max}$ (m/s)	5.05 ± 0.85 (3.83 to 7.24)			0.938	
	$Impacts_{0\text{-}1\ g}$/min	108.06 ± 39.69 (23.3 to 171.77)				−0.892
L_1–L_3	Eigenvalue		3.186	2.481	1.458	1.234
	% variance		31.86	24.82	14.58	12.34
	% cumulative variance		31.86	56.68	71.26	83.60
	$Impacts_{total}$/min	170.57 ± 36.64 (111.87 to 247.79)	−0.843			
	$Impacts_{0\text{-}1\ g}$/min	95.28 ± 38.02 (18.8 to 161.86)	−0.763			
	ApEn (AU)	0.51 ± 0.11 (0.24 to 0.76)	0.725			
	PL/min (AU)	2.78 ± 0.53 (2.16 to 3.88)	0.753			
	PL_{Dif} T_2–T_4–L_1–L_3 (AU)	274.17 ± 251.37 (−306 to 654.66)		0.847		
	$Impacts_{8\text{-}9\ g}$/min	2.16 ± 3.27 (0.32 to 14.68)		0.789		
	$Impacts_{6\text{-}7\ g}$/min	5.93 ± 2.13 (3.04 to 9.39)			−0.677	
	$Impacts_{1\text{-}2\ g}$/min	64.42 ± 13.25 (41.84 to 87.39)			0.781	
	$Impacts_{5\text{-}6\ g}$/min	10.18 ± 3.39 (5.46 to 16.54)				0.933
VL_{right}	Eigenvalue		1.606	1.24	1.081	-
	% variance		32.12	24.81	21.61	-
	% cumulative variance		32.12	56.93	78.55	-
	PL/min (AU)	3.96 ± 0.93 (2.47 to 5.62)	0.823			-
	$Impacts_{7\text{-}8\ g}$/min	3.22 ± 1.1 (1.68 to 5.45)	0.892			-
	$Impacts_{3\text{-}4\ g}$/min	10.74 ± 4.2 (4.72 to 21.75)		0.931		-
	PL_{Dif} VLright–MPright (AU)	52.3 ± 435.04 (−1045.51 to 1002.44)			−0.642	-
	ApEn (AU)	0.46 ± 0.11 (0.32 to 0.67)			0.838	-
VL_{left}	Eigenvalue		1.951	-	-	-
	% variance		65.05	-	-	-
	% cumulative variance		65.05	-	-	-
	PL/min (AU)	3.88 ± 0.88 (2.7 to 5.74)	−0.696	-	-	-
	$Impacts_{5\text{-}6\ g}$/min	5.57 ± 2.04 (2.49 to 10.06)	0.796	-	-	-
	$Impacts_{3\text{-}4\ g}$/min	11.43 ± 4.98 (3.72 to 21.48)	0.913	-	-	-
MP_{right}	Eigenvalue		2.614	1.978	1.238	1.092

		Outcome, M ± SD (95%CI)	PC1	PC2	PC3	PC4
	% variance		32.67	24.72	15.48	13.64
	% cumulative variance		32.67	57.4	72.87	86.52
	$Impacts_{8\text{-}9\ g}$/min	5.77 ± 2.04 (3.11 to 12.12)	0.859			
	PL/min (AU)	4.52 ± 1.03 (3.16 to 6.56)	0.73			
	ApEn (AU)	0.36 ± 0.18 (0.04 to 0.81)	−0.862			
	$Impacts_{total}$/min	115.84 ± 27.91 (74.29 to 163.89)		0.867		
	PL_{Dif} VL_{right}–MP_{right} (AU)	52.3 ± 435.04 (−1045.51 to 1002.44)		0.94		
	$Impacts_{1\text{-}2\ g}$/min	28.83 ± 10.77 (10.43 to 49.95)		0.779		
	$Impacts_{6\text{-}7\ g}$/min	6.95 ± 2.35 (3.32 to 12.12)			−0.845	
	$Impacts_{3\text{-}4\ g}$/min	9.28 ± 4.25 (4 to 16.34)				0.95
	Eigenvalue		2.538	2.206	1.58	1.175
	% variance		28.2	24.51	17.55	13.06
	% cumulative variance		28.2	52.71	70.26	83.32
	PL_{Dif} VL_{left}–MP_{left} (AU)	193.75 ± 0.88 (−1228.3 to 935.59)	-0.766			
	$Impacts_{6\text{-}7\ g}$/min	7.12 ± 2.74 (1.94 to 11.42)	0.754			
	$Impacts_{8\text{-}9\ g}$/min	5.47 ± 1.61 (2.7 to 8.5)	0.888			
MP_{left}	PL/min (AU)	4.53 ± 1.07 (2.95.1 to 7.18)		−0.903		
	$Impacts_{4\text{-}5\ g}$/min	6.9 ± 2.63 (1 to 11.46)		0.887		
	$Impacts_{1\text{-}2\ g}$/min	26.58 ± 8.12 (13.11 to 45.84)			0.88	
	$Impacts_{total}$/min	155.46 ± 20.87 (98.33 to 184.1)			0.842	
	$Impacts_{3\text{-}4\ g}$/min	10.46 ± 4.01 (2.38 to 18.28)				0.92

Note. M: mean; SD: standard deviation; CI: confidence interval; AU: arbitrary units; PC: principal component.

Finally, Table 8 shows the prediction of muscle and kidney injury serum variables change by the workload principal components of each body segment. At pre- vs. -$post_{0h}$, the highest prediction values were found in: sCr by MP_{left} (45%) and L_1–L_3 (27%); sBUN by MP_{right} (40%) and MP_{left} (38%); and sCK by MP_{left} (47%) and L_1–L_3 (40%). At pre- vs. -$post_{24h}$ the highest prediction values was found in: sCr by T_2–T_4 (74%); sBUN by MP_{right} (10%) and T_2–T_4 (10%); and sCK by L_1–L_3 (59%). sALB was not predicted by any of the workload variables.

Table 8. Body segments external workload indicators (principal components) that predicted muscle and kidney injury serum changes (Rojas-Valverde, Sánchez-Ureña, et al., 2019).

	Δ% Pre- vs. -$Post_{0h}$			
Body Segment	**sCr**	**sBUN**	**sALB**	**sCK**
T_2–T_4	$R^2 = 0.23$, β = 44.03 $p < 0.01$ **	$R^2 = 0.22$, β = 51.91 $p < 0.01$ **	$R^2 = 0.18$, β = 100.55 $p = 0.3$	$R^2 = 0.14$, β = 333.97 $p = 0.025$ *

L_1–L_3	R^2 = 0. 27, β = 45.36 p < 0.01 **	R^2 = 0.2, β = 55.99 p = 0.014 *	R^2 = 0.29, β = 112.16 p = 0.286	R^2 = 0.4, β = 350.02 p = 0.019 *
VL_{right}	R^2 = 0.11, β = 42.69 p < 0.01 **	R^2 = 0.33, β = 47.97 p < 0.01 **	R^2 = 0.36, β = 101.28 p = 0.223	R^2 = 0.33, β = 336.79 p = 0.01 **
VL_{left}	R^2 = 0.07, β = 41.63 p < 0.01 **	R^2 = 0.10, β = 48.39 p < 0.01 **	R^2 = 0.16, β = 96.09 p = 0.25	R^2 = 0.2, β = 324.57 p < 0.01 **
MP_{right}	R^2 = 0.2, β = 45.22 p < 0.01 **	R^2 = 0.4, β = 51.51 p = 0.019 *	R^2 = 0.44, β = 119.34 p = 0.243	R^2 = 0.36, β = 373.01 p = 0.024 *
MP_{left}	R^2 = 0.45, β = 47.33 p < 0.01 **	R^2 = 0.38, β = 50.39 p < 0.01 **	R^2 = 0.45, β = 96.35 p = 0.202	R^2 = 0.47, β = 335.28 p < 0.01 **
	Δ% Pre- vs. -$Post_{24h}$			
Body Segment	sCr	sBUN	sALB	sCK
T_2–T_4	R^2 = 0.74, β = 877.57 p = 0.02 *	R^2 = 0.1, β = 39.17 p < 0.01 **	R^2 = 0.29, β = 22.41 p = 0.265	R^2 = 0.3, β = 363.58 p < 0.01 **
L_1–L_3	R^2 = 0.45, β = 5.07 p = 0.229	R^2 = 0.19, β = 37.14 p = 0.057	R^2 = 0.32, β = 19.62 p = 0.529	R^2 = 0.59, β = 493.04 p < 0.01 **
VL_{right}	R^2 = 0.19, β = 6.95 p = 0.077	R^2 = 0.22, β = 36.82 p < 0.01 **	R^2 = 0.18, β = 19.53 p = 0.325	R^2 = 0.22, β = 324.08 p < 0.01 **
VL_{left}	R^2 = 0.002, β = 7.46 p = 0.039 *	R^2 = 0.08, β = 34.9 p < 0.01 **	R^2 = 0.13, β = 13.87 p = 0.41	R^2 = 0, β = 189.63 p = 0.967
MP_{right}	R^2 = 0.56, β = 4.53 p = 0.207	R^2 = 0.23, β = 31.26 p = 0.126	R^2 = 0.5, β = 9.65 p = 0.69	R^2 = 0.27, β = 473.47 p = 0.015 *
MP_{left}	R^2 = 0.12, β = 10.13 p = 0.025 *	R^2 = 0.1, β = 39.93 p < 0.01 **	R^2 = 0.08, β = 13.14 p = 0.524	R^2 = 0.39, β = 413.12 p < 0.01 **

*p< 0.05, ** p< 0.01

Additionally, Table 9 shows the mean differences (lower and upper range) and changes of muscle and kidney injury serum markers by measure moment. Large effect size was found in all kidney (sCr, sBUN and sALB) and muscle (sCK) injury variables between pre- and -$post_{0h}$ or -$post_{24h}$. Regarding kidney injury, the greatest change was found between pre- vs. -$post_{0h}$, while in muscle damage the highest change was shown between pre- vs. -$post_{24h}$. A total of 4/20 (20%) cases met diagnosis criteria [38] for ER and 11/20 (55%) cases met diagnosis criteria [36,37] for AKI_{risk} and 3/20 (15%) AKI_{injury} based on sCr.

Table 9. Mean differences (lower and upper limits) and change delta's percentage in muscle and kidney injury serum makers by measure moment (Rojas-Valverde, Sánchez-Ureña, et al., 2019).

Category Variable	Pre-	-Post$_{0h}$	-Post$_{24h}$	F$_{(2.28)}$ (p)	ω_p^2 rating	Δ% Pre- vs. -Post$_{0h}$	Δ% Pre- vs. -Post$_{24h}$
Kidney Injury							
sCr (mg/dL)	1.22 ± 0.29 (0.66 to 1.7)	1.71 ± 0.4 (1.06 to 2.7) *	1.3 ± 0.29 (0.91 to 1.78) †	19.05 (<0.01)	0.53 large	45.67 ± 42.26 (−1.49 to 171.21)	9.02 ± 12.74 (−14.93 to 31.58)
sBUN (mg/dL)	14.4 ± 4.42 (6 to 24)	19.92 ± 5.2 (8.7 to 29) *	18.88 ± 4.89 (13 to 27) *	14.004 (<0.01)	0.46 large	48.91 ± 68.05 (−15 to 323.1)	37.21 ± 37.41 (−35 to 116.67)
sALB (IU/L)	4.31 ± 1.22 (0.29 to 4.99)	5.01 ± 0.82 (1.71 to 5.84) *	4.67 ± 0.25 (4.16 to 5.06)	4.145 (0.027)	0.17 large	92.55 ± 362.99 (1.2 to 1634.48)	15.6 ± 59.74 (−10.1 to 230.71)
Muscle Damage							
sCK (IU/L)	274.5 ± 384.36 (45 to 1688)	691.05 ± 591.43 (229 to 2695) *	680.87 ± 552.07 (244 to 2400) *	11.021 (<0.01)	0.39 large	322.56 ± 503.01 (42.23 to 2371.1)	337.75 ± 303.25 (−4.56 to 976.23)

sCr: serum creatinine, sBUN: serum ureic blood nitrogen, sALB serum albumin and sCK: serum creatine kinase. * Significant differences with Pre- ($p < 0.01$); † Significant differences with -Post$_{0h}$ ($p < 0.01$). Lower and upper limits were reported in brackets.

C. Specific aim C and D

In the first study There were 17 participants that met AKI criteria (sCr= 1.18 ± 0.26 pre, 1.81 ± 0.35 post, change of 53.4%). There were large differences by measurement (pre **vs.** post) and group (AKI **vs.** No-AKI) in sCr (F= 17.24, p< 0.01, ω_p^2= 0.38 [large]) and sBUN (F= 4.1, p< 0.5, ω_p^2= 0.1 [large]). Pre vs. post differences were found in both AKI (p< 0.01) and No-AKI (p< 0.01) groups in sCr and sBUN. Besides, in sCr post-race differences between AKI and No-AKI groups were found (p=0.03) but no pre-race differences were identified (p=0.34) (see figure 9.a.). Additionally, in sBUN values, there were no pre or post-race differences between AKI and No-AKI groups (see figure 9.b.).

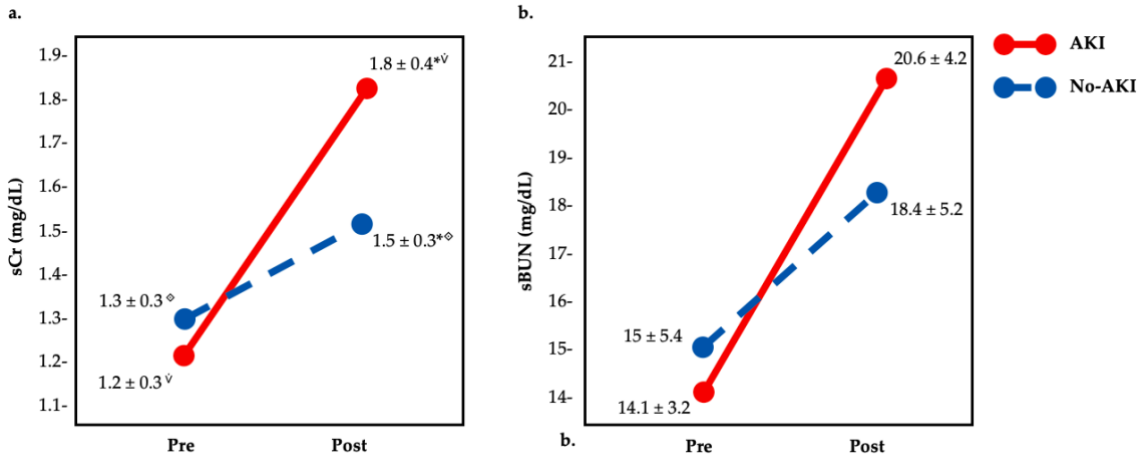


Figure 9. Comparison of pre and post-race a. serum creatinine (sCr), and b. blood ureic nitrogen (sBUN) values of trail runners by the presence or not of acute kidney injury. ◇◊*significant statistical differences (Rojas-Valverde, Olcina, et al., 2020).

The 52.94% of AKI runners presented a significant increase in proteinuria (χ^2= 0.94, p= 0.01) and 47.06% in bilirubinuria (χ^2= 0.94, p= 0.04) when compared pre **vs.** post-race values. No significant increases were found in leucocyturia (17.64%, χ^2= 04.96, p= 0.5), urobilonogenuria (17.64%, χ^2= 0.23, p= 0.63), hematuria (29.41%, χ^2= 0.58, p= 0.13). No nitrituria, glucosuria or ketonuria were found (see table 10).

Table 10. Urinalysis outcomes in participants fulfilling AKI diagnosis criteria (Rojas-Valverde, Olcina, et al., 2020).

Variable (score criteria)	Pre-		-Post0h		$\chi2$	*p*-value
	n*	%	n*	%		
Leucocytes (>1)	0	0	3	17.64	4.96	0.5
Nitrites (>1)	0	0	0	0	-	-
Protein (>1)	0	0	9	52.94	0.94	0.008
Glucose (>1)	0	0	0	0	-	-
Ketones (>1)	0	0	0	0	-	-
Urobilinogen (>1)	0	0	3	17.64	0.23	0.625
Bilirubin (>1)	0	0	8	47.06	0.94	0.039
Erythrocytes (>1)	0	0	5	29.41	0.58	0.125

*Based on AKI group data (n=17).

In the second study, those participants that met AKI diagnosis criteria (33.3% of participants) registered lower number of impacts in 1-2g category (*t*= -2.42, *p*= 0.03, *d*= -1.45, *large effect size*) but higher number of impacts in 14-15g category (*t*= -3.1, *p*= 0.01, *d*= -1.58, *large effect size*) (see figure 10.). No differences we found in 5-6g or 6-7g categories.

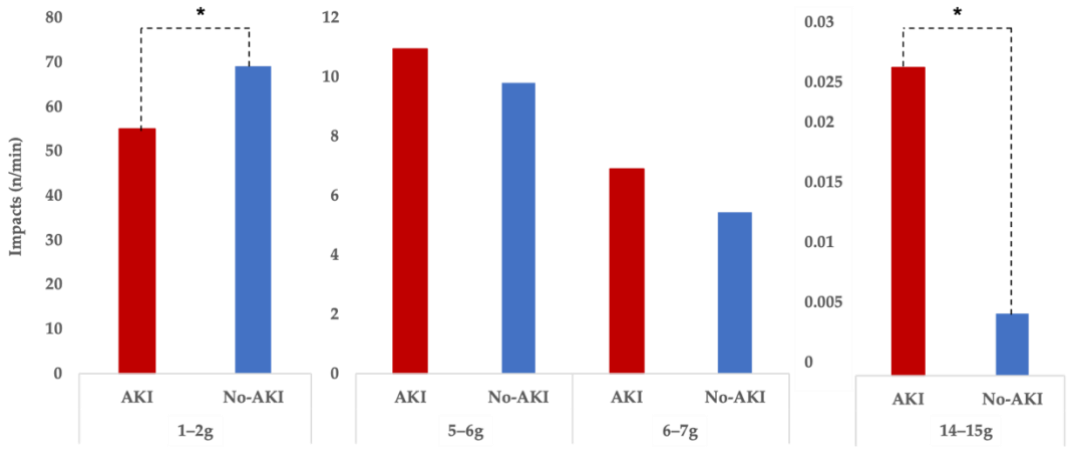
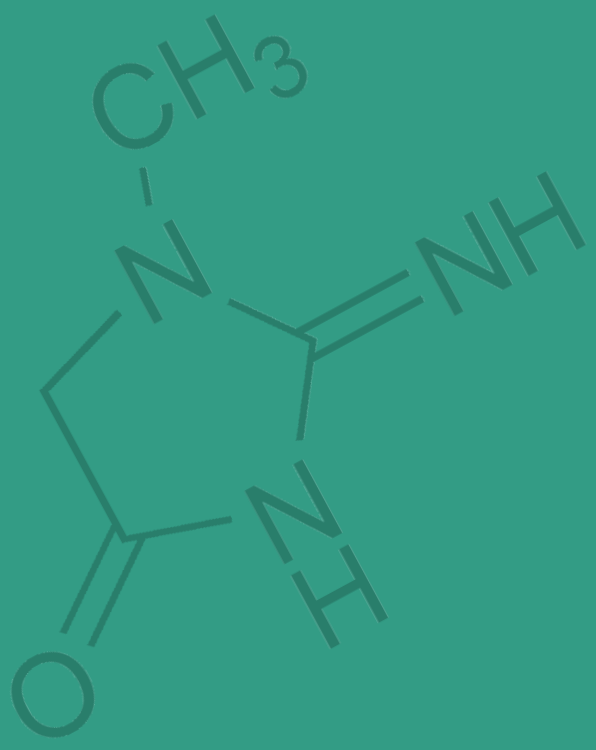


Figure 10. Differences between runners showing signs of AKI (n= 6) and those showing no signs of AKI (n= 12) regarding impacts per minute, grouped in four impact g-force categories. * The biggest difference between the AKI and no-AKI group is that the no-AKI group managed to run "smoother", keeping impacts in the lower impact load ranges, while avoiding higher impacts loads (Rojas-Valverde, Timón, et al., 2020).

There were *large* statistical differences (t= -6.24, p< 0.01, d= -1.47, *large effect size*) between sCr pre-race (1.24 ± 0.28 mg/dL) and sCr post-race (1.74 ± 0.41 mg/dL) and large differences (t= -2.78, p= 0.01, d= -1.47, *large effect size*) in sALB pre-race (4.33 ± 1.29 IU/L) vs post-race (5.01 ± 0.86 IU/L). The Δ% of sCr was predicted by the 4th PC in a 24% (R^2= 0.24, β= 44.03, p< 0.01) and the Δ% of sALB by a 23% (R^2= 0.23, β= 100.55, p= 0.04). Finally, USG as a hydration marker reported no differences between pre and post-race measurements (1.01 ± 0.02 vs. 1.01 ± 0.01; t= 1.02, p= 0.07).



V. DISCUSSION

A. Specific aim A

The systematic review demonstrates evidence of a multifactorial etiology of AKI including dehydration, high metabolic load (external and internal load) and exposure to heat and humidity (heat strain). These three factors may increase the risk of kidney injury (Rojas-Valverde, Sánchez-Ureña, et al., 2019), and in tropical places during endurance running, these three factors are often present.

There is vast evidence that ER and AKI usually worsen when endurance exercise is performed under conditions of high temperature and humidity. The resulted potential high rate of water and electrolyte loss may lead to both hyponatremia (Bruso et al., 2010b; Chlíbková et al., 2015) and dehydration (Bongers et al., 2018). These two conditions also affect renal and muscle function. Due to exercise there is a decrease of renal blood flow increasing the incidence of ER and AKI (Asserraji et al., 2014; D. Kim et al., 2015). These may lead to muscle structural and functional changes, liquid imbalance and alteration in homeostasis processes leading to a decrease of renal function related to dehydration and protein waste product overreaching the kidneys.

The findings of this systematic review suggested that sCr and sCK are the more commonly reported biomarkers for AKI and ER respectively (74.42%). Muscle damage and resulted ER is due to myocyte damage and energy depletion at the cellular level (Hernández-Contreras et al., 2015; Stella & Shariff, 2012). The result of these processes would be an intracellular increase in Na+, which causes a flow of water to the intracellular space, occupying it and an intracellular increase of Ca^{2+}, which causes sustained myofibrillary contractions, which leads to a decrease in ATP (Al-Ismaili et al., 2011) and mitochondrial dysfunction resulting in the production of oxygen radicals increasing cell damage (Patel et al., 2009). The final consequence is a muscle breakdown due to destruction of myofibrillar, cytoskeletal and membrane proteins. The myocyte is destroyed by ischemic reperfusion and inflammation by neutrophils that infiltrate the muscle, destroying the myocyte (Giannoglou et al., 2007).

This may lead to a release of waste products as into the bloodstream as sCK, potassium, phosphorus, MB, AST, ALT, S-LDH, MB and/or Cyst-C (Colombini et al., 2012). This massive protein release could cause athletic pseudo-nephritis and glomerulonephritis. And finally leading to AKI, as a transitory renal dysfunction due to exercise (McVane et al., 2019; Rojas-Valverde, Sánchez-Ureña, et al., 2019). AKI occurs because the release of MB and sCr caused by muscle damage, resulting in a decrease in

nitric oxide (NO), which leads to vasoconstriction, renal hypoperfusion, decrease in eGFR and eventual AKI (Bagley et al., 2007). The systematic review suggested that ER and AKI can be more hazardous when performing strenuous endurance exercise such trail running. This is provoked by a poorly trained athlete, a sudden increase in external workloads, exercises of eccentric predominance, or conditions of high temperature and humidity (Patel et al., 2009).

Considering the reduction in renal function as a functional response to exercise, the subclinical and functional kidney damage differences need to be more in-depth analyze in future studies considering new diagnosis criteria using novel biomarkers (McCullough et al., 2013b).

B. Specific aim B

The MARGs sensors agreement and reliability study have shown that these devices attached to six body segments reported substantial to almost perfect test/retest reliability and good agreement. Two ABELIs, $PL_{RT,}$ and Impacts L_1–L_3, were extracted considering PCA results. During downhill running segments, the greatest external load is demanded due to the high eccentric muscle load required to tolerate repetitive actions, such as changes in direction, acceleration, and deceleration (de Dionisio et al., 2020). When running downhill, the irregular slope can cause the greatest fly time, creating more significant impact to lower limbs. These effects are usually very complex to assess during training and competition, but multiple body segments' measurement represent a potential option.

Based on technological development in the sports science and medicine areas, MARG devices, have been used for external workload assessment in individual and team sports (Gómez-Carmona, Pino-Ortega, et al., 2019). Previous evidence analyzed these sensors' agreement and reliability when attached to different anatomical locations (Gómez-Carmona, Bastida-Castillo, et al., 2019; Gómez-Carmona et al., 2018; Hernández-Belmonte et al., 2018; Pino-Ortega et al., 2018) during lineal and curvilineal running but, no off-road running have been analyzed until now.

In the second study, PL, total impacts, impacts at different ranges and entropy partly explained the total variance in all six body segments. The difference in impacts range between segments is related to the ground-to-ground contact, finding the ranges of higher impacts in lower limb respect to lumbar region and back.

Some studies have reported that greater external workload is present in the nearest segment with the ground-to-ground contact. In trail running, the highest values of external workload were found in downhill segments, while the highest metabolic response was found in uphill segments (Giandolini et al., 2017; Minetti et al., 2002; Vernillo et al., 2017). Again, in downhill segments was found the greatest external workload, where eccentric muscle contractions are critical for trail runner as other necessary actions as changes of direction, accelerations and decelerations (de Dionisio et al., 2020). In downhill segments, the running biomechanics is highly irregular, and require greatest fly time that result in greater impacts.

Moreover, it was found that muscle and kidney injury biomarkers presented moment product large differences (pre < $post_{24h}$ < $post_{0h}$). While, PC's explained 77.5 to 86.5% of total external workload variance, sCK Δ% was predicted in a 40% and a 47% by L_1–L_3 and MP_{left} PC's respectively. Additionally, sCr Δ% was predicted in a 27% and a 45% by the L_1–L_3 and MP_{left} PC's. Also, sBUN Δ% wsa predicted in a 38% and a 40% by MP_{right} and MP_{left} PC's.

When compare pre vs. $post_{0h}$ sCK demonstrated an increase after exertion. In addition, a 20% of total sample met diagnosis criteria for ER after 35 km trail running. These results are in line with other cross sectional studies when compared pre, $post_{0h}$ and $post_{24h}$ (Quinn & Manley, 2012). When the distance of a trail running event increases the sCK rises significantly, but trail running events tend to provoke higher sCK changes when compare to road events. These responses could be due to the higher uphill and downhill segments (de Dionisio et al., 2020), leading to higher number and magnitude of impacts and a resulted higher metabolic workload (de Dionisio et al., 2020). This may suggest frequent slope changes requires higher eccentric muscle contractions (Furman, 2015).

An increase in some AKI related biomarkers was found for functional (sCr and sBUN) and subclinical (sALB) injury. These sCr, and sALB levels increased significantly after the event (-$post_{0h}$), but recovered baselines values after 24h (-$post_{24h}$)., but sBUN higher values in $post_{0h}$ and $post_{24h}$ with large differences respect to pre-. This increase in serum kidney functional biomarkers is widely reported in running endurance events (42km:7.97 mg/dL [43], 89.3 km: 2.99–12.88 mg/dL, or 161 km: 1.1–4.9 mg/dL, 100 km: 17.64 mg/dL and 135 km: 1–1.34 mg/dL) (Rojas-Valverde, Sánchez-Ureña, et al., 2020).

The acute rise in sCr in a 70% of total sample could be interpreted as AKI_{risk} or AKI_{injury} following diagnosis criteria as AKIN, RIFLE and KDIGO (Gameiro et al., 2018; Khwaja, 2012; Lopes & Jorge, 2013). Although there is evidence that the rise in sCr or

sBUN alone should not be considered as AKI due to the lack of link with a subclinical injury, the increase in sALB should suggest transitory functional loss due to potential tubular or glomerular damage (Gameiro et al., 2018). Considering these information and the found mean change of sBUN, sCr and sALB levels of the participants, there was a high incidence of AKI presence after trail running due to functional, physiological and structural changes.

It was hypothesized that the increase of sBUN, sCr and sALB levels could be due to a muscle and kidney mechanical trauma, this last condition has been suggested in other contact sports (Castenfors, 1977) but not deeply analyzed in running sports until present analysis.

The muscle and kidney mechanical trauma hypothesis theory could be supported (Castenfors, 1977) by the results of this study, considering that the sCK Δ% was predicted in 40% and 47% by L_1–L_3 and MP_{left} PC's respectively; sCr Δ% in 27% and 45% by the L_1–L_3 and MP_{left} PC's and sBUN Δ% in 38% and 40% by MP_{right} and MP_{left} PC's. These results may suggest that core muscle resistance, optimal absorption forces, and efficient running economy could be protective factors to avoid greater muscle and kidney mechanical injury. Another consideration about the role of L_1–L_3 in impact absorption could develop mechanical kidney trauma due to kidney shaking and nephritis (Rojas-Valverde, Timón, et al., 2020).

C. Specific aim C and D

The first study responding to the specific aims C and D suggested that AKI could be provoked by some factors as decreased blood flow, direct kidney trauma, blockage of the urinary tract, among others (Bongers et al., 2018). In endurance sports, the physiological mechanisms still unclear, but factors such as heat strain, dehydration, and high metabolic and physical load may boost the risk of AKI and must be seen as primary issues. In this study, dehydration seems to be a factor that did not influence the AKI occurrence in this specific sample.

Under regular conditions, the renal protein excretion in healthy adults is about 150mg per day (Carroll & Temte, 2000). As a consequence of muscle damage as in endurance exercising, excess of proteins are excreted through the urine, known as proteinuria (Shephard, 2016), as found in the present study.

The pathophysiological mechanisms of proteinuria can be partially explained by increasing glomerular capillary permeability to proteins and reduced protein reabsorption capacity in the renal tubules. Still, exercise-induced-proteinuria is not fully understood, but it seems that the renin-angiotensin system and prostaglandins have an essential role in its development (Filha et al., 2019).

Proteinuria and bilirubinuria in endurance sports could be a consequence of a cascade of events in the kidney. In non-contact sports, catecholamines are released by the suprarenal glandules causing a redirection of blood to muscles and restricting kidney blood flow. These events lead to hypoxic nephron damage and an increase in glomerular permeability (Akiboye & Sharma, 2019). Vasoconstriction of the glomerular arteriole is also provoked by catecholamines resulting in decreased glomerular filtration pressure and allowing excretion of some macro and microscopic elements in urine as protein, erythrocytes, albumin, and bilirubin. Other factors contributing to exercise proteinuria could be but are not limited to lactate accumulation, oxidant stress, hormonal changes, and sepsis (Hoffman et al., 2013; Shephard, 2016).

The increase in bilirubin found in this research could be caused by hemolysis and subsequent catabolism of hemoglobin. The proliferation of red blood cell breakdown is caused mainly by free radicals and a mechanical factor (Banfi et al., 2012). Bilirubinuria could also be related to hepatic disturbance during long-distance running (De Paz et al., 1995; Shin et al., 2016). Endurance running may cause a decline in hepatic function related to changes in the liver cells membrane by lipid peroxidation due to blood flow restrictions and free radicals' release. It is known that the liver suffers a temporary decline in its function during prolonged exercises compared to shorter distances running (Shin et al., 2016). A condition called foot strike hemolysis suggests that blood cells' mechanical injury could be related to the consecutive impact during running (Lippi & Sanchis-Gomar, 2019).

Exercise-related proteinuria and bilirubinuria have been related to renal and hepatic dysfunctions. Both conditions could be asymptomatic, transitional, reversible, and usually, it does not need any special care. But endurance athletes could be particularly vulnerable to developing such conditions when exposing to a high level of environmental stress as a hot and humid environment (Gutiérrez-Vargas et al., 2020; Junglee et al., 2013; Rojas-Valverde, Olcina, et al., 2019). These conditions could boost ischemia, hypoxemia, and ATP depletion in renal tubular cells, and considering dehydration, it could be exacerbated by increased sodium reabsorption (Schlader et al., 2019).

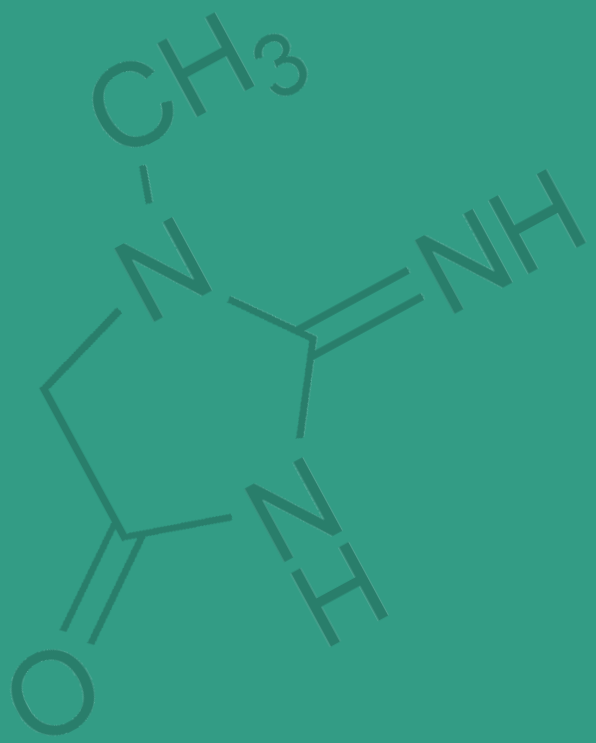
The presence of AKI cases with concomitant proteinuria and bilirubinuria may suggest the potential use of urinalysis as an accessible alternative to early identify AKI cases in the field and monitor training and competition as an outpatient setting. The screening of urine changes could represent an opportunity to identify the potential risk of AKI cases in a simple and fast manner. This result must be analyzed with caution, considering that only 47-52% of AKI runners presented urine changes.

In the second study it was found that renal injury could be caused by an indirect trauma (Kasikcioglu et al., 2004). Urinary trauma could be present in non-contact sports as off-road running (Holmes et al., 2003; Rojas-Valverde, Olcina, et al., 2020; Urakami et al., 2019). This may strengthen the hypothesis that kidney mechanical trauma could mediate in the development of AKI these endurance events (Rojas-Valverde, Sánchez-Ureña, et al., 2019). This could be due to the kidneys' relative mobility during some actions as a downhill run at high speeds, change of directions, falls, and other high g-forces that could affect kidney movements and shaking. This relationship needs to be explored in future studies. The results of the present study indicated that the magnitude and number of impacts (g-forces) could have a potential role in the cumulative mechanical kidney trauma.

Although kidneys are well protected by abdominal and back muscles, ribs, fat, renal pedicle, and ureteropelvic junction and supporting Gerota fascia in the retroperitoneum; they are also susceptible to internal movements (Holmes et al., 2003; Kasikcioglu et al., 2004). In this sense, repeated sudden accelerations and decelerations may provoke renal contusions caused by the collision of kidneys in its surrounding tissues and structures like spine and ribs. These actions could lead to renal vasculatures affections, nephron damage, consequent hematuria and other blood markers findings (Abarbanel et al., 1990; Erlich & Kitrey, 2018; Schmidlin et al., 1998). These accelerations and deceleration could be assessed using the variable impacts as proposed in this study. The results of this study may suggest that both the volume and intensity of the impacts involved during renal contusions play a special role in acute kidney injury. This evidence supports the idea of a new hypothesis of mechanical kidney injury during endurance off-road running based on L1-L3 external workload data (Rojas-Valverde, Sánchez-Ureña, et al., 2019).

The link between the sensors' external load and kidney trauma must be confirmed and discussed in future interventions, considering that the cause of the increase in sCr may be indicative of kidney injury as well as massive muscle damage (Samra & Abcar, 2012). Although elevations in sCr in 33% of participants by itself should not be understood as kidney damage due to physical exercise, the rise in sALB could suggest transitory functional loss due to tubular or glomerular damage. In fact, there is evidence to suggest that proteins released into the bloodstream in high amounts (e.g., rhabdomyolysis) can overload kidney function, resulting in functional or subclinical damage reflected in an increase of sCr and sALB, respectively (Gameiro et al., 2018; Ronco et al., 2012b).

The cumulative effect of micro-trauma during rough exercise such as off-road mountain running may damage the kidney, resulting in AKI. Although there is no clear evidence that cumulative or subsequent AKI events contribute to future renal chronic conditions in athletes (Hoffman & Weiss, 2016; Rojas-Valverde, Sánchez-Ureña, et al., 2020), there is enough evidence to suggest that athletes, coaches, and sports scientists should be concerned with controlling the kidney health of runners, monitoring those variables that can trigger AKI, and thus preventing potential cases of this transitory kidney condition.



VI. CONCLUSIONS

This thesis provides a global perspective of the current situation on the diagnosis, monitoring, and future directions in research related to muscle and kidney damage caused by trail running. This research reasserts that kidney and muscle damage can occur in this sport, and reaffirms the latency and temporality of these condition. It was found that mechanical sensors are a viable option to quantify external load and mechanical damage. It is shown that these load data are related to parameters of both muscle and kidney damage in trail running runners.

A. Specific aim A

Endurance running may impact not only muscle but renal function. ER with concomitant AKI could be asymptomatic and could lead to severe clinical conditions as hospitalization or even death.

Some factors such as hydration, metabolic load (external-internal load), and environmental conditions (humidity, temperature, and thermal index) could increase the possibility of ER+AKI.

The relatively high incidence of ER+AKI in runners may be explained by the increased metabolic load, high exercise duration, and intense exposure to environmental conditions compared to other endurance sports.

B. Specific aim B

$PlayerLoad_{RT}$ and Impacts in L_3-L_4 assessed using MARG sensors are consistent over time (reliable) and show good agreement between measures in a multiple body segment configuration assessment.

This thesis has contributed to a new hypothesis about muscle and kidney mechanical trauma in non-contact sports as trail running due to the high number and magnitude of ground reaction forces, change of direction, acceleration, and deceleration involved during uphill and downhill running.

C. Specific aim C and D

AKI prevalence with concomitant proteinuria and bilirubinuria is relatively uncommon among endurance runners. Although these cases do not represent most of the runner's condition, these findings must be taken with precaution to prevent future complications at clinical level care.

With this, the magnitude and volume of running g-forces monitored with a MARG sensor attached to the lower back of off-road runners could predict the 24% change of sCr and 23% change in sALB.

D. Practical applications and recommendations

To avoid ER and AKI, immediate measures should be taken to prepare, follow-up, and physical evaluation of athletes in endurance events. Actions should include arranging and planning liquid and food intake, monitoring the environmental conditions regularly, and providing the necessary emergency medical attention.

Athletes, coaches, and medical staff should implement specific actions to avoid the possibility of further kidney damage due to participation in endurance events. The frequency of performing prolonged to moderate or high-intensity exercise, exposure to a high rate of thermal stress, and adequate physical preparation to face this type of event should be highly monitored, taking the appropriate actions to avoid or mitigate its adverse effects the health of the athlete.

Also, reducing the amount and magnitude of impacts throughout a session or between sessions can be a way to mitigate the possible collateral damage of AKI during off-road running. Therefore, the preceding considering that monitoring and controlling training external and internal loads is essential for preventing and recovering AKI in off-road runners. In this sense, it is vital to provide constant feedback on running loads behavior, and wearable MARG sensors could be used for these purposes.

Additionally, the support staff for the people participating in this type of events must monitor the different biomarkers of ER and AKI during the different phases of training and competition to prevent new cases and to apply the appropriate recovery protocols (e.g., rest, hydration, ergogenic aids, physical recovery agents, others). When optimal

resources and logistics are available for the early detection of renal function disorders, it may be useful to monitor other markers such as but not limited to NGAL, KIM-1, sAlb, sCyst-C.

If repetitive renal insults as AKI, or severe renal functional loss lead to an accelerated progression of long-term renal issues is currently indefinite. However, the evidence of the link between AKI and chronic renal problems in other populations may turn on an alarm related to the need to continually monitor the different kidney function factors during endurance running training and competition. A more in-depth analysis of the kidney damage indicators is needed before and after the endurance running events, analyzing with caution the outcomes immediately after the event and considering new biomarkers of kidney injury that seem independent of systemic inflammation (e.g., NGAL, KIM-1, Cystatin-C, albumin, PENK) compare to traditional markers (e.g., creatinine, eGFR, blood ureic nitrogen, and urine findings). In addition, it should explore how situational, contextual, and individual factors influence AKI incidence and how this temporary loss of kidney function is related to possible long-term kidney problems.

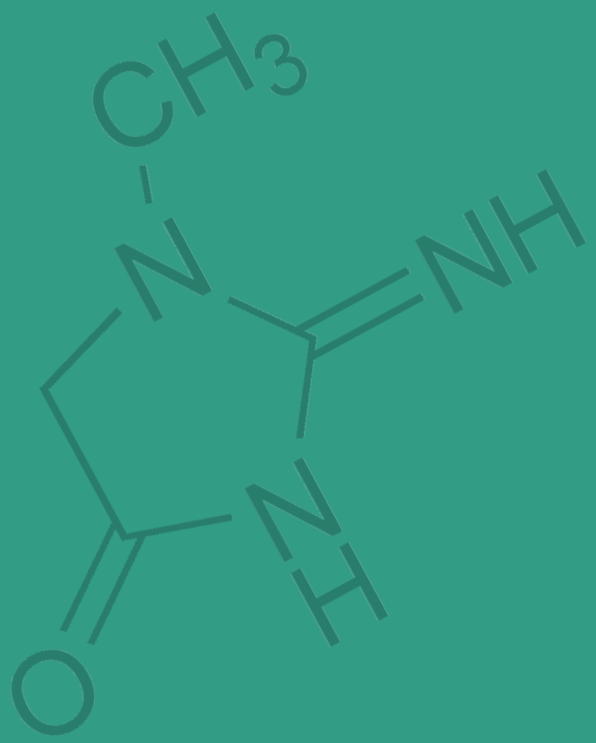
To do this, longitudinal studies are required to observe the behavior of kidney function through a season, multistage races, and, if necessary, investigate how to prevent and treat these acute and chronic kidney conditions promptly for the endurance runners' health. New technological developments such as wearable devices could allow researchers to monitor in real settings and remotely those conditions that could increase the incidence of acute or chronic kidney health problems (e.g., environment temperature and humidity, internal, muscle damage, water balance, sweating rate).

Additionally, clear classifications and guidelines for the diagnosis of conditions such as ER and AKI should be clarified in the scientific community because there is currently an inconsistency between the various ways of classifying and diagnosing these conditions. Additionally, the validity of these diagnostic guidelines for athletes should be explored or, if necessary, to create a specific one for this population considering the physiological responses during exercise.

Based on quantitative results, scientists could overlook the incidence and prevalence of AKI cases with concomitant urine findings. Still, at the clinical level, these results' potential implications may lead stakeholders to deeply analyze those cases, although it could be considered relatively uncommon

Enough is known about the potential risk that we must call on authorities, including universities, ministries, federations, sports committees, and other involved institutions, to

study, and take preventative regulatory actions in the tropical and Mesoamerican region to protect athletes who compete for prolonged periods at high levels of thermal stress. This is of particular urgency given the recent increase in popularity and quantity of this type of event. There is no regional and organizational platform to ensure the implementation of endurance events in a secure manner. Regulation of aspects such as the time of events, heat-exposure, stricter participation criteria, regular medical check-ups, among others, are essential to avoid a regional health problem related to physical exercise.



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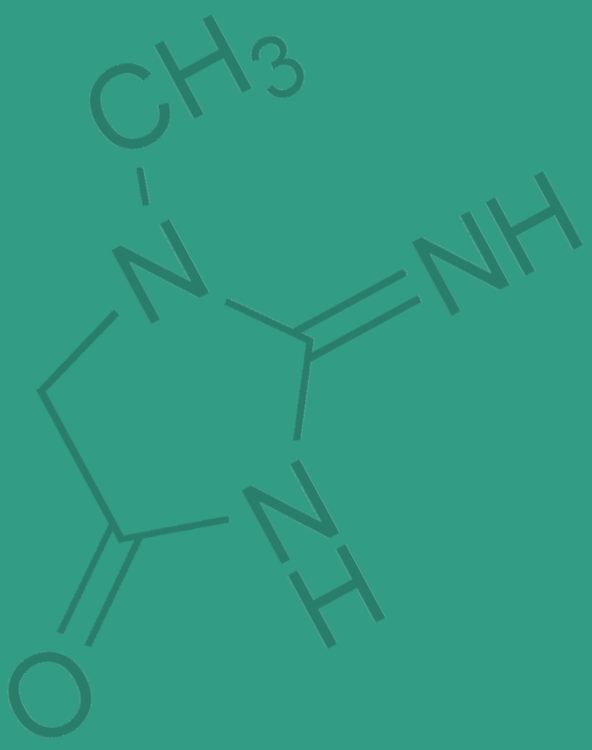
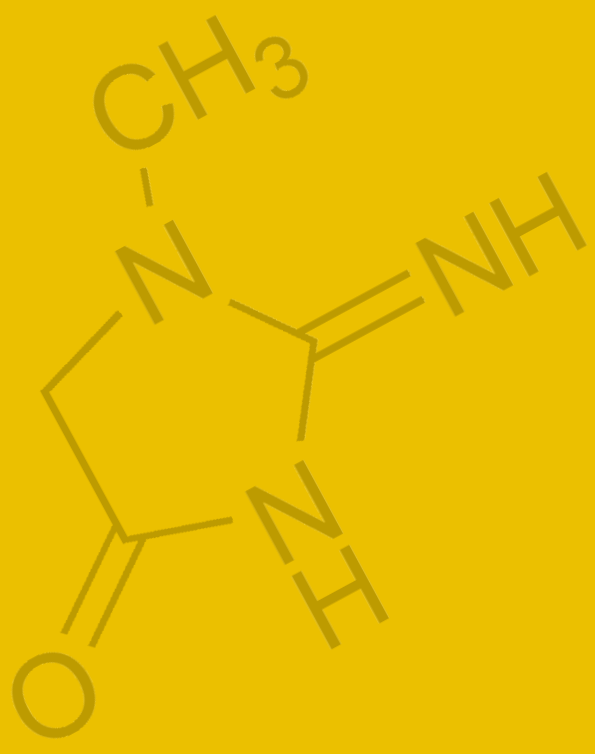


VIII. APPENDIX



A. ACADEMIC PUBLICATIONS

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OPINION
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Heat Strain, External Workload, and Chronic Kidney Disease in Tropical Settings: Are Endurance Athletes Exposed?

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INTRODUCTION

Tropical regions are currently facing a great challenge regarding very high prevalence of chronic kidney disease (CKD). In recent years, a condition called chronic kidney disease of unknown etiology (CKDu), also called CKD of non-traditional origin (CKDnt) or Mesoamerican Nephropathy has been reported (Wesseling et al., 2013, 2015; Wegman et al., 2015). This renal condition has been clearly documented in several so-called hot spots around the globe, typically in low-altitude communities near the western coast of the American continent. Though CKDu presents as typical CKD (Jha et al., 2013), CKDu patients do not present typical risk factors such as obesity, advanced age, hypertension, or diabetes. Instead, they tend to be young, otherwise healthy individuals that often live and work where dehydration and high internal and external physical loads are common. Although the etiology of the disease remains unclear (González-Quiroz et al., 2018; Chapman et al., 2019; Pearce and Caplin, 2019), most researchers agree that CKDu etiology is likely multifactorial and that chronic heat exposure, high external workload, and dehydration are associated with the disease (Wegman et al., 2015; Kupferman et al., 2018). Until now, CKDu in Mesoamerica has been studied mostly in agricultural populations (Crowe et al., 2013, 2015; García-Trabanino et al., 2015; Laws et al., 2016; Wesseling et al., 2016; Butler-Dawson et al., 2018; Kupferman et al., 2018).

Recently, the possibility of elevated rates of CKD in other populations exposed to high external heat and heavy internal heat loads from work/exercise has been raised. These populations could include endurance athletes (Eichner, 2017) in sports such as running, cycling, triathlon, open swimming, adventure races, and other long-duration disciplines. Such athletes usually undertake tremendous physical effort under high heat and humidity during a large number of hours and even consecutive days in multi-stage events in tropical settings (Gutiérrez-Vargas et al., 2018; McDermott et al., 2018; Rojas-Valverde, 2019). Although there are clear differences between CKDu occupational populations and athletes including socio-economic levels, non-optimal working conditions, poor access to health services, lack of recovery time, and low educational levels, there are notable environmental and contextual similarities between both populations.

Since people living and working in agricultural communities often face a wide range of social-economic determinants of health and are exposed to wide variety agents that could feasibly be on the etiologic pathway to CKDu, methodologies to study the effect of heat load on the kidney are complex. The athletes who participate in endurance events represent a privileged sample that could allow the isolation of factors that lead to CKD and provide insights into how external workload, dehydration and heat strain could contribute to the development of adverse renal conditions in athletes as well as populations in more vulnerable populations.

The existing evidence between heat exposure and CKDu in addition to imminent changes in temperature due to global warming have already prompted the question of whether athletes might also be at risk for kidney damage (Eichner, 2017). The sum of external workload and internal factors leading to thermal strain is of great concern, particularly when environmental temperature exceeds normal body temperature. The effect of high thermal stress (e.g., rise in internal temperature, heat loss restriction due to high humidity, and other factors) on the health and performance of athletes has been previously reported (Che Muhamed et al., 2016; Gutiérrez-Vargas et al., 2018; McDermott et al., 2018; Omassoli et al., 2019). Although these conditions are similar to those presented by populations in which CKDu has been diagnosed (see **Figure 1**). AKI leading to CKD has not been directly related to sports practice. However, there is evidence about increasing cases of acute kidney injury (AKI) in endurance sports related to external workload, heat and dehydration (Junglee et al., 2013; Hou et al., 2015; Kao et al., 2015; Bongers et al., 2018), although there remains a lack of information about whether AKI in sports could lead to CKD.

Recently, it has been shown that the practice of endurance sports can cause exertional rhabdomyolysis conditions (Hoffman et al., 2012; Kim et al., 2015), and that this can trigger transitional AKI (Boulter et al., 2011; Chlíbková et al., 2015; Hoffman and Weiss, 2016) due to the release of sarcoplasmic proteins into the bloodstream as a consequence of damage and disintegration of striated muscle during strenuous physical exertion (Bosch et al., 2009; Tietze and Borchers, 2014; Olcina et al., 2018). The preferred biomarkers to diagnose both conditions are serum creatinine and cystatin C levels for kidney function and creatine kinase and lactate dehydrogenase for muscle damage. According to the RIFLE (Risk, Injury, Failure, Loss of Kidney Function, and End-stage kidney disease) categorization, a risk of renal injury exists when serum creatinine (S-Cr) increases 1.5 times; a lesion when S-Cr 2 times, and failure when the S-Cr increases 3 times or values >4 mg/dL (Bellomo et al., 2004). Another classification system, the Acute Kidney Injury Network (AKIN) classification considers AKI to occur when at least one of the following conditions are met in the last 48 h: (a) absolute increase of ≥0.3 mg/dL, (b) increase of 1.5 times above the baseline, or (3) oliguria (urination <0.5 mL/kg per hour per >6 h) (Lopes and Jorge, 2013). In addition, other biomarkers have been recently proposed in order to differentiate functional and subclinical AKI as: cystatin-C, serum albumin, neutrophil gelatinase-associated lipocalin, and kidney injury molecule 1 (McCullough et al., 2013).

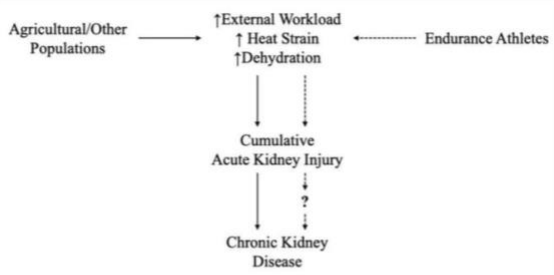

FIGURE 1 | Chronic Kidney Disease common risk factors between agricultural populations and endurance athletes in tropical settings. Dotted line flow needs more longitudinal research and evidence.

Athletes experiencing AKI according to these definitions have been documented to regain baseline renal function in a matter of 1–15 days (Kim et al., 2015; Abbas et al., 2019; Rojas-Valverde et al., 2019), however, documentation also exists of more serious consequences, leading to the death of the athlete in combination to other potential risks (Asserraji et al., 2014). Due to the lack of information about whether the repeated AKI can lead to future CKD (Hoffman and Weiss, 2016), there is a need for studies that shed light on the potential of the combination of environment and physical thermal load in athletes to trigger future CKD, especially in tropical settings, where conditions of high thermal stress exist almost year-round in some regions (Gutiérrez-Vargas et al., 2017, 2018). Unfortunately, in order to observe whether AKI caused by heat exposure and prolonged physical exercise provokes CKD on the long-term requires cohort studies in a long timeframe; such a timeframe could be too late for affected athletes (Eichner, 2017). Other contextual factors that could influence AKI incidence should be explored as internal thermal load indicators, slope variations, age, finish time, carried weight during running, dehydration status and other contextual variables.

DISCUSSION

Existing evidence regarding AKI in hospitalized and occupational (working) populations, as well as the similarities in thermal load experienced by CKDu-affected occupational populations and athlete populations in the tropics, beg the question of whether athletes in hot environments might be experiencing AKI and eventually CKD. Currently available technological tools and methods allow to objectively study cases of AKI and CKD, improving parameters for accurate diagnosis (Clarkson et al., 2006; Stahl et al., 2019) and new markers and methods have been used for their identification (Colombini et al., 2012; McCullough et al., 2013).

Enough is known about the potential risk that we must call on authorities, including universities, ministries, federations, sports committees and other involved institutions, to study, and take preventative regulatory actions in the tropical and Mesoamerican region to protect athletes who compete for prolonged periods at high levels of thermal stress. This is of particular urgency given

the recent increase in popularity and quantity of this type of event and the fact that there is no regional and organizational platform in place to insure implementation of endurance events in a secure manner. Regulation of aspects such as the time of events, heat-exposure, stricter participation criteria, regular medical check-ups, among others, are essential to avoid a regional health problem related to physical exercise.

Through this call for attention, the regional and international scientific community is urged not to wait for the first cases of CKD in athletes related to the combination of three above mentioned factors: heat strain, dehydration, and high external workload, to be documented before acting, since preliminary evidence (i.e., frequent reports of exertional rhabdomyolysis, AKI) are more than enough to demonstrate the need to take timely measures. Since cumulative AKI events have been shown to make individuals more likely to develop CKD in the future (Heung et al., 2016; Hsu and Hsu, 2016), it is highly probable that the same phenomenon is occurring in athletes.

Endurance athletes in tropical regions are exposed to conditions (heat strain, dehydration, and high external workload) similar to those experienced by working populations known to suffer from AKI and CKDu (Gutiérrez-Vargas et al., 2018; Rojas-Valverde et al., 2019), which is why attention should be paid to this factor as a determining point for the monitoring and treatment of this condition in endurance athletes who they carry out long-term activities at moderate to high intensities. We therefore advocate more research in athlete populations in tropical settings in order to (1) protect athletes who may be exposed under the current lack of regulation and (2) provide possible mechanistic insights that might help understand and intervene with CKDu-affected working populations.

AUTHOR CONTRIBUTIONS

DR-V and JC contributed to the conceptualization, the preparation, and the writing of the original draft. DR-V contributed to the literature search. JC, GO, and RG-V critically revised the manuscript and contributed to the supervision. DR-V, JC, GO, and RG-V contributed to the final manuscript approval.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Authors	Daniel Rojas-Valverde, José Pino-Ortega, Rafael Timón Randall Gutiérrez-Vargas, Braulio Sánchez-Ureña, & Guillermo Olcina		
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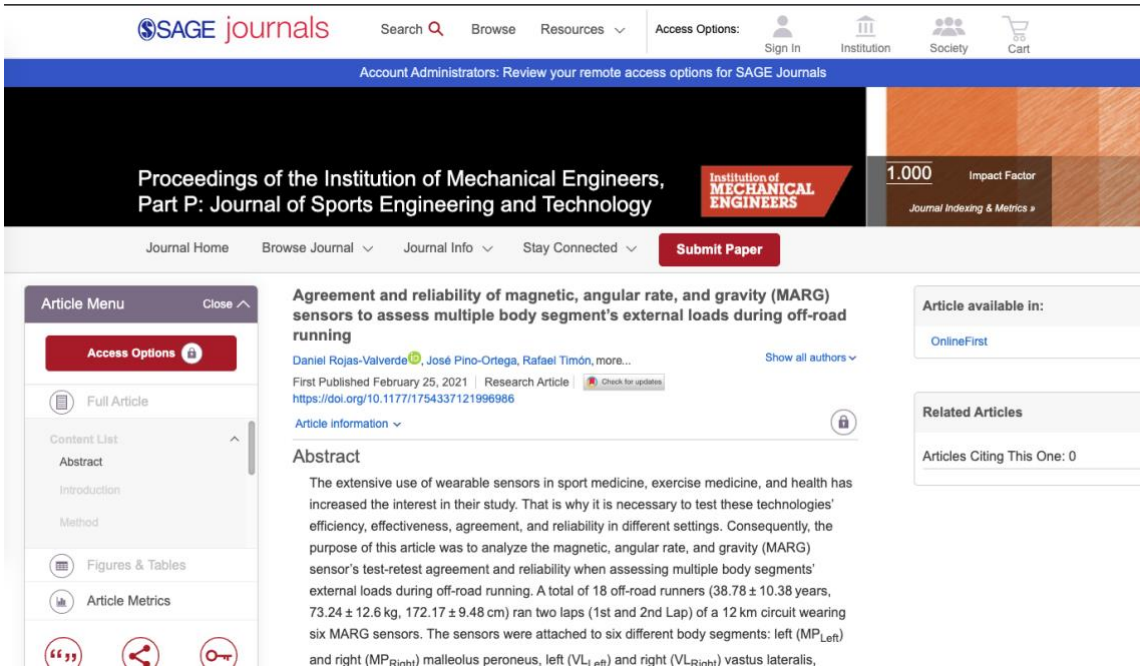

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External Workload Indicators of Muscle and Kidney Mechanical Injury in Endurance Trail Running

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Abstract: Muscle and kidney injury in endurance athletes is worrying for health, and its relationship with physical external workload (eWL) needs to be explored. This study aimed to analyze which eWL indexes have more influence on muscle and kidney injury biomarkers. 20 well-trained trail runners (age = 38.95 ± 9.99 years) ran ~35.27 km (thermal-index = 23.2 ± 1.8 °C, cumulative-ascend = 1815 m) wearing inertial measurement units (IMU) in six different spots (malleolus peroneus [MP_{left}/MP_{right}], vastus lateralis [VL_{left}/VL_{right}], lumbar [L_1–L_3], thoracic [T_2–T_4]) for eWL measuring using a special suit. Muscle and kidney injury serum biomarkers (creatin-kinase [sCK], creatinine (sCr), ureic-nitrogen (sBUN), albumin [sALB]) were assessed pre-, -$post_{0h}$ and $post_{24h}$. A principal component (PC) analysis was performed in each IMU spot to extract the main variables that could explain eWL variance. After extraction, PC factors were inputted in multiple regression analysis to explain biomarkers delta change percentage (Δ%). sCK, sCr, sBUN, sALB presented large differences ($p < 0.05$) between measurements (pre < $post_{24h}$ < $post_{0h}$). PC's explained 77.5–86.5% of total eWL variance. sCK Δ% was predicted in 40 to 47% by L_1–L_3 and MP_{left}; sCr Δ% in 27% to 45% by L_1–L_3 and MP_{left}; and sBUN Δ% in 38%-40% by MP_{right} and MP_{left}. These findings could lead to a better comprehension of how eWL (impacts, player load and approximated entropy) could predict acute kidney and muscle injury. These findings support the new hypothesis of mechanical kidney injury during trail running based on L_1–L_3 external workload data.

Keywords: principal component analysis; mountain sport; acute kidney injury; acute renal failure; exertional rhabdomyolysis

1. Introduction

Recently, inertial measurement units (IMU) composed by different microsensors (gyroscope, accelerometer and magnetometer) have been developed and used for the analysis of human movement [1]. In sports, these types of sensors have been used to quantify the external workload in different team and individual sports [2]. Although the external workload and gait biomechanics

has traditionally analyzed under laboratory conditions using three-dimensional capture systems [3], this microsensors technology have been started to use for the analysis of external workload and biomechanical aspects for the improvement of optimal performance in laboratory conditions [4–7].

Nowadays, there has been a growing interest in quantifying workload in infield settings. In this sense, different researches have assessed by microtechnology the external workload in individual and team sports. The most common used variables were peak acceleration [8], impacts at different ranges [9], accumulated accelerometer load indexes [10–13] and specific events during competition and training sessions such as collisions, jumps or specific events among others [14–17]. In running sports, IMU sensors have been used for the analysis of velocity [18], stride length [18], vertical ground reaction forces [19], body segment kinematics [19], postural stability [4] multi-joints external workload [5,6] and peak accelerations [20], among others. Currently, the usefulness of this type of measurements of external workload has been questioned without having data on the impact they cause at the physiological level, which is why research has been carried out that analyzes both variables to have a clearer understanding of the physical demands and physiological together [21]. The understanding of both internal and external workload variables could better explain the mechanisms of physical damage.

Recently, studies have been carried out in individual and team sports on how this external workload affects muscle function and damage, related to impacts [22,23], jumps [24], changes of direction and speed [25,26]. This muscle damage has been quantified by biochemical (creatine kinase, lactate dehydrogenase, magnesium ant others) [22,27], functional [28] and perceptual methods [29]. On the other hand, the impact that the body receives on each action can also affect the renal function due to the constant mechanical trauma that the kidney can suffer during long-term and moderate to high intensity events, although there is insufficient evidence to relate the external workload and the possible mechanical trauma at the renal level [30]. The most common methods to quantify renal function are cystatin C (sCyst-C), serum creatinine (sCr), estimation of glomerular filtration rate (sGFR), creatinine clearance and blood ureic nitrogen (sBUN) [31–33]; in addition to other novel markers that could suggest subclinical injury as serum albumin (sALB), neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule 1 (KIM-1) [34].

Muscle and kidney injury in endurance athletes has been widely reported [35] and is a point of concern in the health of these types of athletes because the combination of factors such as workload, dehydration and heat strain can trigger acute kidney injury and cause future complications. It has been found that sports that cause a lot of eccentric actions, are carried out for prolonged hours and are exposed to adverse environmental conditions are those that are most likely to cause muscle and kidney injury, this is the case of trail running [33]. Due to the lack of information regarding the external workload indicators that could affect muscle and kidney injury, the purpose of the study was to explore which external workload factors have more influence on the responses of muscle and kidney injury biomarkers in experienced endurance trail runners.

2. Methods

2.1. Design

Participants were assessed -pre (serum test), during (physical external workload), -post$_{0h}$ (serum test) and -post$_{24h}$ (serum test) a trail running event. Participants were asked to run 3 × 11.76 km trail circuit (total distance: ~35.27 km, cumulative positive ascend: 1815 m [from 906 to 1178 m.a.s.l.]). The altimetry of the event and variables with its measurement time can be assessed at Figure 1. The thermal stress index (WetBulb-Globe Temperature [WBGT]) registered throughout the event was 23.2 ± 1.8 °C (temperature: 24.46 ± 2.42 °C and humidity 77.88 ± 10.91%) according to the WBGT (QuestTemp 36, 3M, MN, USA). Final running time was 290.3 ± 54.2 min.

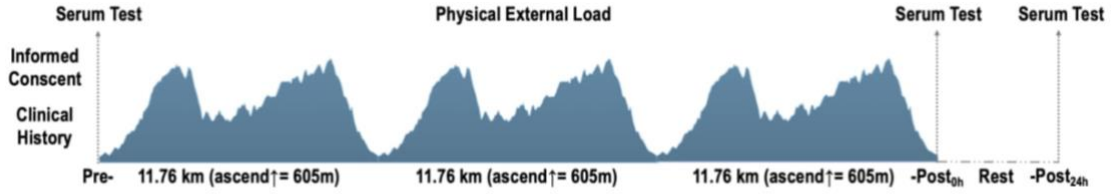

Figure 1. Schematic design of study variables with time measurement and trail altimetry.

2.2. Participants

A total of 20 male runners (age = 38.95 ± 9.99 years, weight = 71.94 ± 12.59 kg, height = 171.15 ± 9.52 cm) took part of the study. Participants were recruited among heat-acclimatized (life and train near event place), trained (running training = 533.1 ± 201.6 min/week) and experienced ultra-endurance runners (years of trail running experience = 6.3 ± 5.8 years). Participants who reported any muscular or metabolic diseases or recent (<6 months) physical injury of the lower limbs were excluded from the study.

Experimental protocol was approved by the Institutional Review Board (Reg. Code UNA-CECUNA-2019-P005). All the participants were informed of the details of the experiment procedures and the associated risks and discomforts. Each subject gave written informed consent, according to the criteria of the Declaration of Helsinki, regarding biomedical research involving human subjects (18th Medical Assembly, 1964, revised in 2013 in Fortaleza).

2.3. Material and Procedures

2.3.1. Serum Markers

A 5 mL of blood was drawn from an antecubital vein directly into a blood collection sterile tube (BD Vacutainer®, New Jersey, NJ, USA) containing spray-coated silica particles activator and a gel polymer for serum separation. Samples were centrifuged at 2000 *g* relative centrifugal force (RCF) for 10 min using tube centrifuge (PLC-01, Gemmy Industrial Corp., Taipei, Taiwan). During sample collecting process, blood samples were stored on ice in a special cooler (45QW Elite, Pelican™, California, CA, USA) until they were stored in a freezer (−20 °C) the same sample extraction day. Sample processing was performed a day after the event under controlled and isolated room using an automatic biochemical analyzer (BS-200E, Mindray, China) by photometry method.

The variables extracted from analysis were serum creatinine (sCr, mg/dL), serum creatine kinase (sCK, IU/L), serum ureic nitrogen (sBUN, mg/dL), serum albumin (sALB, IU/L). Delta percentage of change was calculated for each variable between pre- and -post$_{0h}$ or -post$_{24h}$. Kidney functional loss and Acute Kidney Injury (AKI) was considered and classified following Acute Kidney Injury Network (AKIN), the Risk, Injury, Failure, Loss of kidney Function, and End-stage kidney disease (RIFLE) [36] and the Kidney Disease Improving Global Outcomes (KDIGO) [37] criteria as follow: AKI$_{risk}$ (sCr increase of 150% or acute increase or ≥0.3 mg/dL) and AKI$_{injury}$ (sCr increase of 200%). Additionally, exertional rhabdomyolysis (ER) was considered if sCK level exceeded 1000 UI/L [38].

2.3.2. Physical External Workload

To assess locomotion and kinematic variables, inertial measurement units (IMU) (WIMU PRO™, RealTrack Systems, Almería, Spain) were used in order to register the external workload during running. Six different IMU were attached at six different anatomical spots using a special spandex dark-suit (pat. pending) developed for the research. The suit was made with pockets for each IMU's in six different spots (one IMU at T_2–T_4, one IMU at L_1–L_3; two IMU at right [VL$_{right}$] and left [VL$_{left}$] vastus lateralis muscle bellies and two IMU 3 cm cephalic to right [MP$_{right}$] and left [MP$_{left}$] malleolus peroneus) (see Figure 2a). Suit incorporated dark elastic straps were used to avoid vibrations or non-wanted movements of the devices during running (see Figure 2b).

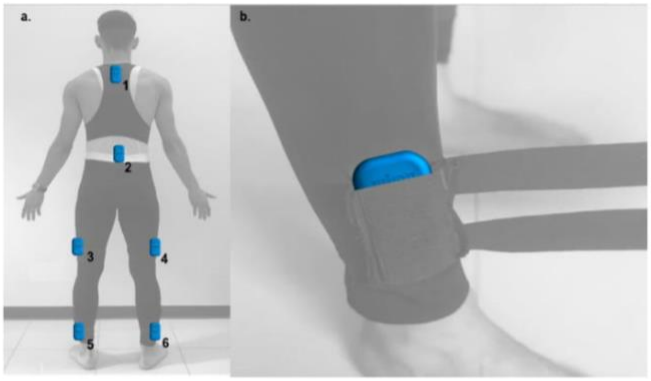

Figure 2. IMU device's setting (**a**) 1: T_2–T_4, 2: L_1–L_3, 3: VL_{left} and 4: VL_{right} vastus lateralis, 5: MP_{left} and 6: MP_{right} malleolus peroneus; (**b**). body attachment anti-vibration straps system.

The IMU's were previously calibrated following protocols for this specific microsensor [39]. This IMU has been used for the assessment of neuromuscular running workload [6] and its reliability had been tested in it use, attached to different body places [39]. Total variables data extracted from IMU software were analyzed using a principal component analysis (PCA) in order to explain total variance [40].

Total variables analyzed were: Player Load per min (AU, PL/min), Player Load difference between segments (AU, PL_{Dif}), approximated entropy (ApEn, AU), maximum acceleration (m/s^{-1}, Acc_{max}), total accelerations (Acc, n/min), total decelerations (Dec, n/min), average acceleration (Acc_{avg}, m/s^2), average deceleration (Dec_{avg}, m/s^2), maximum speed ($Speed_{max}$, m/s), average speed ($Speed_{avg}$, m/s), metabolic power (MP, W/kg), high metabolic load distance (HMLD, m/min), explosive distance (>16 km/h) ($D_{>16\ km/h}$, m/min), maximum heart rate (HR_{max}, bpm), average heart rate (HR_{avg}, bpm), total impacts ($Impacts_{total}$, n/min), and total impacts at 1 g ranges from 0 to >30 g ($Impacts_{total}$, n/min).

2.4. Statistical Analysis

All the variables were reported using the mean, standard deviation and lower and upper limits. Mean significant differences of serum tests variables were explored using a one-way analysis of variance and main differences between time measures were confirmed using Bonferroni method. The magnitude of the differences (effect size) was qualitatively interpreted using partial omega squared (ω_p^2) as follows: >0.01 small; >0.06 moderate and >0.14 large [41]. Change delta's percentage (Δ%) was reported between time measurement in each variable as follow:

$$\Delta\% = \frac{\text{post} - \text{pre}}{\text{pre}} * 100$$

From a total of 458 variables extracted from external workload assessment, only 169 max, average and relative variables were selected for correlation matrix exploration (31 for T_2–T_4, 20 for L_1–L_3, 24 for VL_{right}, 24 for VL_{left}, 35 for MP_{right} and 35 for MP_{left}). A threshold of $r < 0.7$ was used as a criteria selection for extract non correlated variables for running each Principal Component Analysis (PCA). Selected variables were prior scaled and centered (Z-Score) and PCA's were suitable considering Kaiser-Meyer-Olkin values (KMO = 0.61–0.635) and Barleth Sphericity test was significant ($p < 0.01$). After PCA, eingvalues greater than 1 were included for extraction in respective principal component (PC). An orthogonal rotation using VariMax method was used for identification of respective loadings in each PC, then only loadings greater than 0.6 were retained for interpretation and the highest loading was reported when a cross loading was identified between PC's.

After PC's were extracted, multiple lineal regressions (R^2) were performed in order to analyze how the principal components found from each body segment explain both muscle and kidney injury markers change after the event. Alpha was prior set as $p < 0.05$. Data analysis was performed using the Statistical Package for the Social Sciences (SPSS, IBM, SPSS Statistics, v.22.0, Illinois, USA).

3. Results

3.1. Muscle and Kidney Injury Serum Markers

Table 1 shows the mean differences (lower and upper range) and changes of muscle and kidney injury serum markers by measure moment. Large effect size was found in all kidney (sCr, sBUN and sALB) and muscle (sCK) injury variables between pre- and -post$_{0h}$ or -post$_{24h}$. Regarding kidney injury, the greatest change was found between pre- vs. -post$_{0h}$, while in muscle damage the highest change was shown between pre- vs. -post$_{24h}$. A total of 4/20 (20%) cases met diagnosis criteria [38] for ER and 11/20 (55%) cases met diagnosis criteria [36,37] for AKI$_{risk}$ and 3/20 (15%) AKI$_{injury}$ based on sCr.

Table 1. Mean differences (lower and upper limits) and change delta's percentage in muscle and kidney injury serum makers by measure moment.

Category Variable	Pre-	-Post$_{0h}$	-Post$_{24h}$	$F_{(2.28)}$ (p)	ω_p^2 Rating	Δ% Pre- vs. -Post$_{0h}$	Δ% Pre- vs. -Post$_{24h}$
Kidney Injury							
sCr (mg/dL)	1.22 ± 0.29 (0.66 to 1.7)	1.71 ± 0.4 (1.06 to 2.7) *	1.3 ± 0.29 (0.91 to 1.78) †	19.05 (<0.01)	0.53 large	45.67 ± 42.26 (−1.49 to 171.21)	9.02 ± 12.74 (−14.93 to 31.58)
sBUN (mg/dL)	14.4 ± 4.42 (6 to 24)	19.92 ± 5.2 (8.7 to 29) *	18.88 ± 4.89 (13 to 27) *	14.004 (<0.01)	0.46 large	48.91 ± 68.05 (−15 to 323.1)	37.21 ± 37.41 (−35 to 116.67)
sALB (IU/L)	4.31 ± 1.22 (0.29 to 4.99)	5.01 ± 0.82 (1.71 to 5.84) *	4.67 ± 0.25 (4.16 to 5.06)	4.145 (0.027)	0.17 large	92.55 ± 362.99 (1.2 to 1634.48)	15.6 ± 59.74 (−10.1 to 230.71)
Muscle Damage							
sCK (IU/L)	274.5 ± 384.36 (45 to 1688)	691.05 ± 591.43 (229 to 2695) *	680.87 ± 552.07 (244 to 2400) *	11.021 (<0.01)	0.39 large	322.56 ± 503.01 (42.23 to 2371.1)	337.75 ± 303.25 (−4.56 to 976.23)

sCr: serum creatinine, sBUN: serum ureic blood nitrogen, sALB serum albumin and sCK: serum creatine kinase. * Significant differences with Pre- ($p < 0.01$); † Significant differences with -Post$_{0h}$ ($p < 0.01$). Lower and upper limits were reported in brackets.

3.2. External Workload Variables Selected per Body Segment

The external workload variables outcome and principal components of each body segment spot is shown in Table 2. The body segments that explained the highest percentage of variance were MP of both legs and L_1–L_3, while the location that explained the lowest was VL_{left}. Additionally, PL, PL_{Dif} between segments, ApEn, different levels of impacts and impacts$_{total}$/min were the most common variables that explained total variance of the workload.

Table 2. External workload variables outcome and extracted principal components of each body segment spot.

		Outcome, M ± SD (95%CI)	PC1	PC2	PC3	PC4
T_2–T_4	Eigenvalue		3.198	1.352	1.324	1.101
	% variance		35.53	15.02	14.71	12.24
	% cumulative variance		35.53	20.55	65.26	77.5
	PL_{Dif} T_2–T_4–L_1–L_3 (AU)	274.17 ± 251.37 (−306 to 654.67)	−0.818			
	ApEn (AU)	0.43 ± 0.1 (0.26 to 0.64)	0.81			
	$Impacts_{total}$/min	314.77 ± 55.56 (201.29 to 417.54)		0.781		
	Acc_{max} (m/s^{-1})	4.41 ± 1.23 (3.19 to 7.21)		−0.624		
	PL/min (AU)	1.6 ± 0.57 (0.86 to 2.73)		0.766		
	$Speed_{max}$ (m/s)	5.05 ± 0.85 (3.83 to 7.24)			0.938	
	$Impacts_{0\text{-}1\ g}$/min	108.06 ± 39.69 (23.3 to 171.77)				−0.892
L_1–L_3	Eigenvalue		3.186	2.481	1.458	1.234
	% variance		31.86	24.82	14.58	12.34
	% cumulative variance		31.86	56.68	71.26	83.60
	$Impacts_{total}$/min	170.57 ± 36.64 (111.87 to 247.79)	−0.843			
	$Impacts_{0\text{-}1\ g}$/min	95.28 ± 38.02 (18.8 to 161.86)	−0.763			
	ApEn (AU)	0.51 ± 0.11 (0.24 to 0.76)	0.725			
	PL/min (AU)	2.78 ± 0.53 (2.16 to 3.88)	0.753			
	PL_{Dif} T_2–T_4–L_1–L_3 (AU)	274.17 ± 251.37 (−306 to 654.66)		0.847		
	$Impacts_{8\text{-}9\ g}$/min	2.16 ± 3.27 (0.32 to 14.68)		0.789		
	$Impacts_{6\text{-}7\ g}$/min	5.93 ± 2.13 (3.04 to 9.39)			−0.677	
	$Impacts_{1\text{-}2\ g}$/min	64.42 ± 13.25 (41.84 to 87.39)			0.781	
	$Impacts_{5\text{-}6\ g}$/min	10.18 ± 3.39 (5.46 to 16.54)				0.933
VL_{right}	Eigenvalue		1.606	1.24	1.081	-
	% variance		32.12	24.81	21.61	-
	% cumulative variance		32.12	56.93	78.55	-
	PL/min (AU)	3.96 ± 0.93 (2.47 to 5.62)	0.823			-
	$Impacts_{7\text{-}8\ g}$/min	3.22 ± 1.1 (1.68 to 5.45)	0.892			-
	$Impacts_{3\text{-}4\ g}$/min	10.74 ± 4.2 (4.72 to 21.75)		0.931		-
	PL_{Dif} VLright–MPright (AU)	52.3 ± 435.04 (−1045.51 to 1002.44)			−0.642	-
	ApEn (AU)	0.46 ± 0.11 (0.32 to 0.67)			0.838	-
VL_{left}	Eigenvalue		1.951	-	-	-
	% variance		65.05	-	-	-
	% cumulative variance		65.05	-	-	-
	PL/min (AU)	3.88 ± 0.88 (2.7 to 5.74)	−0.696	-	-	-
	$Impacts_{5\text{-}6\ g}$/min	5.57 ± 2.04 (2.49 to 10.06)	0.796	-	-	-
	$Impacts_{3\text{-}4\ g}$/min	11.43 ± 4.98 (3.72 to 21.48)	0.913	-	-	-
MP_{right}	Eigenvalue		2.614	1.978	1.238	1.092
	% variance		32.67	24.72	15.48	13.64
	% cumulative variance		32.67	57.4	72.87	86.52
	$Impacts_{8\text{-}9\ g}$/min	5.77 ± 2.04 (3.11 to 12.12)	0.859			
	PL/min (AU)	4.52 ± 1.03 (3.16 to 6.56)	0.73			
	ApEn (AU)	0.36 ± 0.18 (0.04 to 0.81)	−0.862			
	$Impacts_{total}$/min	115.84 ± 27.91 (74.29 to 163.89)		0.867		
	PL_{Dif} VL_{right}–MP_{right} (AU)	52.3 ± 435.04 (−1045.51 to 1002.44)		0.94		
	$Impacts_{1\text{-}2\ g}$/min	28.83 ± 10.77 (10.43 to 49.95)		0.779		
	$Impacts_{6\text{-}7\ g}$/min	6.95 ± 2.35 (3.32 to 12.12)			−0.845	
	$Impacts_{3\text{-}4\ g}$/min	9.28 ± 4.25 (4 to 16.34)				0.95
MP_{left}	Eigenvalue		2.538	2.206	1.58	1.175
	% variance		28.2	24.51	17.55	13.06
	% cumulative variance		28.2	52.71	70.26	83.32
	PL_{Dif} VL_{left}–MP_{left} (AU)	193.75 ± 0.88 (−1228.3 to 935.59)	−0.766			
	$Impacts_{6\text{-}7\ g}$/min	7.12 ± 2.74 (1.94 to 11.42)	0.754			
	$Impacts_{8\text{-}9\ g}$/min	5.47 ± 1.61 (2.7 to 8.5)	0.888			
	PL/min (AU)	4.53 ± 1.07 (2.95.1 to 7.18)		−0.903		
	$Impacts_{4\text{-}5\ g}$/min	6.9 ± 2.63 (1 to 11.46)		0.887		
	$Impacts_{1\text{-}2\ g}$/min	26.58 ± 8.12 (13.11 to 45.84)			0.88	
	$Impacts_{total}$/min	155.46 ± 20.87 (98.33 to 184.1)			0.842	
	$Impacts_{3\text{-}4\ g}$/min	10.46 ± 4.01 (2.38 to 18.28)				0.92

Note. M: mean; SD: standard deviation; CI: confidence interval; AU: arbitrary units; PC: principal component.

3.3. Prediction of Serum Change by External Workload Variables of Each Body Location

Finally, Table 3 shows the prediction of muscle and kidney injury serum variables change by the workload principal components of each body segment. At pre- vs. -post$_{0h}$, the highest prediction values were found in: sCr by MP$_{left}$ (45%) and L$_1$–L$_3$ (27%); sBUN by MP$_{right}$ (40%) and MP$_{left}$ (38%); and sCK by MP$_{left}$ (47%) and L$_1$–L$_3$ (40%). At pre- vs. -post$_{24h}$ the highest prediction values was found in: sCr by T$_2$–T$_4$ (74%); sBUN by MP$_{right}$ (10%) and T$_2$–T$_4$ (10%); and sCK by L$_1$–L$_3$ (59%). sALB was not predicted by any of the workload variables.

Table 3. Body segments external workload indicators (principal components) that predicted muscle and kidney injury serum changes.

Δ% Pre- vs. -Post$_{0h}$				
Body Segment	**sCr**	**sBUN**	**sALB**	**sCK**
T$_2$–T$_4$	R^2 = 0.23, β = 44.03 p < 0.01 **	R^2 = 0.22, β = 51.91 p < 0.01 **	R^2 = 0.18, β = 100.55 p = 0.3	R^2 = 0.14, β = 333.97 p = 0.025 *
L$_1$–L$_3$	R^2 = 0. 27, β = 45.36 p < 0.01 **	R^2 = 0.2, β = 55.99 p = 0.014 *	R^2 = 0.29, β = 112.16 p = 0.286	R^2 = 0.4, β = 350.02 p = 0.019 *
VL$_{right}$	R^2 = 0.11, β = 42.69 p < 0.01 **	R^2 = 0.33, β = 47.97 p < 0.01 **	R^2 = 0.36, β = 101.28 p = 0.223	R^2 = 0.33, β = 336.79 p = 0.01 **
VL$_{left}$	R^2 = 0.07, β = 41.63 p < 0.01 **	R^2 = 0.10, β = 48.39 p < 0.01 **	R^2 = 0.16, β = 96.09 p = 0.25	R^2 = 0.2, β = 324.57 p < 0.01 **
MP$_{right}$	R^2 = 0.2, β = 45.22 p < 0.01 **	R^2 = 0.4, β = 51.51 p = 0.019 *	R^2 = 0.44, β = 119.34 p = 0.243	R^2 = 0.36, β = 373.01 p = 0.024 *
MP$_{left}$	R^2 = 0.45, β = 47.33 p < 0.01 **	R^2 = 0.38, β = 50.39 p < 0.01 **	R^2 = 0.45, β = 96.35 p = 0.202	R^2 = 0.47, β = 335.28 p < 0.01 **
Δ% Pre- vs. -Post$_{24h}$				
Body Segment	sCr	sBUN	sALB	sCK
T$_2$–T$_4$	R^2 = 0.74, β = 877.57 p = 0.02 *	R^2 = 0.1, β = 39.17 p < 0.01 **	R^2 = 0.29, β = 22.41 p = 0.265	R^2 = 0.3, β = 363.58 p < 0.01 **
L$_1$–L$_3$	R^2 = 0.45, β = 5.07 p = 0.229	R^2 = 0.19, β = 37.14 p = 0.057	R^2 = 0.32, β = 19.62 p = 0.529	R^2 = 0.59, β = 493.04 p < 0.01 **
VL$_{right}$	R^2 = 0.19, β = 6.95 p = 0.077	R^2 = 0.22, β = 36.82 p < 0.01 **	R^2 = 0.18, β = 19.53 p = 0.325	R^2 = 0.22, β = 324.08 p < 0.01 **
VL$_{left}$	R^2 = 0.002, β = 7.46 p = 0.039 *	R^2 = 0.08, β = 34.9 p < 0.01 **	R^2 = 0.13, β = 13.87 p = 0.41	R^2 = 0, β = 189.63 p = 0.967
MP$_{right}$	R^2 = 0.56, β = 4.53 p = 0.207	R^2 = 0.23, β = 31.26 p = 0.126	R^2 = 0.5, β = 9.65 p = 0.69	R^2 = 0.27, β = 473.47 p = 0.015 *
MP$_{left}$	R^2 = 0.12, β = 10.13 p = 0.025 *	R^2 = 0.1, β = 39.93 p < 0.01 **	R^2 = 0.08, β = 13.14 p = 0.524	R^2 = 0.39, β = 413.12 p < 0.01 **

* p < 0.05, ** p < 0.01.

4. Discussion

For our knowledge, this is the first research that explores and analyzes which external workload indicators influence the most on muscle and kidney injury biomarkers during endurance trail runners. It was found that muscle and kidney injury biomarkers presented large differences (ω_p^2: 0.17–0.53; p < 0.01) between measurements (pre < post$_{24h}$ < post$_{0h}$). Extracted PC's explained 77.5 to 86.5% of total external workload variance. sCK Δ% was predicted in 40% and 47% by L$_1$–L$_3$ and MP$_{left}$ PC's respectively; sCr Δ% in a 27% and 45% by the L$_1$–L$_3$ and MP$_{left}$ PC's; and sBUN Δ% in 38% and 40% by MP$_{right}$ and MP$_{left}$ PC's.

The sCK increased when pre vs. post$_{0h}$ (Δ% = 322.56 ± 503; p < 0.01) and pre vs. post$_{24h}$ (Δ% = 337.75 ± 303.25; p < 0.01) with post values between 691.05 and 680.87 UI/L after 35 km trail running with the presence some cases of exertional rhabdomyolysis (20% of total sample met diagnosis criteria). Same similar results that other studies when compared pre, post$_{0h}$ and post$_{24h}$ [42] is also reported in

recent evidence after endurance running in 42.195 km marathon with 131,900 UI/L [43], 89.3 km with 5718-to-54,231 UI/L [44], 100 km ultramarathon with 200,000 IU/L [45], 161 km and 5500 m cumulative climbing with 38,218-to-95,940 UI/L [46] or 1550-to-264,300 UI/L [47].

When sCK rises above 1000 UI/L [38], this could lead to a condition known as exertional rhabdomyolysis, caused by the release of sarcoplasmic proteins into the bloodstream [48] due to the damage and disintegration of striated muscle during strenuous physical exertion [22,49,50]. In the present study 20% of participants presented exertional rhabdomyolysis. There could be observed as expected that when the distance of the event increases the sCK rises significantly, but trail running events tend to provoke greater sCK changes when compare to other road and flat events, due to the higher slope variations (uphill and downhill) [51]. This slope conditions could lead to significant higher impacts and metabolic workload than relative flat events as marathon [51], because the greater effort that should be made in order to absorb impact and constant slope changes that requires higher eccentric muscle contractions [52].

Not only muscle biomarkers have affected, kidney functional (sCr and sBUN) and subclinical (sALB) injury biomarkers also increased. sCr, sBUN and sALB obtaining the highest values -post$_{0h}$. sCr and sALB not presented statistical differences between pre- and -post$_{24h}$, but sBUN maintained equal values in post$_{0h}$ and post$_{24h}$ with large differences respect to pre-. This increase in serum kidney functional biomarkers as sCr have been previously reported in running endurance events during 42.2 marathon with 7.97 mg/dL [43], 89.3 km with 2.99–12.88 mg/dL [44], or 161 km with 5500 m cumulative climbing with 1.1–4.9 mg/dL [39] with clinical symptoms and hospitalization (acute renal dialysis and hyponatremia), or 100 km ultramarathon with 17.64 mg/dL [45] and 135 m ultramarathon with 1–1.34 mg/dL without any clinical symptoms [53].

The found acute rise in sCr ($\Delta\%$ = 45.7, sCr difference $\geq$ 0.3 mg/dL) could be diagnosed as AKI$_{risk}$ or AKI$_{injury}$ following AKIN, RIFLE and KDIGO reference ranges (70% of total sample met criteria) [36,37,54]. Additionally, despite there is evidence that the rise in sCr or sBUN by itself could not be considered as AKI because there is no enough information of subclinical injury, the rise in sALB should suggest transitory functional loss due to tubular or glomerular damage [54]. Considering the above information and analyzing mean change of sBUN, sCr and sALB levels of the participants, this data suggest AKI presence after trail running due to functional, physiological and structural changes. The increase of this serum biomarkers levels could respond to mechanical muscle and kidney trauma, this last condition has been suggested in other contact sports [30] but not deeply explored in running sports until present analysis.

In order to explore the impact of the mechanical muscle and kidney trauma on serum biomarkers, a principal component analysis was performed for each body segment in order to select the main group of variables that could explain external workload variance. This statistical technique has been used in other sports as a data meaning method. PC's extracted explained 77.5–86.5% of total external workload variance. In this study PL, total impacts, impacts at different ranges and entropy partly explained the total variance in all body segments. The difference in impacts range between segments is related to the ground-to-ground contact, finding the ranges of higher impacts in lower limb respect to lumbar region and back.

Real time monitoring using IMU devices have been used in order to explore fatigue, neuromuscular changes and physiological effects of running [22] but there is the first attempts to analyze multi segment external workload in trail running. Previous studies have analyzed multi-segments workload at laboratory conditions through player load and peak accelerations [8]. These studies found greater external workload in the nearest segment with the ground-to-ground contact (ankle) and at a faster speed. In trail running, only two previous researchers analyzed external workload during trail running at different points [51,55], but only one analyzed the workload dynamics during all the race in one body segment (T_2–T_4) through player load, metabolic power, entropy, speed, vertical stiffness and heart rate [51]. The highest values of external workload were found in downhill segments, while the highest metabolic response was found in uphill segments [56–58].

In this sense, if in downhill segments was found the greatest external workload, where eccentric muscle contractions are important for the technical abilities of the trail runner as changes of direction, accelerations and decelerations [51]; it is explainable that impacts, player load and entropy took part in all body segments PCs. The first two variables are calculated from the three axial accelerations of the human body [6,9] and entropy try to explain the regularity of signal dynamics [59]. In downhill segments, the gait biomechanics is more irregular, and the greatest fly time provokes that especially lower limb suffer greater impacts.

Despite the fact that there is enough evidence of the effect of endurance running and trail running on muscle damage [28,42], the rise in muscle damage serum concentration has not been associated with contextual factors as finish time, age, gender, delayed onset muscle soreness or running experience [47]. This could be due to the etiology of serum blood levels increasing is related to muscle damage during endurance running, compared to other activities [42,60]. This is why there is an increasing interest in exploration of which external workload variables could predict serum muscle and kidney changes. It was found that muscle and kidney mechanical trauma hypothesis theory [30] could be supported by the results of the present manuscript, where sCK Δ% was predicted in 40% and 47% by L_1–L_3 and MP_{left} PC's respectively; sCr Δ% in a 27% and 45% by the L_1–L_3 and MP_{left} PC's and sBUN Δ% in 38% and 40% by MP_{right} and MP_{left} PC's. These results may suggest that core muscle resistance, optimal absorption forces, and efficient running economy could be protective factors to avoid greater muscle and kidney mechanical injury. Another consideration about the role of L_1–L_3 in impact absorption could develop mechanical kidney trauma due to kidney shaking and nephritis [30].

While the results of this study have provided valuable information about the influence of external workload variables and its potential prediction of muscle and kidney mechanical injury changes during endurance trail running, some limitations must be acknowledged. Due to the nature of trail running, it was difficulties in the assessment of some serum biomarkers in the middle of the race. Some serum biomarkers as myoglobin and Cyst-C as markers of kidney injury could be assessed in future studies as well as other novel subclinical AKI markers as NGAL and KIM-1 should be assessed in future studies in order to confirm kidney structural damage and early detect AKI [34,61]. Due to the organic exploration of the real setting conditions during and after running, some factors such food intake and liquid intake were no monitored and were led ad libitum. After running recovery strategies were no restricted but registered as internal control. As expected, these results must be addressed considering the specific anthropometric, experience and competitive level of analyzed sample, and should not be extrapolated to other populations that show different kinematical behavior because of their individual characteristics or competitive level.

Finally, future research could explore the impact of contextual factors as slope variations, age, finish time, carried weight during running, dehydration status and other contextual variables on muscle and kidney health during trail running. As well as more exploration of other conditions as medication, dehydration, heat strain and health status that could trigger a potential condition and future exertional rhabdomyolysis or AKI. There is a necessity of new evidence around the efficiency of recovery protocols to maintain or recover serum baseline levels.

5. Conclusions

Based on the previous results, it is confirmed that after an endurance trail running event, there are not only changes in muscle damage markers, but also produced changes in kidney injury biomarkers that could considered as a transitory loss of kidney function. These findings give new evidence that in order to a better understanding of muscle and kidney health, not only pre and post-race serum biomarkers have to be assessed, but other contextual factors such as locomotion variables, temperature, heat strain and dehydration should also be assessed in order to better understand the global muscle and kidney injury phenomenon during trail running.

Considering that this is the first study to address which variables explain the behavior of external workload total variance and it relation to changes in serum biomarkers of kidney and muscle injury;

it is essential to indicate that in the case of endurance trail running, the external workload principal components predicts from 10% to 47% of the serum changes as sCK, sBUN and sCr values.

These findings could lead to a better understanding of how external workload could predict transitory acute kidney injury and exertional rhabdomyolysis in endurance sports. Variables as impacts, player load, approximated entropy and player load difference between segments should be assessed as external workload indicators of mechanical muscle and kidney injury. Additionally, this paper has contributed to a new hypothesis about muscle and kidney mechanical trauma in non-contact sports as trail running, due to high number and magnitude of ground reaction forces, change of direction, acceleration and deceleration involved during uphill and downhill running.

Considering there is enough evidence of the development of AKI during endurance sports and there is data in other populations of how cumulative AKI events could lead to future CKD, why should be different in athletes? Despite there is a lack of evidence around the long term effects of AKI in endurance runners and other kidney injury biomarkers should be assessed, practitioners should address this new information and take action around the optimal physical conditioning, hydration protocols, acclimatization and other considerations in order to reduce kidney injury risk during endurance events.

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Article

Proteinuria and Bilirubinuria as Potential Risk Indicators of Acute Kidney Injury during Running in Outpatient Settings

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Abstract: *Background and objectives*: The purpose of this study was to explore which urinary markers could indicate acute kidney injury (AKI) during prolonged trail running in outpatient settings. *Materials and Methods*: Twenty-nine experienced trail runners (age 39.1 ± 8.8 years, weight 71.9 ± 11 kg, height 171.9 ± 8.3 cm) completed a 35 km event (cumulative positive ascend of 1815 m, altitude = 906 to 1178 m.a.s.l.) under a temperature of 25.52 ± 1.98 °C and humidity of 79.25 ± 7.45%). Two participant groups (AKI = 17 and No-AKI = 12) were made according to AKI diagnosis criteria based on pre- and post-race values of serum creatinine (sCr) (an increase of 1.5 times from baseline). Blood and urinalysis were performed immediately pre- and post-race. *Results*: Pre- vs. post-race differences in sCr and sBUN were found in both AKI and No-AKI groups ($p < 0.01$). Differences in post-race values were found between groups ($p = 0.03$). A total of 52% of AKI runners presented significant increases in proteinuria ($\chi^2 = 0.94$, $p = 0.01$) and 47% in bilirubinuria ($\chi^2 = 0.94$, $p = 0.04$). Conversely, No-AKI participants presented no significant increases in urine markers. *Conclusions*: These study's findings may suggest the potential use of urinalysis as an accessible alternative in the outpatient setting to early identify transitional AKI until a clinical confirmation is performed.

Keywords: urine; biomarkers; renal health; assessment; mountain running; acute renal failure

1. Introduction

Acute kidney injury (AKI) is a condition defined as an injury or damage accompanied in some cases by renal dysfunction over a relatively short period [1–3]. This takes into account that human kidneys have a significant glomerular function reserve and dysfunction may be evident only when more than 50% of the total renal mass is compromised [4]. This sudden episode of kidney damage occurs within a few hours (<48 h), causing a build-up of waste products in the bloodstream, making it difficult for kidneys to maintain the body's fluid balance. This abrupt kidney damage occurs in a wide range of clinical settings. It represents a relatively common but under-recognized problem in sports medicine and science, and AKI's long-term effects on renal function are still unclear [5].

Transitional AKI has been reported as a severe condition with an increasing incidence in endurance sports such as triathlon, open water, swimming, cycling, and running. Approximately 16% to 50% of athletes participating in long-distance events fulfill AKI diagnosis criteria [5], and 97% of these participants are endurance runners [5]. Muscle damage and subsequent inflammatory responses could result from consecutive eccentric–concentric muscle contraction during endurance running and be AKI's potential cause [5–8]. In strenuous and prolonged events, AKI etiology is considered multifactorial, and heat strain, dehydration, high metabolic, and physical load are potential enhancers of this temporal decrease in kidney function [5,9,10].

Some evidence has reported that blood markers' changes seem to be a physiological reaction in sports, and it is overestimated in AKI [11]. Recent epidemiological and experimental studies have demonstrated a real link between AKI and chronic kidney disease (CKD) [12,13]. Repeated AKI episodes, even mild cases, may induce CKD over the long term. The potential link between consecutive AKI episodes and CKD has to be confirmed in sports due to the need for clarification around nature and precise mechanisms leading to AKI in these activities. It is known that prolonged exercising does not impact short-term kidney function, and it is transitional damage that usually requires a few days to recover normal function [5,14].

AKI is usually assessed using markers such as serum creatinine (sCr), albumin, serum blood ureic nitrogen (sBUN), cystatin-C, neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), pro-inflammatory cytokines (e.g., IL-18), and liver-type fatty acid-binding protein [15–18]. This condition is also defined, classified, and stratified based on severity according to some classification systems, such as Risk, Injury, Failure, Loss, and End-Stage (RIFLE) and Acute Kidney Injury Network (AKIN) criteria [3,19]. This diagnosis criterion allows earlier identification of AKI and diagnosis, even in the absence of subsequent kidney dysfunction [4,20].

AKI is also associated with urine alterations [21], such as the high prevalence of proteinuria [11], hematuria [22], albuminuria [23], and creatinuria [24] with a return to baseline after a few days. Urine analysis is usually performed using urine samples analyzed in the laboratory, but urine dipstick readings are generally used as an accessible alternative [25,26]. In endurance sports, it has been shown that long races, compared to short races, present a higher incidence of urine alterations [11], so it is related to duration but not intensity [27]. These changes in urine characteristics are usually associated with hemodynamic adjustments as hemoconcentration and renal hypoperfusion due to dehydration during endurance sports [23].

The increase in wide-spread participation in endurance events has raised concerns around these activities' potential implications for the participant's kidney health [4,10]. Considering that adverse environmental conditions such as high temperature and humidity as in tropical settings increase AKI's risk, renal function must be monitored in endurance sports such as running, mostly when it is practiced in hot regions [28,29]. Additionally, it is fundamental to have more available options to monitor kidney function during training and competition. Based on previous evidence, urinalysis could be an alternative and accessible method to identify AKI early in the field. These measurements could offer some vital information to allow action to be taken on affected athletes in the field, while waiting for subsequent confirmation analysis (e.g., medical imaging or blood tests). This kind of in-field analysis could provide objective data to boost prevention strategies in those diagnosed participants. Since AKI can be highlighted as a severe clinical problem with significant morbidity, the objective of this study was to explore which urinary markers could indicate AKI during prolonged trail running in outpatient settings.

2. Materials and Methods

2.1. Design

This was a retrospective cohort study where participants completed a 35 km event (cumulative positive ascend of 1815 m, altitude = 906 to 1178 m.a.s.l.). The event was held in Mora, San José, Costa

Rica under a temperature of 25.52 ± 1.98 °C and humidity of 79.25 ± 7.45% (QUESTemp^{TM} 36, 3M, Saint Paul, MN, USA). Two participant groups (AKI = 17 and No-AKI = 12) were made according to AKI risk criteria based on pre- and post-race values of sCr (an increase of 1.5 times from baseline) [19]. Both blood and urinalysis were performed immediately pre- and post-race. Serum and urine samples were collected ~15 min before and ~15 min after the race (see Figure 1). Participant's finish time was 4.5 ± 0.3 h.

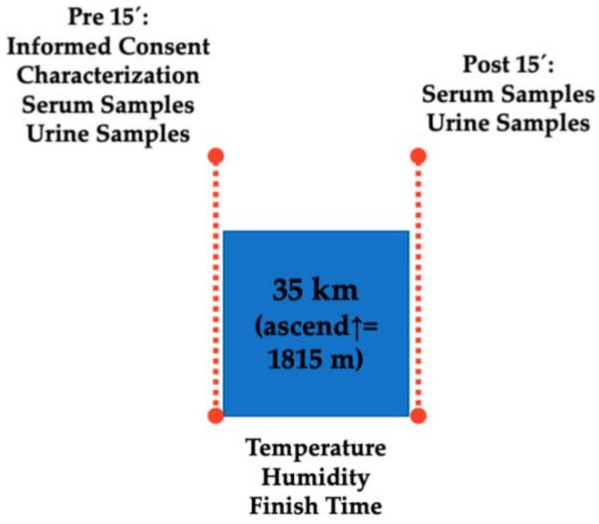

Figure 1. Schematic design of study assessments.

2.2. Participants

Twenty-nine mountain runners (age 39 ± 9.1 years, weight 71.7 ± 10.8 kg, height 172.2 ± 8.3 cm) took part in the study. Participants were selected among >18 years old, voluntary, experienced (5.41 ± 2.79 years), trained (8.8 ± 3.4 h/week), and heat acclimatized (sleep and train in similar study's altitude and weather) ultra-endurance runners. Participants were recruited from a single endurance event and reported no neuromuscular injuries or metabolic disturbances at least six months before the race.

The experimental protocol was approved by the Institutional Review Board of Universidad Nacional de Costa Rica (Reg. Code UNA-CECUNA-2019-P005; 17 June 2019) and Universidad de Extremadura (Reg. Code 139/2020; 25 September 2020). All participants were informed of the details of the experimental procedures and the associated risks and discomforts, as well as their benefits and rights. According to the criteria of the Declaration of Helsinki, each participant gave written informed consent regarding biomedical research involving human subjects (18th Medical Assembly, 1964, revised in 2013 in Fortaleza).

2.3. Materials and Procedures

2.3.1. Serum Test

Blood was extracted in situ from the antecubital vein using a 5 mL blood collection sterile tube (Vacutainer^{TM}, Becton, Dickinson & Company, Franklin Lakes, NJ, USA) containing a spray-coated silica particle activator. Tubes contained a gel polymer to facilitate serum separation during centrifugation (10 min at 2000× *g* relative centrifugal force) using centrifuge tubes (PLC-01, Gemmy Industrial Corp., Taipei, Taiwan). Blood samples were stored on ice in a special cooler (45QW Elite, Pelican Products^{TM}, Torrance, CA, USA) until serum samples were frozen at −20 °C (~5 h after blood extraction). Sample analysis and processing were performed 24 h after data collection in an isolated

and temperature-controlled laboratory using an automatic biochemical analyzer (BS-200E, Mindray, city, China) by photometry method. All procedures were performed under relevant protocols for the handling and disposal of biological materials, according to the manufacturer's instructions for the equipment and reagents used. The analyzed variables were sCr (mg/dL) and sBUN (mg/dL).

2.3.2. Urine Test

Urine samples were collected in situ in a 30 mL polypropylene sterile urine sample container (Nipro Medical Corp., Osaka, Japan). Samples were analyzed using highly sensitive and accurate dipsticks for urine screening (Combur$_{10}$Test M, Roche, Mannheim, Germany) during distance running [25]. Urine dipsticks were examined immediately after collection by two different microbiologists simultaneously using the manufacturer's color scale. In case of disagreement between observers, a consensus was obtained by the opinion of a third researcher. The following parameters were screened: leucocytes, erythrocytes, bilirubin, ketones, nitrites, protein, glucose, and urobilinogen. There were no reported urination problems or difficulties neither before nor after the race. Traces were considered as negatives, and those scores >1 were reported. Urine test interpretation and reporting were made as follows: >1 score was equivalent to leucocytes > 10 cells/μL, erythrocytes > 5 cells/μL, bilirubin > 1, ketones > 1, nitrites +, protein > 30 mg/dL, glucose > 50 mg/dL, and urobilinogen > 1 mg/dL.

2.3.3. Urine Specific Gravity

Urine specific gravity (USG) was assessed as a hydration status marker. Urine solids were assessed, and USG was confirmed and double-checked with a digital valid [30] handheld refractometer (Palm Abbe^{TM}, Misco, Solon, OH, USA). It was classified following the hydration status ranges: well-hydrated < 1.01, minimal dehydration 1.01–1.02, significant dehydration 1.02–1.03, and severe dehydration > 1.03 [31]. The refractometer was cleaned with distilled water and calibrated previously.

2.4. Statistical Analysis

The description of variables was reported using mean, standard deviation, and lower and upper limits. The normality of the data was confirmed using the Shapiro–Wilk test. Participants' basic data and characteristics were compared using an independent *t*-test. Differences between AKI and No-AKI groups in blood biomarkers and USG were explored using a mixed analysis of variance, and post-hoc of Bonferroni was performed to look after specific differences. Omega squared (ω_p^2) was selected to qualify the magnitude of the differences as follows: <0.01 trivial; >0.01 small; >0.06 moderate, and >0.14 large [32].

McNemar's non-parametric test was used to explore the possible change in proportion for the paired data of urinalysis. In those observed cases, the intersection frequency value was <5; the binomial test was performed. The data of urinalysis were paired by measurement moment using a 2 × 2 contingency table. Alpha was set at $p < 0.05$, and all data were analyzed and systematized using the e Statistical Package for the Social Sciences (SPSS, IBM, SPSS Statistics, v.22.0, Chicago, IL, USA).

3. Results

Table 1 shows the comparison between AKI and No-AKI groups based on the participants' basic data and characteristics. There were no differences in this participant's information by group.

Table 1. Participants' basic data and characteristics comparison.

	AKI	No-AKI	*t*	*p*-Value
Age (years)	39.4 ± 8.8	38.1 ± 9.8	0.34	0.74
Weight (kg)	69.2 ± 7.3	76 ± 14.5	−0.99	0.33
Height (cm)	171.6 ± 7.6	173.1 ± 9.6	0.41	0.69
Trail running experience (years)	5 ± 2.6	6.1 ± 3.1	−1.63	0.12
Training (hours)	9.1 ± 2.8	8.5 ± 4.5	−0.45	0.66

There were 17 participants that met AKI criteria (sCr = 1.18 ± 0.26 pre, 1.81 ± 0.35 post, change of 53.4%). There were large differences by measurement (pre vs. post) and group (AKI vs. No-AKI) in sCr ($F = 17.24$, $p < 0.01$, $\omega_p^2 = 0.38$ (large)) and sBUN ($F = 4.1$, $p < 0.5$, $\omega_p^2 = 0.1$ (large)). Pre vs. post differences were found in both AKI ($p < 0.01$) and No-AKI ($p < 0.01$) groups in sCr and sBUN. Moreover, in sCr values, post-race differences between AKI and No-AKI groups were found ($p = 0.03$) but no pre-race differences were identified ($p = 0.34$) (see Figure 2a). Additionally, in sBUN values, there were no pre- or post-race differences between AKI and No-AKI groups (see Figure 2b).

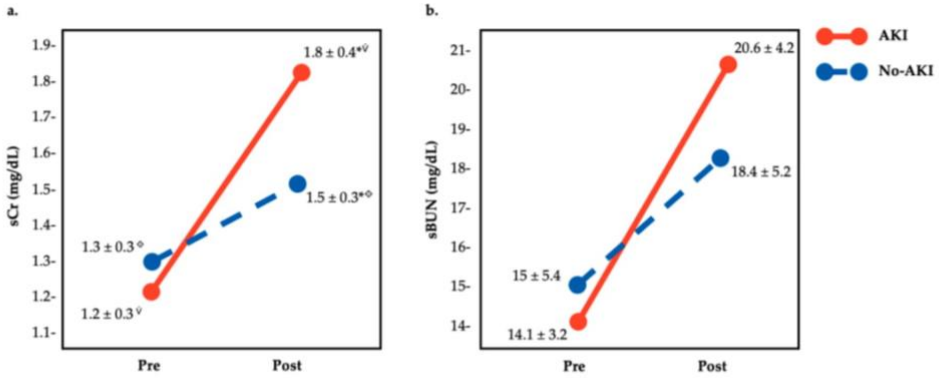

Figure 2. Comparison of pre- and post-race (**a**). serum creatinine (sCr), and (**b**). serum blood ureic nitrogen (sBUN) values of trail runners by the presence or not of acute kidney injury. ◊ ◊ * significant statistical differences.

Of AKI runners, 52.94% presented a significant increase in proteinuria ($\chi^2 = 0.94$, $p = 0.01$) and 47.06% in bilirubinuria ($\chi^2 = 0.94$, $p = 0.04$) when comparing pre- vs. post-race values. No significant increases were found in leucocyturia (17.64%, $\chi^2 = 04.96$, $p = 0.5$), urobilonogenuria (17.64%, $\chi^2 = 0.23$, $p = 0.63$), and hematuria (29.41%, $\chi^2 = 0.58$, $p = 0.13$). No cases of nitrituria, glucosuria, or ketonuria were found (see Table 2).

Table 2. Urinalysis outcomes in participants fulfilling acute kidney injury (AKI) diagnosis criteria.

Variable (Score Criteria)	Pre		Post 0 h		χ^2	*p*-Value
	n *	%	*n* *	%		
Leucocytes (>1)	0	0	3	17.64	4.96	0.5
Nitrites (>1)	0	0	0	0	-	-
Protein (>1)	0	0	9	52.94	0.94	0.008
Glucose (>1)	0	0	0	0	-	-
Ketones (>1)	0	0	0	0	-	-
Urobilinogen (>1)	0	0	3	17.64	0.23	0.625
Bilirubin (>1)	0	0	8	47.06	0.94	0.039
Erythrocytes (>1)	0	0	5	29.41	0.58	0.125

* Based on AKI group data ($n = 17$).

Furthermore, 12 participants did not develop AKI (sCr = 1.28 ± 0.28 pre, 1.5 ± 0.3 post). Proteinuria (33.33%, $\chi^2 = 1.67$, $p = 0.25$), ketonuria (16.66%, $\chi^2 = 0.28$, $p = 1$), bilirubinuria (41.66%, $\chi^2 = 0.74$, $p = 0.63$), urobilonogenuria (8.33%, $\chi^2 = 0.12$, $p = 1$), and hematuria (33.33%, $\chi^2 = 2.59$, $p = 0.5$) were found but with no significant change. No cases of leucocyturia, nitrituria, or glucosuria were presented in the No-AKI group (see Table 3).

Table 3. Urinalysis outcomes in participants without AKI diagnosis.

Variable (Score Criteria)	Pre		Post 0 h		χ^2	*p*-Value
	n *	%	*n* *	%		
Leucocytes (>1)	0	0	0	0	-	-
Nitrites (>1)	0	0	0	0	-	-
Protein (>1)	0	0	4	33.33	1.667	0.25
Glucose (>1)	0	0	0	0	-	-
Ketones (>1)	0	0	2	16.66	0.278	1
Urobilinogen (>1)	0	0	1	8.33	0.123	1
Bilirubin (>1)	0	0	5	41.66	0.741	0.063
Erythrocytes (>1)	0	0	3	33.33	2.59	0.5

* Based on No-AKI group data ($n = 12$).

Finally, USG as a hydration marker showed no significant interaction in AKI vs. No-AKI groups ($F = 0.62$, $p = 0.44$, $\omega_p{}^2 = 0$ (trivial)). There were no pre vs. post differences ($F = 3.1$, $p = 0.09$; pre = 1.018 vs. post = 1.023).

4. Discussion

This study aimed to explore which urinary markers could indicate AKI during prolonged trail running in outpatient settings. The main findings of this study were as follows: (1) Pre- vs. post-race differences in sCr and sBUN were found in both AKI and No-AKI groups ($p < 0.01$); (2) differences in post-race values were found between groups ($p = 0.03$); (3) a total of 52% of AKI runners presented significant increases in proteinuria ($\chi^2 = 0.94$, $p = 0.01$) and 47% in bilirubinuria ($\chi^2 = 0.94$, $p = 0.04$); and, conversely, (4) No-AKI participants presented no significant increases in urine markers.

AKI could be caused by a series of factors such as decreased blood flow, direct kidney trauma, blockage of the urinary tract, among others [33]. In sports, precisely in endurance sports, the mechanisms are still unclear. Some factors such as heat strain, dehydration, and high metabolic and physical load may boost the risk and are primary issues. Dehydration seems to be a factor that did not influence the AKI occurrence in this specific sample, as found in the results. In strenuous exercise, high physical load during prolonged periods has an essential role in AKI development [6,34]. The relative typical rise in sCr values during endurance events could suggest a high muscle damage rate due to the release of sarcoplasmic proteins into the bloodstream. Damage and disintegration of muscle fibers are expected consequences of strenuous physical exertion. Distance running events are one of the most physically demanding sports, and the subsequent structural and functional damage could be exacerbated due to the repetitive concentric–eccentric muscle actions when running uphill and downhill as happen in endurance trail training and competitions. These efforts usually require greater impact absorption and a higher metabolic rate [6,35,36] as compared to other sports.

Protein is one of the main structural components of muscle fibers in the body. Under normal conditions, the kidney's protein excreted in healthy adults is about 150mg per day [37]. As a consequence of muscle damage, an excess of proteins is excreted through the urine, and a condition known as proteinuria could develop. This condition is asymptomatic and associated with intense exercise, also called exercise-induced proteinuria [22], as was found in the present study.

The pathophysiological mechanisms of proteinuria can be partially explained by increasing glomerular capillary permeability to proteins and reduced protein reabsorption capacity in the

renal tubules. Still, exercise-induced proteinuria is not fully understood, but it seems that the renin–angiotensin system and prostaglandins have an essential role in its development [38].

Proteinuria and bilirubinuria in endurance sports could be a consequence of a cascade of events in the kidney. In non-contact sports, catecholamines are released by the suprarenal glandules causing a redirection of blood to muscles and restricting kidney blood flow. These events lead to hypoxic nephron damage and an increase in glomerular permeability [39]. Vasoconstriction of the glomerular arteriole is also provoked by catecholamines, resulting in decreased glomerular filtration pressure and allowing excretion of some macro- and microscopic elements in urine as protein, erythrocytes, albumin, and bilirubin. Other factors contributing to exercise proteinuria could be, but are not limited to, lactate accumulation, oxidant stress, hormonal changes, and sepsis [22,25].

The increase in bilirubin found in this research could be caused by hemolysis and subsequent catabolism of hemoglobin. The proliferation of red blood cell breakdown is caused mainly by free radicals and a mechanical factor [40]. Bilirubinuria could also be related to hepatic disturbance during long-distance running [41,42]. Endurance running may cause a decline in hepatic function related to changes in the liver cells' membrane by lipid peroxidation due to blood flow restrictions and free radicals' release. It is known that the liver suffers a temporary decline in its function during prolonged exercises compared to running over shorter distances [42]. A condition called foot-strike hemolysis suggests that blood cells' mechanical injury could be related to the consecutive impact during running [43].

Exercise-related proteinuria and bilirubinuria have been related to renal and hepatic dysfunctions. Both conditions could be asymptomatic, transitional, reversible, and, usually, they do not need any special care. However, endurance athletes could be particularly vulnerable to developing such conditions when exposing themselves to a high level of environmental stress, such as a hot and humid environment [10,44,45]. These conditions could boost ischemia, hypoxemia, and ATP depletion in renal tubular cells, and considering dehydration, it could be exacerbated by increased sodium reabsorption [29].

The presence of AKI cases with concomitant proteinuria and bilirubinuria may suggest the potential use of urinalysis as an accessible alternative to identify AKI cases early in the field and monitor training and competition as an outpatient setting. The screening of urine changes could represent an opportunity to identify the potential risk of AKI cases in a simple and fast manner. This result must be analyzed with caution, considering that only 47 to 52% of AKI runners presented urine changes.

Based on quantitative results, scientists could overlook the incidence and prevalence of AKI cases with concomitant urine findings. Still, at the clinical level, these results' potential implications may lead stakeholders to deeply analyze those cases, although it could be considered relatively uncommon [5]. Finally, there is no clear link between AKI and more severe complications such as chronic kidney disease in endurance sports. Some actions must be addressed to prevent future health issues in athletes. Managing fluid intake and restoring electrolytes prior to, during, and after endurance events may contribute to the reduction of the number or lessen the severity of AKI cases. Avoiding repeated participation at endurance events without the required rest and recovery between exhaustive efforts could be protective against AKI.

These findings must be seen in the light of some limitations. Some contextual factors such as liquid intake, food consumption, and supplements during running should be controlled in future studies. Despite limited access, it may be interesting to assess some novel AKI indicators as Cyst-C, NGAL, and KIM-1 as subclinical AKI markers. It is fundamental to develop a cohort follow-up to confirm the potentiality of cumulative AKI events leading to CKD. Unfortunately, assessing blood and urine samples of a large cohort for research purposes during a long-distance event as trail running is not always feasible; future studies must include a greater sample size.

Homogeneity between groups and diagnosis criteria of AKI made it difficult to interpret why there were no differences between AKI vs. No-AKI groups, considering there was an occurrence

of proteinuria in 33% of No-AKI cases as well as differences in sCr and sBUN between pre- and post-race assessments. This may suggest that, during clinical evaluation of AKI during endurance sports, patients may be analyzed individually to explore these findings' real relevance and potential runner's health impairment.

Additionally, it should be considered that dipstick analysis is usually used in most outpatient settings to semi-quantitatively measure the urine protein concentration but not the type or total amount. These tests are a crude estimation of urine protein concentration, so this is an initial approach for AKI that seems to correlate with AKI markers. Runners with persistent proteinuria should undergo a quantitative measurement of protein excretion using, for example, a 24-h urine specimen (urine protein/Cr).

5. Conclusions

Endurance trail running could lead to an increase in some blood and urine samples related to transitory AKI. This study found pre- vs. post-race differences in sCr and sBUN in both AKI and No-AKI groups, differences in post-race values between groups ($p = 0.03$), and a total of 52% of AKI runners presented significant increases in proteinuria ($\chi^2 = 0.94$, $p = 0.01$) and 47% in bilirubinuria ($\chi^2 = 0.94$, $p = 0.04$).

These results may suggest that AKI prevalence with concomitant proteinuria and bilirubinuria is relatively uncommon among endurance runners. Although these cases do not represent most of the runner's condition, at clinical level care, these findings must be taken with precaution to prevent future complications. Furthermore, although there is insufficient evidence that links AKI to other future complications, these markers should be monitored during training and competition to prevent potential future damage.

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Potential Use of Wearable Sensors to Assess Cumulative Kidney Trauma in Endurance Off-Road Running

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Abstract: (1) Background: This study aimed to explore wearable sensors' potential use to assess cumulative mechanical kidney trauma during endurance off-road running. (2) Methods: 18 participants (38.78 ± 10.38 years, 73.24 ± 12.6 kg, 172.17 ± 9.48 cm) ran 36 k off-road race wearing a Magnetic, Angular Rate and Gravity (MARG) sensor attached to their lower back. Impacts in g forces were recorded throughout the race using the MARG sensor. Two blood samples were collected immediately pre- and post-race: serum creatinine (sCr) and albumin (sALB). (3) Results: Sixteen impact variables were grouped using principal component analysis in four different principal components (PC) that explained 90% of the total variance. The 4th PC predicted 24% of the percentage of change (Δ%) of sCr and the 3rd PC predicted the Δ% of sALB by 23%. There were pre- and post-race large changes in sCr and sALB ($p \leq 0.01$) and 33% of participants met acute kidney injury diagnosis criteria. (4) Conclusions: The data related to impacts could better explain the cumulative mechanical kidney trauma during mountain running, opening a new range of possibilities using technology to better understand how the number and magnitude of the g-forces involved in off-road running could potentially affect kidney function.

Keywords: renal health; wearable devices; technology; acute kidney injury; inertial measurement units (IMU)

1. Introduction

Acute kidney injury (AKI) is a relatively uncommon condition in sports. This condition has been reported in prolonged and repetitive strenuous exercises [1]. It is understood as a transitional decrease in renal function, expressed by a reduction in glomerular filtration rate, increase in serum creatinine (sCr) and albumin (sALB), and alterations of other novel AKI-related urine and blood biomarkers during a relatively short period (1–3 days) [2].

The evidence of AKI cases in both contact and non-contact sports has been increased, but with clear different etiological backgrounds [3–5]. In contact sports like football, boxing, and rugby, AKI cases have been related to kidney contusion or trauma (grade I in American Association for Surgery of

Trauma classification) during tackles, punches, or other high-intensity actions with direct impact to the body [6,7]. On the other hand, in non-contact sports (e.g., endurance running and cycling), AKI has been related to the high number of muscle eccentric-concentric contractions leading to muscle damage [8,9].

In endurance running and mainly off-road running [8,10], some evidence has been published regarding the impact of external workload (e.g., impacts) as an additional factor that may contribute to AKI incidence, next to other known factors like dehydration, heat strain, and high metabolic activity [11]. Within this multifactorial etiology, high physical internal and external load seems to be a discernible contributing factor to the transitory decrease in renal function in endurance runners [12]. It could be due to muscle damage in response to high eccentric actions and its effect on inflammatory and hemodynamic responses that may affect the kidney [13]. New evidence has also highlighted the cumulative mechanical trauma that affects the kidney during off-road running as a potential cause of AKI [9]. Although kidneys are very well protected structures, there is relative mobility that could lead to injury even when no direct trauma occurred [14], for example, during downhill running or change of directions during training or competition.

Monitoring physical load is critical in endurance sports, such as off-road running, due to the high number of actions involved [15]. This is why non-invasive tools as wearable sensors could be an accessible option to assess potential cumulative mechanical kidney trauma, indirectly analyzing the mobility of anatomical structures near the kidneys, such as the lower back. These wearable sensors are used to monitor physical load during exercise in different parts of the body, such as the wrist, waist, and trunk [16–18]. It has also been determined that there is a relationship between the increase in serum blood factors related to kidney damage and the quantified load in the lower back [9]. Therefore, this study aimed to explore the potential use of wearable Magnetic, Angular Rate and Gravity (MARG) sensors to assess cumulative mechanical kidney trauma during off-road running.

2. Materials and Methods

2.1. Design

Participants were asked to perform three loops of a 12 km (+ascend = 600 m) circuit (total distance = 36 km and total +ascend = 1800 m), under 25° Celsius of temperature, and 80% of humidity (Wet Bulb globe Temperature, 3M, USA). Runners wore a MARG sensor attached to the lower back during the race, and variables of time-related impacts were extracted. Two blood samples were collected pre- and post-race to assess serum creatinine (sCr) and albumin (sALB). An analysis was made to explore a model based on impact variables that explained sCr and sALB increases between pre- and post-race.

2.2. Participants

Eighteen experienced mountain runners participated in this study (age 38.78 ± 10.38 years, weight 73.24 ± 12.6 kg, height 172.17 ± 9.48 cm). They had 4.78 ± 2.42 years of experience competing in ultra-endurance events. Participant's mean finish time was 4.2 ± 0.21 h. No neuromuscular, metabolic, or structural injuries were reported at least six months before the study. The participants were asked to avoid intense endurance exercise at least a week before the event.

All participants were notified of the study's aim, protocol details and the potential risks and rights during their participation. The study´s protocol followed all biomedical guidelines based on the Declaration of Helsinki (2013) and it was reviewed and approved by the Institutional Review Boards of Universidad Nacional (Reg. Code 2019-P005) and Universidad de Extremadura (Reg. Code 139/2020).

2.3. Materials and Procedures

Sixteen different time-related impacts (*n*/min, g forces) variables were assessed using a Magnetic, Angular Rate and Gravity (MARG) sensor (WIMU PRO^{TM}, RealTrack Systems, Almería, Spain). The devices were attached to the lower back (~L1–L3) [9] of each participant with a special spandex

dark belt adjusted with elastic straps to avoid device´s unwanted vibrations or movements (see Figure 1). The MARG´s integrate four 3-axis microelectromechanical systems accelerometers (2x ± 16 g, 1x ± 32 g, and 1x ± 400 g), gyroscope, and magnetometer. All MARG´s calibration and setting were developed following published guidelines [19,20], its reliability for neuromuscular running physical load assessment has been proven [21] and its reliability has been tested in different body parts [22]. The variables extracted were total impacts per min ($Impacts_{Total}$/min) and fifteen progressively scaled categories of g-force magnitude, each 1 g wide ($Impacts_{1–15\ g}$/min).

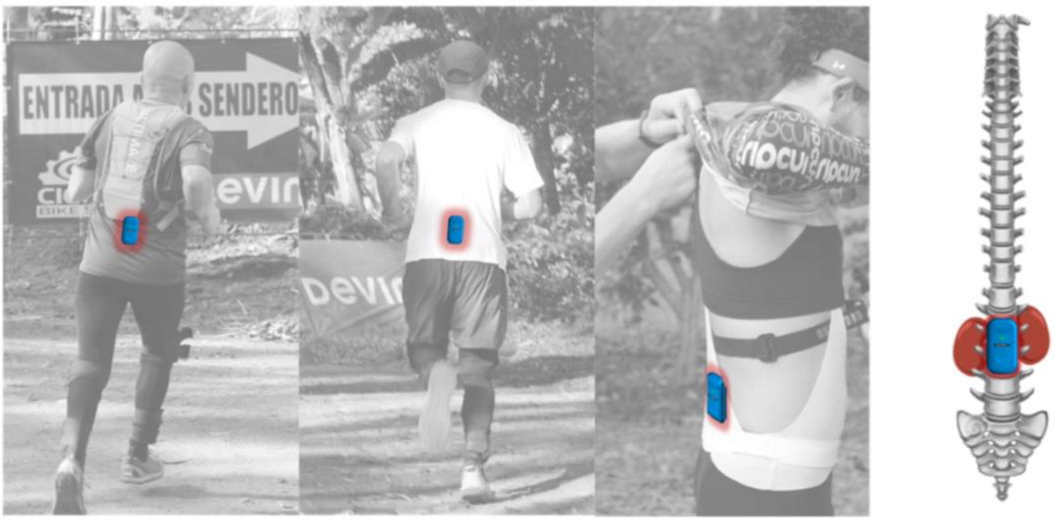

Figure 1. Inertial measurement unit attachment at runner´s lower back (L1–L3).

Blood serum samples were collected using 5 mL blood spray-coated silica tubes (BD Vacutainer®, Franklin Lakes, NJ, USA). After centrifugation (10 min at 2000 *g*), samples were stored at −20 °C. After 24 h, the samples were processed by the photometry method using an automatic biochemical analyzer (BS-200E, Mindray, China). The variable analyzed was serum creatinine (sCr, mg/dL) and serum albumin (sALB, IU/L). Acute kidney injury (sCr baseline in mg/dL *1.5) was considered following established diagnosis criteria [23]. Two groups were made based on AKI diagnosis as follows: those participants that met AKI diagnosis (AKI) and the ones that did not (No-AKI), in order to explore differences in the number of impacts reported.

Urine specific gravity (USG) was assessed as a hydration status marker. USG was confirmed and double-checked with a digital valid [24] handheld refractometer (Palm AbbeTM, Misco, Solon, OH, USA). It was classified following the hydration status ranges: well-hydrated <1.01, minimal dehydration 1.01–1.02, significant dehydration 1.02–1.03, and severe dehydration >1.03 [25]. The refractometer was cleaned with distilled water and calibrated previously. There were no reported urination problems or difficulties neither before nor after the race.

2.4. Statistical Analysis

All sixteen impact variables were grouped using a Principal Component Analysis (PCA) following previous studies guidelines [9,26]. PCA was suitable, according to Kaiser-Meyer-Olkin (*KMO* = 0.63) values and the Barleth Sphericity test ($p < 0.01$). Eigenvalues (EV) > 1 were considered for the extraction of each Principal Component (PC). A VariMax-orthogonal rotation method was used to identify the high correlation of components. A threshold of 0.6 was set to retain loadings. The highest loading was used when a cross-loading was found between PCs. PCA procedure followed standard quality criteria [27], meeting 21 out of 21 of the quality items.

A paired t-test was used to explore sCr and sALB changes between pre- and post-race data and the Change delta´s percentage (Δ%) was calculated as follows: ((sCr post-race–sCr pre-race)/sCr

pre-race)*100. An unpaired t-test was performed to explore potential differences in the number of impacts between those participants who met AKI diagnosis and those who did not. USG data were analyzed using a repeated measure *t*-test. The magnitude of the differences was calculated using Cohen´s *d*.

Finally, a stepwise regression model (R^2) was applied to resulted factor scores obtained from impact´s PCA using the Δ% of sCr and sALB as the dependent variable. This statistical technique was applied to identify which impact´s PC could predict the Δ% of sCr, and Δ% of sALB.

All variables were presented in mean ± standard deviation. Alpha was set at $p < 0.05$ and all analyses were made using the Statistical Package for Social Science (v.22, SPSS, Chicago, IL, USA).

3. Results

Participants experienced a total of 170.57 ± 34.42 impacts per minute. Figure 2 shows the mean number of impacts per minute in relation to the associated magnitude of g-force (see Figure 2).

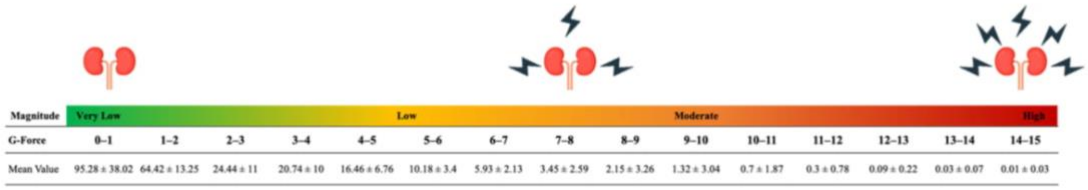

Figure 2. Mean values of impacts per minute associated with 15 g-force categories during off-road mountain running.

All sixteen impact-related variables were grouped in four different PC´s, explaining the 90.39% of total impacts cumulative variance. The 1st PC explained the 50.5% (EV = 8.08) of total variance, 2nd PC the 17.58% (EV = 2.81), 3rd PC the 13.05% (EV = 2.09), and 4th PC the 9.27% (EV = 1.48). Grouped variables and loadings are presented in Figure 3.

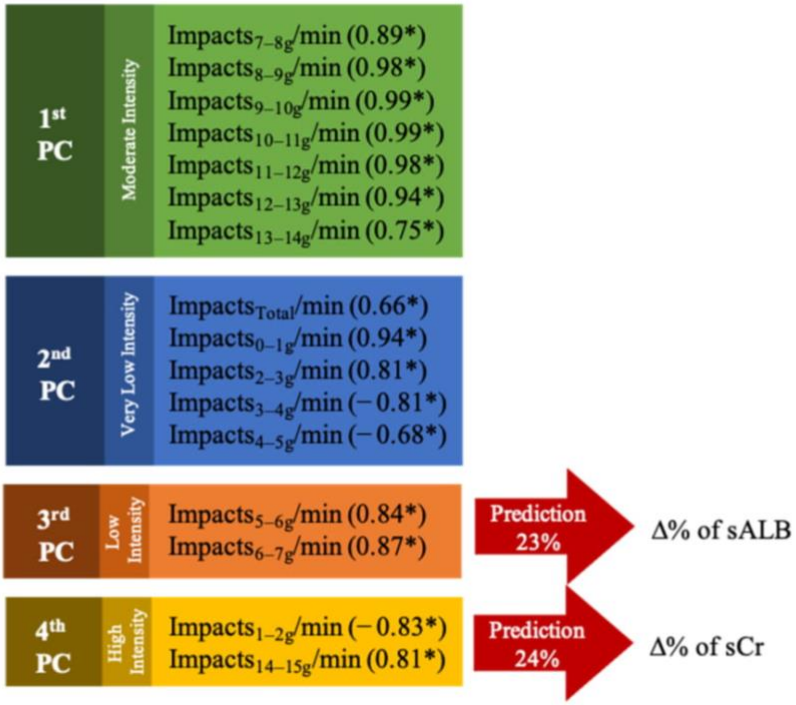

Figure 3. Principal component analysis extracted variables and loadings. * Loadings values.

In follow up to the abovementioned PCA results, those participants that met AKI diagnosis criteria (33.3% of participants) registered lower number of impacts in the 1–2 g category ($t = -2.42$, $p = 0.03$, $d = -1.45$, large effect size) but higher number of impacts in the 14–15 g category ($t = -3.1$, $p = 0.01$, $d = -1.58$, large effect size) (see Figure 4.). No differences we found in the 5–6 g or 6–7 g categories.

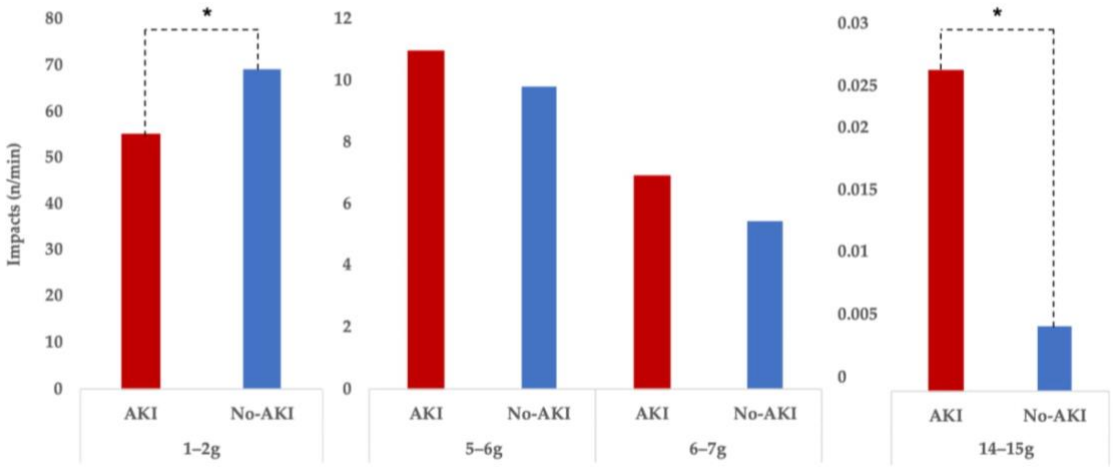

Figure 4. Differences between runners showing signs of AKI ($n = 6$) and those showing no signs of AKI ($n = 12$) regarding impacts per minute, grouped in four impact g-force categories. * The biggest difference between the AKI and no-AKI group is that the no-AKI group managed to run "smoother," keeping impacts in the lower impact load ranges, while avoiding higher impacts loads.

There were large statistical differences ($t = -6.24$, $p < 0.01$, $d = -1.47$, large effect size) between sCr pre-race (1.24 ± 0.28 mg/dL) and sCr post-race (1.74 ± 0.41 mg/dL), and large differences ($t = -2.78$, $p = 0.01$, $d = -1.47$, large effect size) in sALB pre-race (4.33 ± 1.29 IU/L) vs. post-race (5.01 ± 0.86 IU/L). The Δ% of sCr was predicted by the 4th PC in a 24% ($R^2 = 0.24$, $\beta = 44.03$, $p < 0.01$) and the Δ% of sALB by a 23% ($R^2 = 0.23$, $\beta = 100.55$, $p = 0.04$). Finally, USG as a hydration marker reported no differences between pre- and post-race measurements (1.01 ± 0.02 vs. 1.01 ± 0.01; t = 1.02, $p = 0.07$).

4. Discussion

Renal injury provoked by an indirect trauma has been reported in previous cases with no symptoms other than lumbar pain but with radiological findings such as subcapsular renal hematoma [14]. Some evidence suggests that urinary trauma could be present in non-contact sports such as off-road running [4,5,28]. It has been hypothesized that kidney mechanical trauma could mediate in the development of acute kidney injury after running [9]. This could be due to the kidneys' relative mobility during some actions as a downhill run at high speeds, change of directions, falls, and other high g-forces that could affect kidney movements and shaking. This relationship needs to be explored in future studies. The results of this study suggest that the 4th PC and 3rd PC of impact-related variables explained the Δ% of sCr and sALB between 23 to 24%. These findings indicate that the magnitude and number of impacts (g-forces) could have a potential role in the cumulative mechanical kidney trauma.

Despite kidneys being well protected by abdominal and back muscles, ribs, fat, renal pedicle, and ureteropelvic junction and supporting Gerota fascia in the retroperitoneum, they are also susceptible to internal movements [14,28]. Repeated sudden accelerations and decelerations may lead to renal contusions caused by the collision of kidneys in its surrounding tissues and structures like spine and ribs. These actions could lead to renal vasculatures affections, nephron damage, consequent hematuria, and other blood markers findings [29–31]. These accelerations and deceleration could be assessed using the variable impacts as proposed in this study. The impacts between 5–7 g explained

the pre-post increase of sALB and the impacts of 1–2 g and 14–15 g explained the rise in sCr. Based on the literature [9], these results may suggest that both the volume and intensity of the impacts involved during renal contusions play a special role in acute kidney injury. It has been found that the Δ% of blood markers as serum creatine kinase and sCr could predict the external workload of wearable devices placed in L1–L3 by 40% and 27%, respectively [9]. This evidence supports the idea of a new hypothesis of mechanical kidney injury during endurance off-road running based on L1–L3 external workload data [9].

The results of the present study showed that MARG sensors could be used to register the impacts and g-forces that affect the lower back, which is the kidney´s nearest external structure of the body. MARG sensors could register vertical, anterior-posterior, and mediolateral forces using the integration of accelerometer, gyroscope, and magnetometer data. The g-forces provoked by sudden accelerations and decelerations may affect the kidneys. The number and magnitude of these impacts could be monitored using MARGs attached to the kidney´s nearest external structure of the body, the lower back. Kidneys typically extend from T12 to L3 and weigh 135–150 g, so the MARG positioning should be at this level despite a slight position change due to the kidney's free mobility resulting from both body positions and respiration [32].

The link between the sensors' external load and kidney trauma must be confirmed and discussed in future interventions. Previously, considering the cause of the increase in sCr may be indicative of kidney injury as well as massive muscle damage [33]. Although elevations in sCr in 33% of participants by itself should not be understood as kidney damage due to physical exercise, the rise in sALB could suggest transitory functional loss due to tubular or glomerular damage. In fact, there is evidence to suggest that proteins released into the bloodstream in high amounts (e.g., rhabdomyolysis) can overload kidney function, resulting in functional or subclinical damage reflected in an increase of sCr and sALB, respectively [34,35].

The cumulative small injuries during rough exercises as off-road mountain running might damage the kidney, resulting in AKI. Although there is no clear evidence that cumulative or subsequent AKI events contribute to future renal chronic conditions in athletes [1,36], there is enough evidence to suggest that athletes, coaches, and sports scientists should be concerned with controlling the kidney health of runners, monitoring those variables that can trigger AKI, and thus, preventing potential cases of this transitory kidney condition. Some preventive strategies have been proposed to endurance athletes such as optimal fluid and food intake, appropriate physical loading, rest, and acceptable recovery between efforts [3]. Monitoring physical load is essential and those external and internal variables that could affect not only kidney health but also general well-being should be assessed. Dehydration seems to be a factor that did not influence the AKI occurrence in this specific sample, as found in the results.

MARG units as wearable devices containing accelerometers, gyroscopes, and magnetometers allow trainers, athletes, and medical staff to monitor and control the physical external and internal loads involved during the off-road running. The information obtained would allow us to provide feedback on the kinematic behavior of the runner in an objective manner [37] and would facilitate the programming and prescription of training loads, preventing and mitigating the impact of AKI on the runner's health and performance.

These findings must be seen in light of some limitations. Considering that the cause of acute kidney injury is multifactorial, future studies may confirm the contribution of mechanical kidney damage in the increase of blood markers related to AKI. A global analysis of heat strain, metabolic responses, and dehydration should be made to explore the role of kidney mechanical trauma on AKI. The link of impacts assessed in the periphery of the body and mechanical trauma of hard connective tissues must be confirmed in future studies.

Also, it must be explored how much does prolonged massive g-forces impact runners during rough running (e.g., downhill, off-road, mountain) and produce kidney damage compared to similar heavy muscular exercise, but without the massive g-forces. Consequently, it should be explored if

downhill running, sudden change of direction, falls, or other similar high magnitude actions produce greater damage than other running actions (e.g., uphill and flat running). Finally, there is a need to use other blood markers (e.g., Cystatin-C, NGAL, KIM-1) that allow researchers to differentiate AKI´s and extreme muscular exercise´s signs and symptoms. There is a need to review AKI's diagnosis criteria and its validity when applying it to sport sciences and medicine.

5. Conclusions

The results suggest that the magnitude and volume of running g-forces monitored with a MARG sensor attached to the lower back of off-road runners could predict the 24% change of serum creatinine and 23% change in serum albumin. These results must be confirmed in future research comparing similar heavy exercise with lower shock loads to the back and kidneys. Although these results may appear promising regarding the potential use of wearable devices to monitor cumulative mechanical kidney trauma in the future, greater understanding is required in the interaction of internal load (e.g., physiological responses) and external load (e.g., accelerations, impacts, decelerations) during prolonged exposure to vigorous repetitive exercise.

The results suggest that a decrease in the amount and magnitude of impacts throughout a session or between sessions can be a way to mitigate the possible collateral damage of acute kidney damage during off-road running. The foregoing considers, therefore, that the monitoring and control of training external and internal loads is essential for the prevention and recovery of AKI in off-road runners. In this sense, it is essential to provide constant feedback on running loads behavior and wearable MARG sensors could be used for these purposes.

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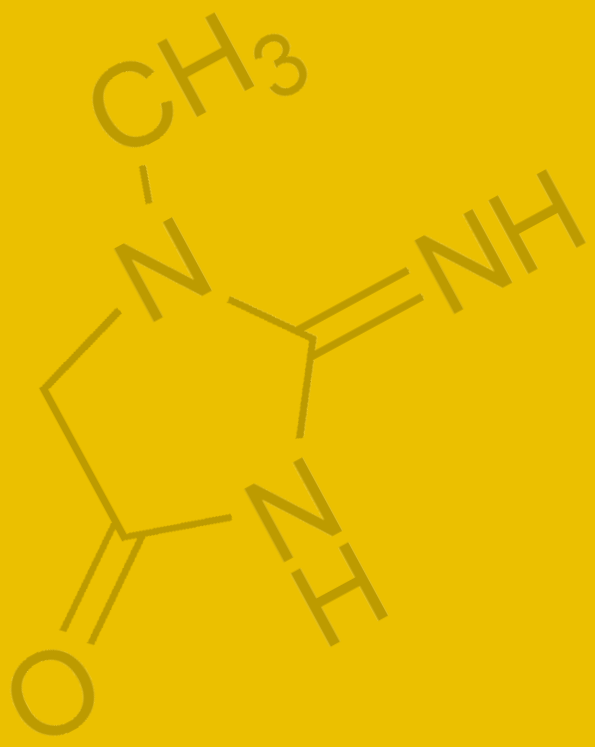

B. CONFERENCES PAPERS

February 4, 2020

Poster Presentation Notification

Please read all details carefully.

We are pleased to inform you that your abstract entitled "**Muscle Damage And Acute Kidney Injury In Endurance Mountain Running**" has been accepted for presentation in a **Free Communication/Poster** session at the 2020 Annual Meeting, World Congress on Exercise is Medicine®, and World Congress on the Basic Science of Exercise in Regenerative Medicine of the American College of Sports Medicine being held at the Moscone Center West and San Francisco Marriott Marquis in San Francisco, California, May 26-30, 2020. Your abstract will be published in *Medicine and Science in Sports and Exercise*, Volume 52:5 Supplement.

2020 Poster Session and Presentation Time Changes - please pay close attention to your poster session and presentation times as these are different from past years.

Your presentation date and time are as follows:

Author Block: Daniel Rojas-Valverde[1], Braulio Sanchez-Ureña[1], Guillermo Olcina[2], Jose Pino-Ortega[3], Randall Gutierrez-Vargas[1], Rafael Timón[4], Jeffrey M. Mjaanes, FACSM[5]. *[1]National University, Heredia, Costa Rica. [2]University of Extremadura, Cáceres, Spain. [3]University of Murcia, Murcia, Spain. [4]University of Extremadura, Caceres, Spain. [5]Northwestern University, Evanston, IL.* (Sponsor: Jeffrey M. Mjaanes, FACSM)
Session Title: Acute Exercise
Session Number: E-26
Session Viewing Date/Time: Friday May 29, 2020 9:30 AM - 12:00 PM
Presentation Time: 10:30am - 12:00pm

You will receive an additional email notification in April that will include your assigned poster board number and additional reminders.

Session Details
Attendees consider the poster sessions an important and valuable part of the educational program of the Annual Meeting, World Congress on Exercise is Medicine®, and World Congress on the Basic Science of Exercise in Regenerative Medicine. Therefore, the Program Committee has determined the following viewing times of the posters:

25TH ANNIVERSARY CONGRESS

OCTOBER 28-30, 2020

EUROPEAN COLLEGE OF SPORT SCIENCE

Aachener Str. 1053 -1055
50858 Cologne

GERMANY

VAT-ID: DE251715668 - St.Nr.: 223/5905/0216
register of associations: VR12508

Cologne, 08.09.2020 - 21:35:50

Letter of Acceptance

This is to certify that the following title has been accepted at the 25th Virtual Congress of the European College of Sport Science between 28 - 30 October 2020:

Daniel Rojas-Valverde

Universidad Nacional de Costa Rica
Ave6 Calles 10 y12, Heredia, Costa Rica
3000 Heredia, Costa Rica

Abstr.-ID: 2743
Title: Proteinuria and bilirubinuria as indicators of acute kidney injury in endurance mountain runners
Authors: Rojas-Valverde, D.,Olcina, G., Martínez-Guardado, I., Sánchez-Ureña, B., Timón, R., Institution: Universidad Nacional de Costa Rica; Universidad de Extremadura
Presentation format: Oral, YIA: No

European College of Sport Science

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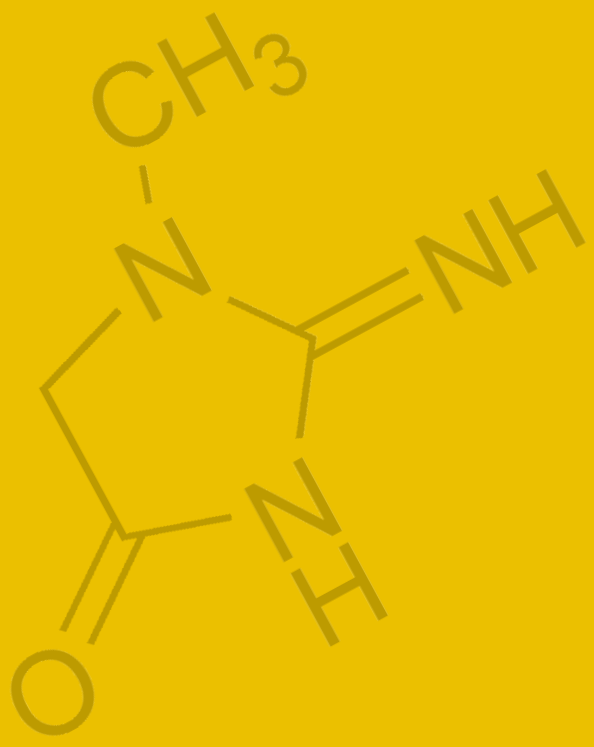

C. RESEARCH INTERNSHIPS AND TRAINEESHIPS

Deutsche
Sporthochschule Köln
German Sport University Cologne

Psychologisches Institut
Institute of Psychology

Deutsche Sporthochschule Köln · 50927 Köln

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Research Internship certificate - Daniel Rojas-Valverde

Köln, 15.04.2021

To whom it may concern,

Hereby I declare that Daniel Rojas-Valverde, personal ID No. 402050569, has made a research internship under the mobility project ¨Grants to Support the Initiation of International Collaboration¨, funded by the Deutsche Forschungsgemeinschaft and the Costa Rican National Council of University Rectors (CONARE).

The abovementioned research internship began on November 29th, 2019 and ended on December 30th, 2019. During the internship, as a research partner of the Institute of Psychology of the German University of Cologne, I provided the technical facilities and academic support that were required to develop the project goals.

Here are the tasks that Daniel Rojas-Valverde realized during his research internship:

a. Development, review, discussion and analysis of a research protocol to be submitted to the Institutional Review Board of the Universidad Nacional under the title: Cortical and autonomic effects of exposure in natural and virtual environments at rest and exercise.

b. Development of at least four individual discussion workshops with Dr. Sylvain Laborde to review the protocol abovementioned.

c. Group analysis session with the staff of the Institute of Psychology regarding the presentation of the study design and

specific methodology considerations for the execution of the joint investigation.

d. Coordination to continue with the GSU-UNA student mobility project with Dr. Gerard King.

Kind Regards

Dr. Sylvain Laborde

ESCOLA DE CIÊNCIAS E TECNOLO
DEPARTAMENTO DE DESPORTO E S

Évora, 17 January 2021

TRAINEESHIP CERTIFICATE

Name of the trainee: Daniel Rojas-Valverde
Personal ID nº: 402050569

Hereby we declare that the above-mentioned person has completed a traineeship within our organisation, *Departamento de Desporto e Saúde*, University of Évora, located in *Prolongamento da Rua de Reguengos de Monsaraz, 14, 7000, PORTUGAL.*

The traineeship was carried out between on 16/11/2020 and 16/01/2021 with an overall length of 2 months. The trainee has fulfilled the traineeship programme proposed for the host organisation by performing the following activities:

- Learning and handling of the BIODEX isokinetic dynamometer.
- Tensomiography seminar
- Seminar on thermography
- Bibliographic review on thermography
- Preparation and submission to the journal "XX" of the scientific article entitled "Short-term skin temperature responses to endurance exercises: a systematic review of methods and future challenges in the use of infrared thermography".

FOR THE HOST ORGANISATION

Name of the mentor: Nuno Miguel Prazeres Batalha

Position: Department Director

Signatura and Stamp:

¨Last but not least, I wanna thank me,
for believing in me, for doing all this hard work,
for having no days off, for never quitting,
for always being a giver, and tryna give more than I receive,
for tryna do more right than wrong ¨

Snoop Dogg