

TESIS DOCTORAL



ESCUELA INTERNACIONAL DE DOCTORADO

Programa de Doctorado Ciencias de la Salud

Efectos de la suplementación con nitrato y citrulina malato en jugadoras de fútbol profesional: estudio experimental y análisis complementario del perfil antropométrico, composición corporal, rendimiento y ciclo menstrual

Autora:

Marta Ramírez Munera

Directores:

Dr. D. Javier Marhuenda Hernández

Dr. D. Raúl Arcusa Saura

Murcia, septiembre de 2025

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La presente tesis doctoral adopta el formato de compendio e incluye cuatro artículos, tres de ellos ya publicados en revistas científicas con revisión por pares y un cuarto pendiente de aceptación. Las referencias de dichos artículos se detallan a continuación:

ARTÍCULO 1

Ramírez-Munera M, Arcusa R, López-Román FJ, Victoria-Montesinos D, García-Muñoz AM, Ávila-Gandía V, Pérez-Piñero S, Marhuenda J. Anthropometric and Body Composition Changes during Pre-Season of Spanish Professional Female Soccer Players According to Playing Position. *Nutrients*. 2024 Aug 22;16(16):2799. doi: 10.3390/nu16162799.

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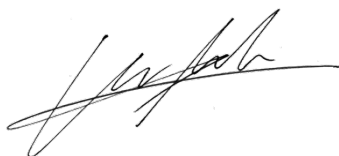
Los resultados de esta tesis doctoral también han sido presentados como comunicaciones orales en eventos científicos nacionales e internacionales:

- Marta Ramírez-Munera; Raúl Arcusa; Francisco Javier López-Román; Vicente Ávila-Gandía; Silvia Pérez-Piñero; Javier Marhuenda. *Cambios antropométricos y de composición corporal durante la pretemporada según la posición de juego en jugadoras españolas de fútbol profesional*. X Jornadas de Investigación y Doctorado “Bienestar Psicológico en los Estudios de Doctorado: repercusión en la Calidad de los Programas”, 2024, Murcia, España.
- Marta Ramírez-Munera; Raúl Arcusa; Francisco Javier López-Román; Desirée Victoria-Montesinos; Ana María García-Muñoz; Vicente Ávila-Gandía; Silvia Pérez-Piñero; Javier Marhuenda. *Relación entre el perfil antropométrico, la composición corporal y el rendimiento físico en jugadoras españolas de fútbol profesional al inicio de la pretemporada*. International Congress “Women-UP Mujeres en el Deporte”, 2024, Murcia, España.

AUTORIZACIÓN DEL DIRECTOR DE LA TESIS PARA SU PRESENTACIÓN

El Dr. D. Javier Marhuenda Hernández y el Dr. D. Raúl Arcusa Saura como Directores (1) de la Tesis Doctoral titulada “Efectos de la suplementación con nitrato y citrulina malato en jugadoras de fútbol profesional: estudio experimental y análisis complementario del perfil antropométrico, composición corporal, rendimiento y ciclo menstrual” realizada por D^a. Marta Ramírez Munera en el Programa de Doctorado en Ciencias de la Salud, autoriza su presentación a trámite dado que reúne las condiciones necesarias para su defensa.

Lo que firmo, para dar cumplimiento al Real Decreto 99/2011 de 28 de enero, en Murcia a 08 de septiembre de 2025.



Dr. Javier Marhuenda Hernández



Dr. Raúl Arcusa Saura

⁽¹⁾ Si la Tesis está dirigida por más de un Director tienen que constar y firmar ambos.

RESUMEN

El fútbol femenino profesional atraviesa una etapa de expansión sin precedentes que ha incrementado las exigencias competitivas y la necesidad de generar conocimiento científico específico. La pretemporada constituye un momento estratégico dentro del calendario, ya que concentra las cargas más elevadas de entrenamiento y marca el punto de partida del rendimiento. No obstante, la evidencia disponible sobre esta fase en futbolistas profesionales es todavía muy limitada, lo que dificulta la aplicación de estrategias basadas en datos.

El objetivo general de esta tesis doctoral fue investigar cómo optimizar la preparación y recuperación de jugadoras de fútbol profesional durante la pretemporada, integrando la composición corporal, el rendimiento físico, la suplementación con precursores de óxido nítrico (NO) y el ciclo menstrual. Para ello, se desarrolló una investigación en futbolistas profesionales, estructurada en un compendio de cuatro artículos que abordan dimensiones complementarias con el objetivo de optimizar la pretemporada.

Los resultados obtenidos mostraron que la composición corporal de las futbolistas tiende a mejorar durante la pretemporada, aunque con diferencias en función de la posición de juego. Además, se identificaron asociaciones claras entre el perfil corporal y los resultados en pruebas de fuerza explosiva, potencia anaeróbica y capacidad aeróbica, confirmando la relevancia de la masa muscular en el desempeño físico y el efecto negativo de la masa grasa sobre estas capacidades. En paralelo, la suplementación crónica con nitrato (NO_3^-) y citrulina malato (CM) durante cuatro semanas se asoció con una evolución más favorable de la carga externa en pretemporada, reflejada en una mayor velocidad máxima ($V_{\text{máx}}$) en entrenamientos y partidos y en una menor pérdida de rendimiento el día posterior a un partido, aportando evidencia novedosa sobre el potencial de esta estrategia nutricional en el fútbol femenino. Por último, se observaron variaciones en la carga externa de los entrenamientos en función de las fases del ciclo menstrual estimadas mediante una aplicación móvil, lo que sugiere la influencia de ritmos fisiológicos propios de la mujer sobre la respuesta al entrenamiento y refuerza la necesidad de integrar la salud menstrual en la planificación.

En conjunto, esta tesis doctoral aporta una visión multidimensional sobre la pretemporada en fútbol femenino profesional, destacando la importancia de un seguimiento individualizado que contemple la evolución de la composición corporal, el perfil posicional, la suplementación con precursores de NO y el estado menstrual. Estos hallazgos amplían la escasa literatura existente y ofrecen aplicaciones prácticas para optimizar la preparación, la recuperación y el rendimiento de las futbolistas profesionales en un contexto caracterizado por crecientes exigencias competitivas.

PALABRAS CLAVE

fútbol femenino; pretemporada; composición corporal; antropometría; rendimiento; precursor de óxido nítrico; ciclo menstrual

ABSTRACT

Professional women's soccer is undergoing unprecedented growth, which has raised competitive demands and highlighted the need for sport-specific scientific knowledge. Pre-season represents a decisive stage in the calendar, as it concentrates the highest training loads and sets the baseline for subsequent performance. Despite its relevance, research on this phase in professional female players remains scarce, limiting the application of evidence-based strategies.

The overall aim of this doctoral thesis was to investigate how to optimize preparation and recovery during pre-season in professional female soccer players, considering body composition, physical performance, nutritional supplementation with nitric oxide (NO) precursors, and the menstrual cycle. The research was structured as a compendium of four articles, each addressing a complementary dimension of this common objective.

Findings showed that players' body composition generally improved across the pre-season, although changes differed by playing position. Clear associations were also identified between body profile and outcomes in explosive strength, anaerobic power, and aerobic capacity, underscoring the positive role of muscle mass and the detrimental effect of fat mass on these physical qualities. In addition, four weeks of chronic supplementation with nitrate (NO_3^-) and citrulline malate (CM) were linked to a more favorable progression of external load, including higher maximum velocity (V_{max}) in both training and matches, together with a smaller decline in performance the day after a competitive game. This represents novel evidence on the potential of this nutritional strategy in women's soccer. Finally, variations in external load were observed across menstrual cycle phases estimated through a mobile application, suggesting the influence of female-specific physiological rhythms on training responses and reinforcing the importance of incorporating menstrual health into performance monitoring.

Taken together, this thesis provides a multidimensional perspective on pre-season in professional women's soccer. It highlights the value of individualized

monitoring that integrates changes in body composition, positional demands, nutritional strategies, and menstrual status. These findings expand the limited body of literature available and offer practical applications for optimizing preparation, recovery, and performance in professional female players within an increasingly demanding competitive environment.

KEYWORDS

female soccer; pre-season; body composition; anthropometry; performance; nitric oxide booster; menstrual cycle

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"If you truly pour your heart into what you believe in, even if it makes you vulnerable, amazing things can and will happen". Emma Watson.

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SIGLAS Y ABREVIATURAS

ATP,	Adenosín trifosfato
BIA,	Bioimpedancia
CM,	Citrulina malato
CMJ,	<i>Countermovement Jump</i>
CMO,	Contenido mineral óseo
DXA,	Absorciometría dual de rayos X
FIFA,	<i>Fédération Internationale de Football Association</i>
GPS,	Sistema de posicionamiento global
HIR,	Carrera de alta intensidad
ISAK,	<i>International Society for the Advancement of Kinanthropometry</i>
MIR,	Carrera de máxima intensidad
NO,	Óxido nítrico
NO₂⁻,	Nitrito
NO₃⁻,	Nitrato
NOS,	Enzima óxido nítrico sintasa
RSA,	Capacidad de repetir <i>sprint</i>
TD,	Distancia total
V_{máx},	Velocidad máxima
VO₂ máx,	Consumo máximo de oxígeno
WAnT,	<i>Wingate Anaerobic test</i>
Yo-Yo IR1,	<i>Yo-Yo Intermittent Recovery (Level 1) Test</i>

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ÍNDICE DE FIGURAS

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I – INTRODUCCIÓN

I - INTRODUCCIÓN

1.1. EVOLUCIÓN DEL FÚTBOL FEMENINO

En la última década, el fútbol femenino ha experimentado un crecimiento sostenido a nivel global, con avances significativos en participación, visibilidad y profesionalización. Según el informe anual de la *Fédération Internationale de Football Association* (FIFA) de 2024 (1), más de 30 millones de mujeres practican fútbol en todo el mundo, y el Mundial de 2023, celebrado en Australia y Nueva Zelanda, registró cifras sin precedentes, con casi dos millones de asistentes en los estadios y más de 2.000 millones de telespectadores acumulados. Este impulso internacional ha favorecido la implementación de estructuras estables en numerosas federaciones y una inversión creciente en el desarrollo del fútbol practicado por mujeres.

En España, esta evolución se ha materializado en varios hitos recientes. La aprobación oficial de la profesionalización del fútbol femenino por parte del Consejo Superior de Deportes en 2021 (2) permitió el nacimiento de la Liga F como competición profesional a partir de la temporada 2022/23, marcando un punto de inflexión en su consolidación institucional. Este avance ha ido acompañado de un crecimiento significativo en la participación: durante la temporada 2023/24 se superaron por primera vez las 100.000 licencias federativas (3), lo que representa un incremento del 23 % respecto al año anterior. A ello se suman los éxitos deportivos recientes, como el título mundial de la selección española en 2023, la conquista de la *UEFA Women's Nations League* en 2024 y el subcampeonato europeo alcanzado en 2025, que han reforzado su visibilidad mediática y el creciente interés social por el fútbol femenino en España.

Este proceso de expansión también ha tenido repercusión en el ámbito académico, con un aumento progresivo en la producción científica centrada en el fútbol femenino. Sin embargo, desde el año 2000, solo el 20 % de los estudios publicados sobre fútbol se han enfocado en la modalidad femenina (4), lo que evidencia el claro predominio de investigaciones centradas en el fútbol masculino. Además, aproximadamente la mitad de esos trabajos se han concentrado en los últimos cuatro años (5), lo que refleja una evolución aún incipiente. En cuanto al

enfoque metodológico, la mayoría de las publicaciones disponibles son de carácter descriptivo, con escasa presencia de diseños experimentales (6). En este contexto, la limitada disponibilidad de datos específicos y de estudios aplicados en entornos profesionales sigue siendo uno de los principales retos para avanzar en el conocimiento científico del fútbol femenino.

1.2. CARACTERÍSTICAS Y EXIGENCIAS DEL FÚTBOL

1.2.1. Aspectos generales

El fútbol es un deporte colectivo en el que se enfrentan dos equipos con once jugadores cada uno. En cuanto a las posiciones se distingue a diez jugadores de campo que actúan principalmente con las extremidades inferiores para controlar y desplazar el balón, pudiendo utilizar también otras partes del cuerpo salvo manos y brazos, y un portero que puede usar las manos dentro del área y viste un color distinto al de sus compañeros. A su vez, los jugadores de campo se diferencian en distintas posiciones, cada una asociada a un rol específico entre las que se encuentran defensas, centrocampistas y delanteros. El objetivo final de este deporte es marcar mayor número de goles que el adversario, y para ello se debe introducir el balón en la portería contraria (7). A diferencia de otros deportes que cuentan con descansos o tiempos muertos, cada encuentro se disputa en dos tiempos de 45 minutos, separados por un descanso de 15 minutos (8). No obstante, la constante evolución del mundo del deporte ha dado lugar a pequeños cambios en las reglas del fútbol. Entre dichos cambios destacan, la incorporación de un minuto de hidratación en cada tiempo de 45 minutos, así como la regla de los cinco cambios. En cuanto al minuto de hidratación, se utilizó por primera vez durante la Copa Mundial de la FIFA de 2014 en Brasil. Esta medida se implementó debido a las altas temperaturas y la humedad, con el objetivo de garantizar la seguridad y el bienestar de los jugadores (9, 10). Respecto a la regla de los cinco cambios, fue introducida por la FIFA en 2020 como medida temporal en respuesta a la pandemia de COVID-19. Esta regla permite a los equipos realizar hasta cinco sustituciones durante un partido, en lugar de las tres habituales. Afortunadamente para la salud de los futbolistas, estas medidas temporales se han mantenido (11-14).

La duración total puede ampliarse con tiempo añadido o una prórroga de dos tiempos de 15 minutos, según lo requiera el desarrollo del juego. En cuanto al terreno de juego, este debe tener forma rectangular y puede presentar distintas dimensiones dentro de un margen reglamentario: entre 90 y 120 metros de largo y entre 45 y 90 metros de ancho. En competiciones internacionales, estos valores se ajustan a un intervalo más reducido, con longitudes entre 100 y 110 metros y anchuras entre 64 y 75 metros, con el fin de garantizar una mayor uniformidad entre sedes (7).

Es importante remarcar que el fútbol, a diferencia de otros deportes, posee las mismas reglas para mujeres y hombres. De hecho, el balonmano, el hockey sobre hielo, el voleibol y el baloncesto tienen normas y reglamentos diferentes para los dos sexos, como el uso de pelotas de menor tamaño/peso u otras adaptaciones de las normas. Por lo que, en el fútbol, las jugadoras tienen que hacer frente a exigencias relativas mucho más exigentes que los hombres (15).

1.2.2. Bases fisiológicas del rendimiento

El músculo esquelético obtiene energía a partir de dos vías principales: el metabolismo oxidativo en las mitocondrias (metabolismo aeróbico) y la fosforilación a nivel de sustrato en el sarcoplasma (metabolismo anaeróbico). Ambos procesos generan adenosín trifosfato (ATP), principal fuente de energía para la contracción muscular. El metabolismo aeróbico requiere oxígeno y se caracteriza por una elevada capacidad para sostener el esfuerzo siempre que exista disponibilidad de sustratos. En cambio, el metabolismo anaeróbico no depende del oxígeno y permite una resíntesis de ATP mucho más rápida, aunque limitada en el tiempo. La intensidad del ejercicio determina la contribución relativa de cada sistema, de modo que a intensidades bajas o moderadas predomina el metabolismo aeróbico, mientras que a intensidades muy elevadas la mayor parte del ATP procede de las vías anaeróbicas (16-18) .

Como ocurre en otros deportes de equipo, el fútbol se caracteriza por un patrón intermitente que combina esfuerzos repetidos de alta intensidad con fases de baja intensidad (19, 20). Desde el punto de vista fisiológico, esto implica una participación simultánea de los sistemas energéticos aeróbico y anaeróbico.

La evidencia señala que el metabolismo aeróbico representa la principal fuente de energía en este deporte (19), especialmente por la duración total de los encuentros y el elevado grado de activación del sistema cardiorrespiratorio durante el juego. De hecho, las jugadoras pueden llegar a recorrer entre 9 a 11 km por partido (21-26), con una frecuencia cardiaca media que se sitúa entre el 84% y el 89% de la frecuencia cardiaca máxima (23, 27, 28).

Sin embargo, muchas de las acciones más determinantes en un partido son los esfuerzos de muy alta intensidad, como *sprints*, saltos o cambios de dirección, dependientes del metabolismo anaeróbico (19, 29). Siendo la capacidad de repetir *sprint* (RSA) una de las cualidades físicas más importantes en el fútbol (30-32). En un partido, entre un 22 % y un 28 % de la distancia total recorrida (TD) corresponde a carreras de muy alta intensidad y *sprints* (24). Además, se han llegado a registrar más de 1300 cambios en los patrones de actividad, así como más de 400 aceleraciones y desaceleraciones (33), lo que evidencia la elevada carga neuromuscular asociada a este tipo de esfuerzos repetidos.

Así, aunque el fútbol es una actividad predominantemente aeróbica, la alternancia constante de esfuerzos intensos y breves exige también un elevado aporte energético procedente de la vía anaeróbica. En este contexto la capacidad de recuperación frente a esfuerzos repetidos de alta intensidad está estrechamente ligada al desarrollo de la capacidad aeróbica (34, 35). Esta se define como la capacidad para sostener una determinada intensidad de trabajo durante un tiempo prolongado (36) y se evalúa habitualmente mediante pruebas como el *Yo-Yo Intermittent Recovery (Level 1) Test* (Yo-Yo IR1) (37).

De manera complementaria, la eficacia en las acciones explosivas depende de la capacidad anaeróbica del sistema neuromuscular, especialmente de la fuerza explosiva y la potencia anaeróbica de las extremidades inferiores (38). Estas variables se evalúan habitualmente mediante pruebas como el *Countermovement Jump* (CMJ) (39-41) y el *Wingate Anaerobic test* (WAnT) (42).

1.2.3. Demandas de entrenamiento y partido

La monitorización de las futbolistas resulta cada vez más relevante para comprender las respuestas individuales a la carga de entrenamiento y competición, con el fin de optimizar el rendimiento físico y reducir el riesgo de lesiones (43, 44).

En la actualidad, la mayoría de los equipos profesionales emplean diferentes tecnologías para controlar la carga externa, siendo el sistema de posicionamiento global (GPS) una de las más utilizadas (45-47). Estos dispositivos permiten cuantificar variables como la velocidad máxima ($V_{\text{máx}}$), la TD, la distancia cubierta a diferentes velocidades alcanzadas durante entrenamientos y partidos (48, 49).

En el caso de los entrenamientos la literatura en fútbol femenino sigue siendo limitada (50), la revisión sistemática de Costa et al. (51) señala que se han llegado a registrar TD en torno a 3.000–6.500 m por sesión, de las cuales una fracción corresponde a carreras de alta velocidad (≈ 200 –1.500 m). Aunque estos valores muestran una elevada variabilidad entre estudios y dependen de la organización del microciclo reflejan que las sesiones reproducen parcialmente las demandas competitivas. En conjunto, la principal función del entrenamiento es preparar a las jugadoras para las exigencias del partido, favoreciendo las adaptaciones fisiológicas necesarias (52). En cuanto a las demandas de partido, la revisión sistemática de Rodríguez-Porras et al. señala que las jugadoras pueden llegar a recorrer entre 8.800 y 10.800 m por encuentro, con un rango que puede variar entre 7.800 y 11.200 m. De esa distancia, aproximadamente 900–1.500 m corresponden a carreras por encima de 16 km/h y unos 300–400 m a esfuerzos superiores a 20 km/h. En conjunto, estos valores ponen de manifiesto la elevada exigencia física de la competición en fútbol femenino.

En conjunto, los datos disponibles muestran que tanto los entrenamientos como los partidos implican una elevada exigencia física para las futbolistas. Ello pone de relieve la importancia de seguir profundizando en los factores que condicionan el rendimiento en el fútbol femenino, con el fin de optimizar la preparación y responder a las demandas específicas de este deporte.

1.3. IMPORTANCIA DE LA PRETEMPORADA

La pretemporada en el fútbol es el período preparatorio que precede al inicio de la competición oficial y tiene lugar tras la pausa de final de temporada. Su principal objetivo es generar adaptaciones tanto a nivel individual como en el funcionamiento del equipo, con el fin de afrontar en condiciones óptimas el comienzo de la temporada competitiva (53). Su duración varía en función de las

exigencias del calendario de cada equipo, aunque habitualmente se extiende entre cuatro y seis semanas (54).

Al comenzar la pretemporada, las jugadoras presentan diferentes niveles de condición física. La etapa previa, correspondiente al *off-season*, suele estar marcada por una reducción o incluso ausencia de estímulo de entrenamiento, así como por una mayor flexibilidad en los hábitos nutricionales, lo que puede traducirse en cambios desfavorables en la aptitud física y composición corporal (55). Conscientes de estos efectos, cada vez más jugadoras optan por mantener cierto nivel de actividad durante este periodo mediante entrenamientos voluntarios o programas individuales no estructurados (56). Aun así, no existe un patrón único: la forma en que cada jugadora gestiona el *off-season* (en cuanto a entrenamiento, descanso o alimentación) da lugar a un punto de partida muy variable al inicio de la pretemporada. Esta heterogeneidad refuerza la necesidad de realizar evaluaciones individuales, no solo para ajustar la carga de entrenamiento, sino también para orientar las decisiones nutricionales, médicas y de planificación general del equipo.

La pretemporada representa además uno de los periodos de mayor exigencia dentro del calendario competitivo del fútbol profesional (57). Durante estas semanas se aplican habitualmente las cargas de entrenamiento más elevadas del año, combinando un volumen alto de trabajo físico con un aumento progresivo de la intensidad (58, 59). Esta exigencia se ve incrementada por factores contextuales cada vez más frecuentes, como la participación en giras promocionales. En muchos casos estas giras concentran varios partidos en pocos días y requieren desplazamientos, a menudo demasiado cerca del inicio oficial de la temporada (53).

Precisamente por su intensidad, su brevedad y su impacto funcional, la pretemporada constituye un momento estratégico para estudiar factores que pueden influir en el rendimiento y explorar posibles acciones orientadas a optimizar la recuperación y la preparación física de las jugadoras.

1.4. ANTROPOMETRÍA Y COMPOSICIÓN CORPORAL

La definición de antropometría deriva de las raíces *anthropos* (especie humana) y *metrein* (medir), definiéndose así como una rama de ciencias que se dedica al estudio del cuerpo humano (60). Los estudios antropométricos se realizan a partir de distintas medidas y dimensiones corporales tomadas de forma

estandarizada y siguiendo un protocolo definido. Estos valores se aplican en ecuaciones específicas que permiten estimar la composición corporal (masa grasa y masa musculoesquelética) así como el somatotipo (61). Todo en relación con el rendimiento deportivo, la nutrición y el crecimiento (62). Este procedimiento suele realizarse siguiendo el protocolo de la *International Society for the Advancement of Kinanthropometry* (ISAK) (61), lo que aporta el grado más alto de precisión y fiabilidad a los datos.

En el fútbol profesional, estas evaluaciones se utilizan junto con pruebas de condición física para valorar el estado de preparación competitiva. Además, permiten monitorizar cómo los cambios en el entrenamiento y la dieta afectan a la composición corporal (63), con el fin de optimizarla y favorecer una mejora del rendimiento a lo largo de la temporada. A pesar de la importancia que se otorga a la evaluación y optimización de la composición corporal en el deporte de élite, no existe un método de referencia universalmente aceptado. En la práctica, la elección de la técnica suele depender de factores como la disponibilidad de recursos o la facilidad de aplicación (64).

Las características antropométricas desempeñan un papel relevante en función de la posición específica que ocupa la jugadora en el terreno de juego (65). La magnitud y el tipo de esfuerzo requerido no es uniforme entre todas las jugadoras, sino que depende en gran medida de la posición específica, que condiciona tanto el patrón de movimiento como las exigencias físicas asociadas. En un equipo de fútbol se pueden distinguir diferentes posiciones, como delanteras, centrocampistas, defensas y porteras. Por ejemplo, las delanteras y las extremos (un tipo de defensa que actúa por los flancos y realiza numerosos *sprints* a lo largo del partido) suelen llevar a cabo esfuerzos intensos de velocidad y agilidad, lo que exige potencia explosiva y rápida aceleración para superar a la defensa rival. Las centrocampistas, por su parte, recorren habitualmente grandes distancias durante un partido, lo que demanda una elevada resistencia física para contribuir eficazmente tanto en defensa como en ataque. Las defensas combinan fuerza, agilidad y visión de juego para neutralizar los ataques contrarios e iniciar la construcción ofensiva. Por último, las porteras requieren agilidad, reflejos y capacidad de impulso para realizar paradas decisivas (66). Las particularidades de cada posición pueden originar diferencias en la composición corporal y en las

características físicas de los jugadoras, derivadas de los patrones de movimiento y de las exigencias de rendimiento propias de su función en el campo (67).

Debido a estas diferencias, resulta especialmente útil identificar qué variables antropométricas o de composición corporal deberían priorizarse en función de las capacidades físicas y exigencias específicas de cada posición. Disponer de información precisa sobre cómo estas variables se relacionan con componentes del rendimiento, como la fuerza explosiva, la potencia anaeróbica o la capacidad de recuperación, puede aportar un valor aplicado clave para orientar los entrenamientos y las estrategias nutricionales según el perfil y las demandas de cada jugadora. No obstante, los estudios que han explorado estas asociaciones en jugadoras profesionales siguen siendo escasos (68-70).

1.5. CICLO MENSTRUAL

La monitorización del estado de las jugadoras es un elemento clave a la hora de planificar y evaluar el rendimiento en el fútbol profesional (71). Este seguimiento debe tener en cuenta también ciertos ritmos fisiológicos propios de las mujeres, como el ciclo menstrual (72). Este proceso está regulado por el eje hipotálamo-hipófisis-ovario (73) y se caracteriza por variaciones cíclicas en la secreción de hormonas sexuales, principalmente estrógenos y progesterona (74). La literatura científica ha descrito cómo estas hormonas pueden influir en funciones cardiovasculares, en el uso de los sustratos energéticos y en la termorregulación (75-77).

En mujeres con ciclos menstruales regulares, conocidas como eumenorreicas, la duración habitual del ciclo varía entre 21 y 35 días, situándose la media en torno a los 28 días (78). De forma general, este proceso se divide en dos grandes fases: la fase folicular (días 1 a 14), en la que los niveles de estrógenos son elevados y los de progesterona bajos, y la fase lútea (días 15 a 28), donde se invierte este patrón hormonal (77, 79, 80). Aunque existen modelos más específicos para dividir el ciclo en fases (81, 82), actualmente no hay un acuerdo unificado en el ámbito de la ciencia del ejercicio y el deporte sobre la terminología ni sobre los criterios hormonales que deberían emplearse para dicha clasificación (79).

En los últimos años, el interés por comprender la influencia del ciclo menstrual en el rendimiento deportivo ha aumentado de forma considerable. A

pesar de los avances metodológicos en investigación experimental, su aplicación práctica en deportes de equipo sigue presentando importantes limitaciones (83). En este contexto, el uso de herramientas digitales como las aplicaciones de seguimiento se ha consolidado como una alternativa accesible y no invasiva para monitorizar el estado menstrual en entornos reales (83-85). Si bien estas estimaciones no permiten una clasificación hormonal precisa, su integración en el seguimiento de las jugadoras puede aportar información valiosa para interpretar la respuesta al entrenamiento, gestionar la fatiga y apoyar un acompañamiento individualizado en el deporte de alto nivel.

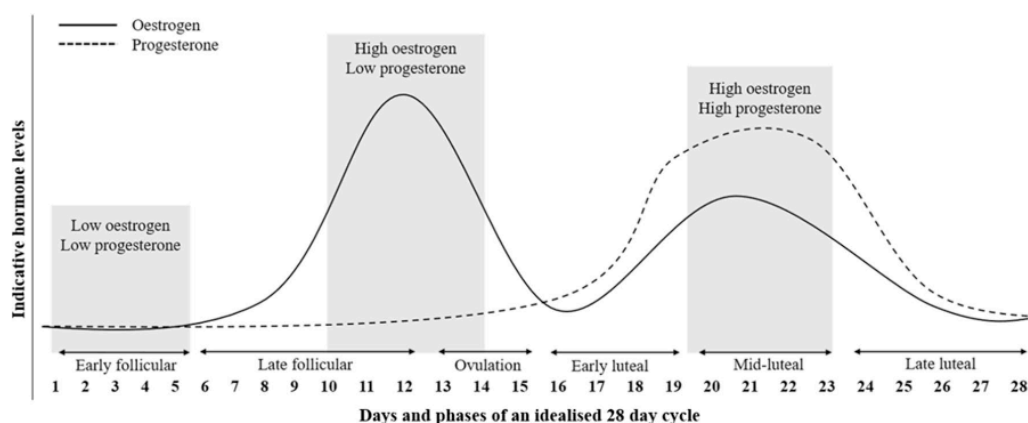


Figura 1. Esquema de las fluctuaciones hormonales a lo largo de un ciclo menstrual idealizado de 28 días, con la ovulación en el día 14. Extraída de McNulty y cols (81).

1.6. SUPLEMENTACIÓN CON PRECURSORES DE ÓXIDO NÍTRICO

En el deporte, tanto a nivel profesional como en otras categorías competitivas, es cada vez más habitual recurrir a suplementos dietéticos con potencial ergogénico como parte de la estrategia nutricional (86). Su uso se plantea como una herramienta complementaria para apoyar las adaptaciones al entrenamiento, mejorar la recuperación o facilitar la respuesta fisiológica ante esfuerzos exigentes (87). En contextos donde las diferencias entre deportistas son mínimas, este tipo de intervenciones puede marcar una diferencia práctica. Entre los suplementos que han ganado visibilidad en los últimos años se encuentran los precursores de óxido nítrico (NO), ampliamente disponibles en el mercado y con una creciente presencia en el ámbito de la suplementación deportiva (88).

El NO es una molécula señalizadora implicada en diversas funciones fisiológicas, como la vasodilatación, la respiración y biogénesis mitocondrial, la angiogénesis, la captación de glucosa en el músculo y la regulación del calcio en el retículo sarcoplasmático (89-91). Como consecuencia de estos efectos, el NO se ha asociado con una mayor eficiencia muscular (92, 93), una menor demanda de oxígeno durante el ejercicio y una mejor recuperación neuromuscular (94), lo que lo convierte en un potencial recurso para optimizar el rendimiento físico.

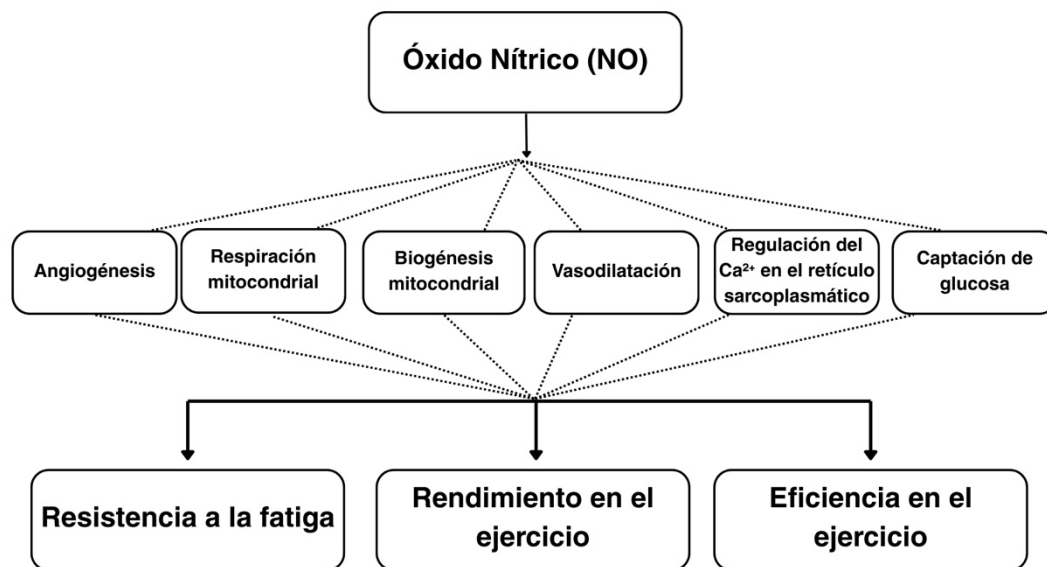


Figura 2. Resumen de los efectos atribuidos al NO que podrían contribuir a mejorar el rendimiento físico. Adaptado de Nyakayiru (95).

1.6.1. Vías de producción de óxido nítrico

El cuerpo humano genera NO a través de dos vías complementarias (96). La primera es la vía citrulina-arginina-NO, en la que el NO se sintetiza de forma endógena a partir de L-arginina y oxígeno mediante las enzimas óxido nítrico sintasa (NOS), produciendo L-citrulina como subproducto (97). Este proceso se conoce comúnmente como la vía dependiente de NOS. La segunda vía es parcialmente exógena, ya que depende de nitrato (NO_3^-) y nitrito (NO_2^-) obtenidos tanto por la oxidación endógena del NO (98) como por fuentes dietéticas ricas en NO_3^- (96), como las espinacas, la remolacha y la rúcula (99). Tras su ingestión, el

NO_3^- es absorbido en el tracto gastrointestinal superior y entra en la circulación sistémica (100). Aunque parte se elimina por vía urinaria (101), hasta un 25% es captado activamente por las glándulas salivales mediante el transportador sialina (102). Esto permite que su concentración en la saliva sea hasta 20 veces superior a la plasmática (103). Una vez en la cavidad bucal, una parte del NO_3^- salival es reducido a NO_2^- por bacterias anaerobias facultativas, ubicadas principalmente en el dorso de la lengua (104-106). Posteriormente cuando se ingiere, una parte del NO_2^- se reduce a NO en el ambiente ácido del estómago (107), mientras que el resto entra en la circulación sistémica. Este NO_2^- se distribuye rápidamente en la sangre y otros tejidos, incluida la vasculatura periférica y el músculo esquelético (98), y puede ser reducido fácilmente a NO mediante la ganancia de un electrón (88). Esta reacción se ve potenciada en condiciones de hipoxia (108) y acidez (109), como las que se observan en el músculo esquelético durante la contracción (110). Esta ruta se conoce como la vía NO_3^- - NO_2^- -NO o vía independiente de NOS.

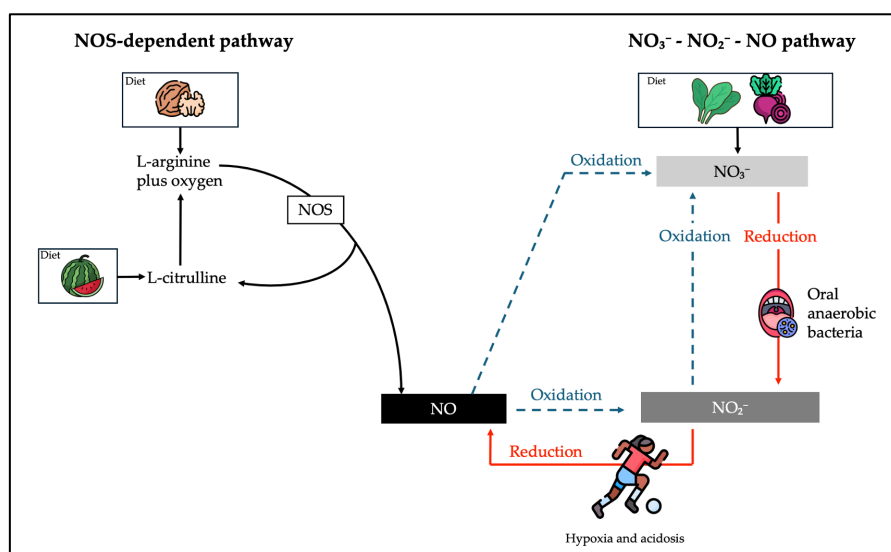


Figura 3. Vías de producción de NO. Adaptado de Jones y cols (96).

1.6.2. Citrulina

La L-citrulina es un aminoácido no esencial y no proteinogénico, presente de forma natural en alimentos como la sandía, considerada su fuente dietética principal (98). Sin embargo, la cantidad de este aminoácido que puede obtenerse a

través del consumo de sandía es muy limitada (111), ya que para alcanzar una dosis diaria de 3 gramos sería necesario ingerir entre 1 y 1,5 kilos de sandía fresca (112-114). Esto hace que la principal fuente de L-citrulina en el organismo, sea su síntesis endógena, que ocurre principalmente en los enterocitos del intestino delgado a partir de otros aminoácidos (115, 116) como la glutamina y la L-arginina (117, 118).

Una vez sintetizada, la L-citrulina se libera a través de la membrana basolateral hacia la circulación portal (116, 118, 119) y es transportada al riñón, donde se convierte en L-arginina. Este aminoácido es el principal precursor endógeno del NO (97). Sin embargo, la administración oral de L-arginina presenta una biodisponibilidad limitada, ya que una parte significativa es degradada por la arginasa hepática antes de alcanzar la circulación sistémica (120).

En este contexto, la suplementación con L-citrulina ha emergido como una alternativa más eficaz para aumentar los niveles plasmáticos de L-arginina (121). A diferencia de esta última, la L-citrulina no es metabolizada en el hígado, lo que le permite alcanzar la circulación sistémica y ser transportada directamente al riñón, donde es convertida en L-arginina (122), aumentando así la disponibilidad de sustrato para la síntesis del NO.

En el ámbito de la suplementación, la L-citrulina se administra habitualmente en forma de citrulina malato (CM) (123), una combinación de L-citrulina con ácido málico, un compuesto que participa como intermediario en el ciclo de Krebs (124, 125). Esta formulación no solo apoya la síntesis de NO, sino que podría favorecer la producción de ATP y facilitar la eliminación del amoníaco, especialmente en esfuerzos repetidos de alta intensidad (121, 126). Estos efectos adicionales resultan de especial interés en modalidades intermitentes como el fútbol, donde se alternan acciones explosivas con breves periodos de recuperación (29), y se requiere una activación constante de los sistemas energéticos aeróbico (38, 127) y anaeróbico (34, 35).

1.6.3. Nitrato

El NO_3^- es un compuesto químico inorgánico derivado del nitrógeno que se encuentran de forma natural en el medio ambiente (128). En particular se encuentra en altas concentraciones principalmente en verduras de hoja verde, como las espinacas y la rúcula, así como en hortalizas como la remolacha (129).

La suplementación con NO_3^- se ha asociado con mejoras en el rendimiento aeróbico, la resistencia muscular y la potencia en esfuerzos de alta intensidad (130). Se considera que estas mejoras están relacionadas con adaptaciones fisiológicas mediadas por el NO (89-93). No obstante, la falta de homogeneidad metodológica entre estudios, junto con la limitada representación de mujeres deportistas, pone de relieve la necesidad de continuar investigando en contextos deportivos aplicados (130-133).

Por otro lado, en los últimos años se ha propuesto que las fibras musculares tipo II (reclutadas principalmente en acciones explosivas y de alta intensidad (110)) podrían actuar como reservorios de NO_3^- , favoreciendo la producción localizada de NO en condiciones de hipoxia y acidosis, lo que contribuiría a la regulación del pH muscular a través de un efecto tampón intramuscular (134, 135).

Estas observaciones refuerzan la hipótesis de que el músculo esquelético podría funcionar como un reservorio fisiológico de NO_3^- , adaptando su contenido en función de la disponibilidad dietética. Estudios en modelos animales han mostrado que el agotamiento de NO_3^- reduce los depósitos intramusculares, mientras que su reintroducción permite una recuperación rápida e incluso una acumulación por encima de los niveles fisiológicos, lo que sugiere un posible mecanismo adaptativo que respaldaría el interés por estrategias de suplementación crónica (136).

En cuanto a las formas de administración, el zumo de remolacha ha sido la fuente de NO_3^- más investigada hasta la fecha (137). Sin embargo, en los últimos años han surgido otras opciones dietéticas como el amaranto, que han despertado interés como posibles alternativas, aunque los estudios sobre estas siguen siendo escasos (138-140).

1.6.4. Combinación de citrulina malato y nitrato

Teniendo en cuenta las distintas rutas implicadas en la síntesis de NO, se ha propuesto que la combinación de precursores como la CM y NO_3^- podría potenciar su biodisponibilidad (141). Hasta el momento, la literatura científica se ha centrado principalmente en analizar sus efectos de forma independiente (123, 125, 126, 130, 142, 143) o en la combinación de NO_3^- con L-citrulina (144-150), mientras que la evidencia sobre la suplementación conjunta de NO_3^- y CM es prácticamente nula.

II – JUSTIFICACIÓN

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El crecimiento y la progresiva profesionalización del fútbol femenino han incrementado de forma notable las exigencias competitivas. En este contexto, la pretemporada adquiere un papel decisivo, ya que concentra las cargas de entrenamiento más elevadas y marca el punto de partida de la temporada. Una preparación adecuada en estas semanas iniciales resulta esencial para que las jugadoras alcancen un nivel óptimo de rendimiento. No obstante, la evidencia científica disponible sobre este proceso en fútbol femenino es todavía escasa, particularmente en España, lo que limita la aplicación de estrategias fundamentadas en datos y resalta la necesidad de avanzar en la investigación en este ámbito.

El compendio de publicaciones que conforma esta tesis doctoral se articula en torno a un mismo eje temático: la optimización de la pretemporada en jugadoras de fútbol profesional. Cada artículo aborda una dimensión complementaria de este objetivo común. En primer lugar, se analizan las características antropométricas y de composición corporal y su evolución a lo largo de la pretemporada, lo que permite identificar patrones específicos según la posición de juego y orientar estrategias individualizadas de entrenamiento y nutrición. En segundo lugar, se examina la relación entre estas características y diversas pruebas de rendimiento físico el primer día de pretemporada, aportando información clave para comprender qué atributos resultan más determinantes en la preparación inicial de las jugadoras. En tercer lugar, se evalúa la suplementación con NO_3^- y CM en el contexto de la pretemporada como estrategia potencial para afrontar la elevada exigencia de entrenamientos y partidos, así como para favorecer la recuperación posterior. Este trabajo constituye el primer estudio que analiza la combinación de estos precursores de NO, aportando evidencia inicial que abre una línea de investigación novedosa en el campo de la nutrición aplicada al deporte de alto nivel. Finalmente, se explora la influencia del ciclo menstrual en la carga externa de entrenamiento a través de un sistema de monitorización no invasivo basado en una aplicación móvil, lo que permite obtener datos en un entorno real y aporta información práctica para el cuerpo técnico.

En conjunto, el estudio se desarrolla íntegramente en jugadoras profesionales y en condiciones de campo, empleando herramientas y pruebas de uso habitual en la práctica deportiva. La unidad temática de los artículos del compendio reside en su contribución conjunta a mejorar la comprensión científica de la pretemporada en fútbol femenino profesional, integrando la perspectiva antropométrica y de composición corporal, el rendimiento físico, la suplementación y el seguimiento del ciclo menstrual. Los resultados obtenidos amplían el conocimiento en un ámbito en el que la literatura es todavía muy escasa y ofrecen una base para orientar futuras aplicaciones en el deporte femenino de alto nivel.

III – OBJETIVOS

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3.1. OBJETIVO PRINCIPAL

- Analizar estrategias para optimizar el rendimiento y la recuperación de jugadoras profesionales de fútbol durante la pretemporada, integrando la composición corporal, el perfil posicional, la suplementación con precursores de NO y la influencia del ciclo menstrual.

3.2. OBJETIVOS ESPECÍFICOS

- Describir las características antropométricas y de composición corporal de las futbolistas profesionales y evaluar sus variaciones a lo largo de la pretemporada en función de la posición de juego.
- Determinar la relación entre el perfil antropométrico, la composición corporal y el rendimiento físico al inicio de la pretemporada.
- Comparar el rendimiento físico entre posiciones de juego, considerando las demandas específicas del fútbol femenino.
- Evaluar los efectos de la suplementación crónica con 500 mg de NO_3^- procedente de extracto de amaranto y 8 g de CM durante cuatro semanas sobre la carga externa y la recuperación tras la competición.
- Analizar las variaciones de la carga externa de entrenamiento en relación con las fases del ciclo menstrual estimadas mediante la aplicación FitrWoman.

IV – COMPENDIO DE ARTÍCULOS

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4.1. ARTÍCULO 1

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Article

Anthropometric and Body Composition Changes during Pre-Season of Spanish Professional Female Soccer Players According to Playing Position

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Abstract: Background: In professional soccer, body composition analysis is crucial to assess preparation and optimize performance. Different playing positions have different physical demands, which can lead to variations in body composition. However, there are few studies on women's soccer that consider the playing position. This study aims to fill that gap by examining position-specific differences in anthropometric and body composition characteristics among Spanish professional female soccer players at the beginning and end of pre-season. Furthermore, it investigates the possible changes during the pre-season period between positions and correlates the data obtained from anthropometric equations with bioimpedance (BIA) measurements. Methods: Thirty-four female soccer players: 8 midfielders, 12 defenders, 11 forwards, and 3 goalkeepers (age: 23.06 ± 4.29 years, height: 164.15 ± 5.84 cm, weight: 58.39 ± 6.62 kg, and $\sum 6$ skinfolds: 74.57 ± 18.48 mm) completed the study that lasted 4 weeks (pre-season) where they were measured anthropometrically and by bioimpedance twice. Results: Goalkeepers showed greater wingspan (176.60 ± 7.06 p < 0.05) compared to other positions. Regarding differences during pre-season, midfielders had the greatest decrease in $\sum 6$ skinfolds compared to other positions ($\Delta -12.10 \pm 5.69$ p < 0.05). There was a correlation of % fat between Faulkner's equation and BIA (Pearson's $r = 0.817$). Conclusions: It seems that there are no significant differences in terms of positions and body composition, except for the wingspan and ankle diameter. During pre-season, midfielders are the ones who improve their body composition the greatest. The anthropometric equation for body fat that shows the highest correlation with BIA is Faulkner's equation, followed by Durnin's equation.

Keywords: female soccer; anthropometry; bioimpedance; performance

1. Introduction

In recent years, there has been a significant surge in interest towards women's soccer [1]. According to data from the Fédération Internationale de Football Association (FIFA), the number of women's clubs in 2023 was around 55,600, 59% of which were located in Europe [2]. In Spain, the number of federation licenses for women's football in recent years has increased considerably, exceeding the figure of 87,000 in 2022, with a growth rate of 30.8% from 2021 to 2022 [3]. Moreover, research about women's soccer has also increased [4], although it still remains limited for female players compared to their male counterparts [5].

Anthropometric studies allow the estimation of body composition, the study of morphology, dimensions and proportionality in relation to sports performance and nutrition [6].

One aim of analyzing body composition is to identify and measure different body compartments. In professional soccer, these assessments are used alongside fitness tests to assess readiness for competition and to track how training and diet changes affect body composition [7], and in this way, optimize body composition in order to achieve an improvement in sporting performance throughout the season.

Different positions can be distinguished in a soccer team such as forwards, midfielders, defenders, and goalkeepers. For example, forwards and wingers (type of defender who plays on the flank and carries out numerous sprints while playing) often make intense bursts of speed and agility, requiring explosive power and rapid acceleration to penetrate opposing defenses. Midfielders, meanwhile, often cover extensive distances during a match, requiring exceptional stamina and endurance to contribute both defensively and offensively. Defenders focus on strength, agility, and spatial awareness to disrupt opposing attackers and initiate build-up play. Goalkeepers require agility, reflexes and explosive movement to make decisive saves [8]. These distinct roles may contribute to differences in players' body composition and physical attributes due to the specialized movements and performance expectations associated with their positions [9].

In Spain, there is limited research that provides some information on body composition or anthropometric values in women's soccer [10–18]. Most of the data are about body composition without absolute anthropometric data, or with anthropometric data limited to the measurement of skinfolds. In addition, there are only four studies that compare values between playing positions: one of them is performed on adolescent players [13], another one is performed on elite players (however, the absolute differences for elite players were not available as values were combined with non-elite players) [11], and finally, to our knowledge, there are only two research studies with body composition data of Spanish professional players comparing between playing positions, published in 2009 [10] and more recently in 2024 [18]. Therefore, there is a clear need for updated data of Spanish professional players by playing position. In the same way, at the international level, positional differences of elite female players have been assessed in a small number of studies [1].

The pre-season period in the Spanish Women's Football League lasts between 5 and 7 weeks, depending on each team's schedule. This period is seen as the final opportunity for coaches to enhance the technical, tactical, and physical capabilities, including body composition, of their teams before starting the competitive period [19], usually by systematically increasing the training load and providing individualized dietary regimes [20]. To the best of our knowledge, this research appears to be the first to analyze how anthropometric and body composition parameters change across different playing positions in female soccer players during pre-season. Previous studies have explored changes in these parameters throughout the season, but they typically focused on the entire team without considering positional differences [16,19,21,22].

Due to the lack of scientific literature on women's football and anthropometric data, the aim of the present research is to contribute to identify position-specific differences in the body composition characteristics of Spanish professional female football players at the beginning and end of pre-season and, as a secondary aim, to examine the correlation of different anthropometric equations with bioimpedance (BIA).

2. Materials and Methods

2.1. Study Design

A prospective study was carried out during pre-season with the aim of evaluating changes in anthropometric and BIA variables in professional female soccer players. All determinations were performed on all players at two different times: at the beginning and at the end of pre-season (4 weeks). The protocol was approved by the Institutional Review Committee of the Catholic University San Antonio of Murcia (UCAM) (code CE052204). This research was carried out following the Standards of Good Clinical Practice and was conducted according to the Declaration of Helsinki. The clinical trial was registered

at www.clinicaltrials.gov (identifier NCT05525871). The study was carried out at the Department of Exercise Physiology of the Universidad Católica de Murcia (UCAM). The current European legislation on the protection of personal data (Regulation (EU)2016/679) was complied with.

2.2. Participants

The study was carried out in collaboration with two soccer teams from the first and second Spanish Women's Football League. In total, 34 female players (age: 23.06 ± 4.29 years) were distributed according to the usual playing positions: 8 midfielders (age: 25.00 ± 3.46 years), 12 defenders (age: 23.08 ± 3.15 years), 11 forwards (age: 22.27 ± 5.64 years), and 3 goalkeepers (age: 20.67 ± 4.50 years).

The inclusion criteria were as follows: (a) be a healthy subject with medical authorization for the practice of a federated sport and (b) belong to a team of the first or second division of the Spanish Women's League. The exclusion criteria for the study were as follows: (a) changing teams during pre-season and (b) a history of drug, alcohol or other substance abuse, or other factors limiting their ability to cooperate during the study. All players were previously informed of the objectives and method of the research, signing informed consent forms before starting the research. In the case of those players who were minors, it was their parents who signed the consent.

2.3. Anthropometric Measurements and Bioimpedance Analysis

The determinations were measured as follows: four basic measurements (body mass, height, sitting height, and arm span), eight skinfolds (biceps, triceps, subscapular, suprailiac, supraspinal, abdominal, front thigh, and medial calf), six breadths (humerus, femur, biacromial, bi-styloid, bi-iliocristal, and bimalleolar), and six perimeters (arm relaxed, arm flexed and tensed, waist, hip, thigh, and calf).

Body mass measurement and BIA analysis were determined with Tanita BC 420 S MA Class III, tetra polar system and single frequency (50 kHz). Height was measured using a scale with a stadiometer (Seca, Hamburg, Germany) to the nearest 0.1 cm; a SECA 217 detachable portable stadiometer (SECA, Germany) to the nearest 0.1 cm and anthropometry box were used to measure sitting height; a CESCORF inextensible metal tape (CESCORF, Porto Alegre, Brazil) was used to measure perimeters and arm span; a Harpenden skinfold caliper to the nearest 0.1 mm was used to measure skinfolds; and large and small sliding calipers (CESCORF, Porto Alegre, Brazil) were used to measure breadths. All anthropometric measurements were measured two or three times by an anthropometrist who was level 2 accredited by International Society for the advancement of the Kinanthropometry (ISAK), following the procedures established by the ISAK [23].

Body composition was determined using the equations described in the consensus document of the Spanish Group of Kinanthropometry of the Spanish Federation of Sports Medicine [6], following the four-component model (muscle mass (MM), fat mass (FM), bone mass (BM), and residual mass (RM)). The following equations were used: the Withers [24], Slaughter [25], Carter [26], Faulkner [27], Durnin [28], and Jackson and Pollock [29] equations to calculate FM expressed in percentage (%) and kilograms (kg); the Poortmans [30] and Lee [31] equations to calculate MM expressed in kg and %; and Rocha's equation [32] to calculate BM expressed in kg and %. The sum of 3, 6, and 8 skinfold measurements were also calculated. The sum of 8 skinfolds includes all measured skinfolds, the sum of 6 skinfolds excludes the bicipital and suprailiac skinfolds, while the sum of 3 skinfolds are located near the abdominal area (suprailiac, supraspinal, and abdominal).

The Heath-Carter [33] method was used to estimate anthropometric somatotypes (endomorphy, mesomorphy, and ectomorphy).

2.4. Statistical Analyses

SPSS Statistics 27 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Descriptive statistics were calculated and used to describe the anthropometric and BIA characteristics of the players.

The statistics were reported as mean $\pm$ standard deviation (SD). One-way analysis of variance (ANOVA) was used to determine differences between the anthropometric measures and BIA analysis among the four playing positions. Tukey's post hoc test was used to determine which variables differed significantly. The level of statistical significance was set at $p \leq 0.05$. Pearson's correlation coefficient (r) was used to determine the possible correlation between body composition obtained by anthropometric equations and body composition obtained by BIA.

3. Results and Discussion

Table 1 shows the baseline demographic characteristics of the players; for this reason, no p -values have been included. Table 2 includes the p -values at baseline since the deviation was residual as they were bone diameters. Furthermore, from Table 3 onwards, it was only considered to include the p -value of the variation during pre-season (end of pre-season—baseline) because no significant changes were found in the values at the beginning and the end of pre-season regarding playing positions. According to these findings, the similarity between playing positions is consistent with some previous studies in the literature [10,34–37]. Other studies did report significant differences between positions, but it is important to mention the diversity of the study populations, because the studies reporting differences have not studied populations composed exclusively of elite or professional players. One study involves a mix of Spanish elite and non-elite players [11], while another study includes Chilean professional and collegiate players [38]. Additionally, there are studies where all players are from South African semi-professional or collegiate clubs [39], and another study, despite the team being national, specifies that the players compete in semi-professional teams in South Africa [40]. In studies on professional or elite female players, minor differences were reported [41–43].

Table 1. Demographic data of the players at baseline: mean and standard deviations (mean $\pm$ SD).

	Mean $\pm$ SD
n	34
Gender	Female
Age	23.06 $\pm$ 4.29
Height (cm)	164.15 $\pm$ 5.84
Weight (kg)	58.39 $\pm$ 6.62
BMI (kg/m ²)	21.64 $\pm$ 1.77
Σ 6 Skinfolds (mm)	74.57 $\pm$ 18.48

Σ 6 Skinfolds (triceps, subscapular, supraspinal, abdominal, front thigh, and medial calf).

Table 2 shows the descriptive statistics for the different body dimensions, such as standing and sitting height, wingspan, bone diameters, bone mass, and age by both playing and total positions. These variables were only considered to be measured at baseline, as bone diameters and lengths do not change in such a short period of time (4 weeks).

The variation of these body dimensions showed statistically significant differences ($p < 0.05$) in only two variables (wingspan and ankle diameter). Both variables showed differences between goalkeepers and the rest of the positions. No differences were observed between the rest of the positions ($p > 0.05$). Regarding wingspan, it was observed that goalkeepers were taller than the rest of the positions (goalkeepers vs. midfielders: $\Delta = 15.30$ cm, $p = 0.004$) (goalkeepers vs. defenders: $\Delta = 13.11$ cm, $p = 0.012$) (goalkeepers vs. forwards: $\Delta = 12.26$ cm, $p = 0.021$). That high wingspan diameter in goalkeepers can be explained by the need to effectively block shots and defend the goal against the opposing team's attempts. Comparing with other studies, these findings are in line with results

from studies on Spanish female players aged 16 and 18 [13]. That study also showed the greatest wingspan in goalkeepers, but it was not specified if it was statistically significant compared with the rest of the playing positions. Nonetheless, a study of Ecuadorian female players [44] reported similar values for wingspan between playing positions. It is important to note that in this study, players were divided into eight different categories with only two goalkeepers included, which may limit the robustness of these findings. On the contrary to wingspan, ankle diameter showed lower values in goalkeepers (5.80 ± 0.89 cm) compared to midfielders (6.93 ± 0.38 cm), defenders (6.70 ± 0.28 cm), and forwards (6.66 ± 0.56 cm), with a p -value of 0.012. This contrasts with a study on South African sub-elite female players [39], which reported ankle diameters of 6.4 ± 0.4 cm for forwards, 6.4 ± 0.3 cm for midfielders, 6.6 ± 0.4 cm for defenders, and 6.7 ± 0.3 cm for goalkeepers, with a p -value of 0.034. Although the South African study found statistically significant differences, the practical relevance was unclear due to wide confidence intervals and small effect sizes. The discrepancies between the two studies could be attributed to differences between categories. The present study involves professional players who have different training intensities and physical demands compared to the sub-elite players [45]. Additionally, ethnicity [7], genetic and environmental factors [46], as well as potential variations in measurement methods, might contribute to these differences. It is important to highlight that there is a limited number of studies that have examined or considered both wingspan and ankle diameter in female soccer players in different playing positions.

Regarding height, goalkeepers were shown to be the tallest players. That fact agrees with the observations reported for Tunisian [41], Croatian [47,48], and Chilean [37] first division players, sub-elite and national team South African players, and Chilean players, including both national team members [42] and professional and elite college players [38]. Other studies reported slight differences. For example, a study revealed that the Norwegian first and second division goalkeepers [34] were, along with the defenders, the tallest players on the team. Similarly, in the national team of Montenegro [35], the tallest players were again the goalkeepers, but in this case, their height was similar to that of the forwards. The height reported in the present study for goalkeepers (171.73 ± 8.39) is higher than that observed in other studies [34,37–41], including the study conducted in 2009 on Spanish first division players (1.64 ± 0.6) which reported the defenders as the tallest players [10], but is similar to a study in Croatian first division players (172.5) [47] (172.5 ± 4.6) [48] and the national teams of Chile (172.5 ± 6.6) [42] and Montenegro (170.5 ± 4.2) [35].

Continuing with height, but analyzing the lowest values, midfielders were shown to be the players with the shortest heights. This finding is concordant with observations in Danish [49] and Croatian [47] first division players, as well as sub-elite South African players [39]. It is also worth mentioning that in the national team of Montenegro [35], midfielders were the shortest, along with defenders. However, other studies reported the short height for forwards [34,36,38,40,42,43,48], leading to a wide distribution of height within different playing positions. To understand these differences observed in the different studies, it would be interesting to consider both the playing style of the different teams or divisions, as well as the criteria of the researchers to consider a player within a specific category of playing position. For example, it is not the same to consider a skilled player who plays on the wings (that is usually a small player) as a lateral midfielder or as a winger, since in the first case she would be defined as a midfielder, and in the second, as a forward.

Table 2. Descriptive statistics for the different body dimensions of the players: mean and standard deviations (mean $\pm$ SD).

Variable	Position	Value	p -Value
Age	MF	25.00 ± 3.46	0.415
	DF	23.08 ± 3.15	
	FW	22.27 ± 5.64	
	GK	20.67 ± 4.51	
	Total	23.06 ± 4.29	

Table 2. Cont.

Variable	Position	Value	p-Value
Height (cm)	MF	161.85 ± 5.48	0.089
	DF	164.11 ± 4.37	
	FW	163.79 ± 5.89	
	GK	171.73 ± 8.39	
	Total	164.15 ± 5.84	
Sitting height (cm)	MF	161.30 ± 5.78	0.506
	DF	163.49 ± 5.35	
	FW	164.34 ± 6.80	
	GK	176.60 ± 7.06	
	Total	164.41 ± 7.06	
Wingspan (cm)	MF	161.30 ± 5.78	0.008 *
	DF	163.49 ± 5.35	
	FW	164.34 ± 6.80	
	GK	176.60 ± 7.06 *	
	Total	164.41 ± 7.06	
Humerus (cm)	MF	6.31 ± 0.26	0.372
	DF	6.28 ± 0.25	
	FW	6.26 ± 0.29	
	GK	6.60 ± 0.61	
	Total	6.31 ± 0.30	
Femur (cm)	MF	8.94 ± 0.46	0.617
	DF	8.96 ± 0.24	
	FW	8.87 ± 0.30	
	GK	9.23 ± 1.01	
	Total	8.95 ± 0.41	
Biacromial (cm)	MF	34.84 ± 1.78	0.146
	DF	36.22 ± 1.50	
	FW	36.14 ± 2.43	
	GK	37.83 ± 1.75	
	Total	36.01 ± 2.01	
Wrist (bistyloid) (cm)	MF	5.03 ± 0.212	0.474
	DF	4.98 ± 0.18	
	FW	5.01 ± 0.25	
	GK	5.20 ± 0.27	
	Total	5.04 ± 0.22	
Bi-iliocristal (cm)	MF	26.71 ± 1.10	0.215
	DF	26.73 ± 1.03	
	FW	26.32 ± 1.63	
	GK	28.50 ± 3.50	
	Total	26.75 ± 1.58	
Ankle (bimalleolar) (cm)	MF	6.93 ± 0.38	0.012 *
	DF	6.70 ± 0.28	
	FW	6.66 ± 0.56	
	GK	5.80 ± 0.89 *	
	Total	6.66 ± 0.53	
Bone mass (kg) (Rocha)	MF	9.10 ± 0.74	0.134
	DF	9.25 ± 0.63	
	FW	9.27 ± 0.73	
	GK	10.45 ± 1.96	
	Total	9.33 ± 0.88	
%Bone mass (Rocha)	MF	15.81 ± 1.26	0.159
	DF	16.35 ± 1.54	
	FW	15.83 ± 0.86	
	GK	16.25 ± 0.46	
	Total	16.04 ± 1.20	

MF: midfielders; DF: defenders; FW: forwards; GK: goalkeepers. * Significant differences between positions at baseline ($p < 0.05$).

Table 3 shows the descriptive statistics at the beginning and end of pre-season, as well as their evolution for body mass in kg, BMI, and the different perimeters. In this research, the heaviest players were goalkeepers. This finding coincides with observations in Spanish [10,18], Chilean [37], Tunisian [41], and Croatian [47,48] first division players. This is also consistent with observations made in the national teams of Montenegro [35], Chile [42], and South Africa [40], as well as in Chilean professional and elite college play-

ers [38] and South African sub-elite players [39]. In turn, a recent review of the literature reveals that there is an almost equal number of studies reporting that forwards are the lightest [34,36,38,42,43] as those reporting that midfielders [18,35,39,41,47–49] or defenders [10,35,37,40,48] are the lightest. Consequently, the present research neither contradicts nor supports the existing studies on this matter, as there is no clear predominance of one position being the lightest. In terms of evolution during pre-season, the most noticeable fact was the decrease observed in the relaxed arm perimeter ($p < 0.05$), showing a reduction in all the positions except for goalkeepers. Comparing playing positions, goalkeepers and midfielders were the only ones that showed statistically significant differences ($p = 0.017$).

Table 3. Descriptive statistics at baseline and at the end of pre-season, and variation of body mass, BMI, and perimeters: mean and standard deviations (mean $\pm$ SD).

Variable	Position	Baseline	Final	Δ Final— Baseline	p-Value
Body mass (kg)	MF	57.91 $\pm$ 6.43	57.18 $\pm$ 6.98	−0.74 $\pm$ 1.40	0.257
	DF	56.83 $\pm$ 4.25	57.03 $\pm$ 4.31	0.21 $\pm$ 1.37	
	FW	58.77 $\pm$ 6.53	58.51 $\pm$ 6.70	−0.26 $\pm$ 0.99	
	GK	64.53 $\pm$ 13.90	65.33 $\pm$ 12.14	0.80 $\pm$ 1.76	
	Total	58.39 $\pm$ 6.62	58.28 $\pm$ 6.67	−0.12 $\pm$ 1.32	
BMI (kg/m ²)	MF	22.07 $\pm$ 1.90	21.78 $\pm$ 2.01	−0.29 $\pm$ 0.54	0.228
	DF	21.12 $\pm$ 1.76	21.20 $\pm$ 1.71	0.07 $\pm$ 0.51	
	FW	21.86 $\pm$ 1.61	21.76 $\pm$ 1.69	−0.09 $\pm$ 0.35	
	GK	21.68 $\pm$ 2.44	21.99 $\pm$ 1.84	0.31 $\pm$ 0.59	
	Total	21.63 $\pm$ 1.76	21.59 $\pm$ 1.73	−0.04 $\pm$ 0.49	
Relaxed arm (cm)	MF	27.39 $\pm$ 2.14	26.90 $\pm$ 2.35	−0.49 $\pm$ 0.40 *	0.027 *
	DF	26.23 $\pm$ 1.84	26.15 $\pm$ 1.71	−0.08 $\pm$ 0.64	
	FW	26.94 $\pm$ 1.37	26.72 $\pm$ 1.52	−0.22 $\pm$ 0.44	
	GK	27.13 $\pm$ 2.20	27.77 $\pm$ 1.63	0.63 $\pm$ 0.57	
	Total	26.81 $\pm$ 1.78	26.65 $\pm$ 1.80	−0.16 $\pm$ 0.58	
Contracted arm (cm)	MF	28.91 $\pm$ 2.29	28.03 $\pm$ 2.06	−0.87 $\pm$ 0.81	0.105
	DF	27.29 $\pm$ 1.65	27.21 $\pm$ 1.61	−0.07 $\pm$ 0.53	
	FW	28.01 $\pm$ 1.35	27.83 $\pm$ 1.26	−0.18 $\pm$ 0.74	
	GK	28.83 $\pm$ 2.30	28.86 $\pm$ 1.15	0.03 $\pm$ 1.32	
	Total	28.04 $\pm$ 1.83	27.75 $\pm$ 1.60	−0.28 $\pm$ 0.78	
Waist (cm)	MF	69.05 $\pm$ 3.38	68.03 $\pm$ 4.04	−1.01 $\pm$ 1.13	0.525
	DF	70.42 $\pm$ 4.26	69.94 $\pm$ 4.22	−0.48 $\pm$ 1.29	
	FW	70.41 $\pm$ 3.90	69.74 $\pm$ 3.91	−0.67 $\pm$ 1.44	
	GK	71.40 $\pm$ 7.45	71.70 $\pm$ 6.15	0.30 $\pm$ 1.51	
	Total	70.18 $\pm$ 4.11	69.58 $\pm$ 4.17	−0.60 $\pm$ 1.31	
Hip (cm)	MF	97.46 $\pm$ 4.70	96.78 $\pm$ 5.10	−0.67 $\pm$ 2.09	0.356
	DF	95.99 $\pm$ 4.24	97.03 $\pm$ 3.81	1.04 $\pm$ 2.92	
	FW	97.58 $\pm$ 4.02	97.30 $\pm$ 4.58	−0.27 $\pm$ 1.24	
	GK	102.33 $\pm$ 11.00	102.23 $\pm$ 9.09	−0.10 $\pm$ 2.47	
	Total	97.41 $\pm$ 5.08	97.52 $\pm$ 4.90	0.11 $\pm$ 2.26	
Thigh (cm)	MF	51.03 $\pm$ 2.93	50.42 $\pm$ 2.67	−0.61 $\pm$ 0.87	0.106
	DF	49.73 $\pm$ 3.30	50.01 $\pm$ 2.91	0.28 $\pm$ 1.05	
	FW	51.60 $\pm$ 2.41	51.44 $\pm$ 2.45	−0.16 $\pm$ 0.63	
	GK	50.20 $\pm$ 5.02	50.73 $\pm$ 4.61	0.53 $\pm$ 0.68	
	Total	50.68 $\pm$ 3.06	50.63 $\pm$ 2.79	−0.05 $\pm$ 0.91	
Calf (cm)	MF	36.13 $\pm$ 1.64	36.06 $\pm$ 1.98	−0.07 $\pm$ 0.60	0.905
	DF	34.40 $\pm$ 1.69	34.38 $\pm$ 1.61	−0.01 $\pm$ 0.88	
	FW	35.46 $\pm$ 1.46	35.60 $\pm$ 1.35	0.13 $\pm$ 0.25	
	GK	36.50 $\pm$ 2.95	36.53 $\pm$ 2.30	0.03 $\pm$ 0.66	
	Total	35.33 $\pm$ 1.81	35.36 $\pm$ 1.78	0.02 $\pm$ 0.62	

MF: midfielders; DF: defenders; FW: forwards; GK: goalkeepers. * Significant differences between positions in pre-season variation ($p < 0.05$).

Regarding the type of skinfold and the position on the field, Table 4 shows a significant reduction in the triceps, front thigh, and medial calf. Midfielders exhibited the lowest values, with a significant decrease compared to defenders, especially in the triceps ($p = 0.031$), front thigh ($p = 0.003$), and medial calf ($p = 0.005$). Compared to other positions, midfielders showed a significant reduction in the medial calf compared to forwards ($p = 0.025$) and

in the triceps compared to goalkeepers ($p = 0.041$). In this research, it is observed that the skinfolds with the highest millimeters are the front thigh, suprailiac, and triceps, followed by the abdominal and medial calf. The skinfolds with the lowest measurements are found in the biceps, subscapular, and supraspinal regions. This is consistent with observations from a study involving more than 700 female athletes from various disciplines, which shows that, aside from having higher skinfold measurements than their male counterparts, fat accumulation tends to occur more in the lower body and trunk areas [50].

Regarding the different sums of skinfolds, midfielders were also the position that showed the most pronounced decreases. The sum of the 3 skinfolds showed significant differences between positions ($p = 0.041$). The sum of 6 skinfolds indicated differences between midfielders and defenders ($p = 0.001$) and between midfielders and forwards ($p = 0.030$). Finally, the sum of 8 skinfolds (which includes all the measured skinfolds) revealed significant differences between midfielders and defenders ($p = 0.004$). Continuing with the sum of 6 skinfolds, goalkeepers were shown to be the players with the highest values, and forwards shown to be the players with the lowest values. A possible explanation for this is that goalkeepers cover less distance during the game and thus use less energy, while other players, such as forwards, are generally lighter to be able to cover more ground during the match. This finding about goalkeepers is concordant with observations in South African sub-elite players [39,40] and Chilean [38] professional and elite collegiate players. However, this differs from the observations in the Chilean first division [37] and national team [42] goalkeepers. In these studies, the players with the highest sum of 6 skinfolds were central defenders and defenders, respectively. In terms of values, the sum of 6 skinfolds at the end of pre-season for goalkeepers in this research was lower (78.57 ± 19.45) than those found in sub-elite South African [39,40] (89 ± 5.23) (125.6 ± 45.9) and professional and elite collegiate level Chilean players [38] (104.2 ± 36.2), but higher than those found in Chilean first division [37] (62.5 ± 22.0) and national team [42] (67.78 ± 13.3) players. In summary, the results of the present research for the sum of 6 skinfolds across positions are lower than those observed in semi-professional or sub-elite players, but higher than those observed in players from national teams, which can be understood due to the difference in levels between the players from the different studies.

Table 4. Descriptive statistics at baseline and at the end of pre-season, and variation of skinfolds and sum of skinfolds: mean and standard deviations (mean $\pm$ SD).

Variable	Position	Baseline	Final	Final—Baseline	p-Value
Triceps (mm)	MF	15.19 $\pm$ 5.01	13.01 $\pm$ 4.06	−2.18 $\pm$ 1.45 *	0.017 *
	DF	12.86 $\pm$ 2.85	12.23 $\pm$ 2.75	−0.63 $\pm$ 0.88	
	FW	13.06 $\pm$ 3.13	12.19 $\pm$ 2.64	−0.86 $\pm$ 1.28	
	GK	14.17 $\pm$ 5.96	14.20 $\pm$ 5.38	0.03 $\pm$ 0.67	
	Total	13.59 $\pm$ 3.75	12.58 $\pm$ 3.20	−1.01 $\pm$ 1.31	
Subscapular (mm)	MF	8.51 $\pm$ 1.95	7.63 $\pm$ 1.81	−0.89 $\pm$ 0.56	0.206
	DF	8.19 $\pm$ 1.36	8.03 $\pm$ 1.21	−0.16 $\pm$ 1.01	
	FW	8.07 $\pm$ 1.34	7.86 $\pm$ 1.63	−0.22 $\pm$ 0.90	
	GK	9.07 $\pm$ 2.34	9.17 $\pm$ 2.20	0.10 $\pm$ 0.36	
	Total	8.31 $\pm$ 1.56	7.98 $\pm$ 1.56	−0.33 $\pm$ 0.88	
Biceps (mm)	MF	6.28 $\pm$ 3.47	5.73 $\pm$ 3.32	−0.55 $\pm$ 0.71	0.659
	DF	4.75 $\pm$ 1.35	4.60 $\pm$ 1.28	−0.15 $\pm$ 0.51	
	FW	5.27 $\pm$ 2.10	5.13 $\pm$ 1.98	−0.15 $\pm$ 1.33	
	GK	7.03 $\pm$ 4.30	7.17 $\pm$ 3.68	0.13 $\pm$ 0.81	
	Total	5.48 $\pm$ 2.49	5.26 $\pm$ 2.33	−0.22 $\pm$ 0.90	
Suprailiac (mm)	MF	14.90 $\pm$ 6.49	11.68 $\pm$ 4.96	−3.23 $\pm$ 3.08	0.066
	DF	13.24 $\pm$ 3.68	12.77 $\pm$ 2.99	−0.48 $\pm$ 2.53	
	FW	14.43 $\pm$ 3.87	11.81 $\pm$ 3.50	−2.62 $\pm$ 1.98	
	GK	15.27 $\pm$ 5.81	15.23 $\pm$ 2.84	−0.03 $\pm$ 3.71	
	Total	14.19 $\pm$ 4.54	12.42 $\pm$ 3.66	−1.78 $\pm$ 2.79	

Table 4. Cont.

Variable	Position	Baseline	Final	Final— Baseline	p-Value
Supraspinal (mm)	MF	7.66 ± 3.21	6.89 ± 3.20	−0.78 ± 1.09	0.826
	DF	7.36 ± 2.64	6.87 ± 1.77	−0.49 ± 1.29	
	FW	7.42 ± 1.77	6.93 ± 2.00	−0.49 ± 0.99	
	GK	8.60 ± 3.12	8.53 ± 2.44	−0.07 ± 1.07	
	Total	7.56 ± 2.48	7.04 ± 2.24	−0.52 ± 1.10	
Abdominal (mm)	MF	12.84 ± 4.99	10.93 ± 4.56	−1.91 ± 1.16	0.123
	DF	12.15 ± 2.70	11.26 ± 3.35	−0.89 ± 1.70	
	FW	12.79 ± 3.77	11.17 ± 3.00	−1.62 ± 1.22	
	GK	13.03 ± 1.82	13.23 ± 3.35	0.20 ± 1.68	
	Total	12.60 ± 3.51	11.33 ± 3.45	−1.27 ± 1.50	
Front thigh (mm)	MF	25.50 ± 9.07	21.00 ± 6.71	−4.50 ± 3.51 *	0.004 *
	DF	19.13 ± 4.19	18.73 ± 4.41	−0.40 ± 1.85	
	FW	19.62 ± 4.09	17.69 ± 3.44	−1.93 ± 1.30	
	GK	24.33 ± 8.10	20.67 ± 4.94	−3.67 ± 3.17	
	Total	21.24 ± 6.33	19.10 ± 4.79	−2.15 ± 2.75	
Medial calf (mm)	MF	13.14 ± 5.64	11.29 ± 4.51	−1.85 ± 1.65 *	0.003 *
	DF	10.08 ± 3.96	10.00 ± 3.84	−0.08 ± 0.50	
	FW	10.34 ± 4.18	9.97 ± 4.19	−0.36 ± 0.44	
	GK	14.50 ± 5.46	12.77 ± 3.25	−1.73 ± 2.20	
	Total	11.27 ± 4.66	10.54 ± 4.00	−0.735 ± 1.27	
Sum of Skinfolds					
Σ3 skinfolds	MF	35.40 ± 13.33	29.49 ± 12.13	−5.91 ± 3.82	0.041 *
	DF	32.75 ± 7.84	30.89 ± 7.08	−1.86 ± 4.67	
	FW	34.64 ± 9.00	29.91 ± 8.10	−4.73 ± 2.61	
	GK	36.90 ± 10.41	37.00 ± 8.42	0.10 ± 3.16	
	Total	34.35 ± 9.54	30.78 ± 8.73	−3.57 ± 4.14	
Σ6 skinfolds	MF	82.84 ± 26.24	70.74 ± 23.06	−12.10 ± 5.69 *	0.002 *
	DF	69.77 ± 12.24	67.12 ± 12.28	−2.65 ± 5.84	
	FW	71.29 ± 15.39	65.81 ± 14.45	−5.48 ± 1.80	
	GK	83.70 ± 24.85	78.57 ± 19.45	−5.13 ± 5.75	
	Total	74.57 ± 18.48	68.56 ± 16.21	−6.01 ± 5.87	
Σ8 skinfolds	MF	104.01 ± 35.38	88.14 ± 30.58	−15.88 ± 9.08 *	0.007 *
	DF	87.76 ± 15.31	84.48 ± 14.92	−3.28 ± 8.08	
	FW	90.99 ± 20.04	82.75 ± 19.14	−8.25 ± 3.56	
	GK	106.00 ± 33.83	100.97 ± 24.39	−5.03 ± 9.88	
	Total	94.24 ± 24.27	86.24 ± 21.11	−8.00 ± 8.54	

MF: midfielders; DF: defenders; FW: forwards; GK: goalkeepers. * Significant differences between positions in pre-season variation ($p < 0.05$).

Tables 5 and 6 show the descriptive statistics at the beginning and end of pre-season, as well as their evolution for absolute and relative values of FM, respectively, showing a general decrease. However, the reduction observed for goalkeepers was only related to equations that only consider folds in the full body, but not for those that only consider the skin folds of the upper body exclusively (Faulkner and Durnin).

Table 5 displays the kg of fat obtained by the different equations ($p < 0.05$). In all the equations, the lowest values were observed for midfielders, especially compared with defenders—Withers ($p = 0.013$), Slaughter ($p = 0.010$), Carter ($p = 0.007$), and Jackson and Pollock ($p = 0.010$)—and versus goalkeepers, with Faulkner ($p = 0.030$). In contrast, Durnin's equation also revealed significant differences between positions ($p = 0.036$). Despite the specific positions not being revealed, the two positions that showed the greatest variation in this parameter were the midfielders (-1.18 ± 0.61 kg) and goalkeepers (0.25 ± 0.99 kg).

The % fat followed a similar trend as that observed for the kg of fat. Table 6 shows the lowest values in midfielders and significant decreases were observed versus defenders, Withers ($p = 0.003$), Slaughter ($p = 0.003$), Carter ($p = 0.001$), Faulkner ($p = 0.038$), and Jackson and Pollock ($p = 0.003$); versus forwards, Withers ($p = 0.023$), Slaughter ($p = 0.015$), and Carter ($p = 0.030$); and versus goalkeepers, Withers ($p = 0.036$), Faulkner ($p = 0.016$) and Durnin ($p = 0.030$). It is notable that positions with significant differences in skinfolds, such as defenders, lead to a minor improvement potential. Similarly, for the sum of 6 and 8 skinfolds, defenders started with the lowest values and, therefore, lower fat values

while goalkeepers had the highest, followed by midfielders. This trend could explain the significant difference in progression between midfielders and defenders. Defenders had the lowest values at the beginning of pre-season, followed by forwards, midfielders, and goalkeepers. The differences observed in midfielders compared to defenders could be attributed to the initial differences in body composition. Similarly, while midfielders and goalkeepers often had similar baseline values, midfielders consistently demonstrated greater improvements. This difference in improvement might be linked to the specific training regimens for each position. This situation can also be observed later with other data such as somatotype or BIA values.

The lowest fat values were obtained using Carter’s equation, followed by Faulkner, Withers, Jackson and Pollock, and Slaughter, with the highest fat values provided by Durnin’s equation. Interpreting results requires understanding which skinfolds are considered in the anthropometric equations estimating body fat. For example, Carter uses an equation that includes the sum of 6 skinfolds. In contrast, other equations, such as Durnin and Faulkner, only consider upper body skinfolds. This can lead to an overestimation or underestimation of body fat percentage, as the lower body, a common site for fat accumulation in female athletes, is not accounted for [50]. Comparing with other studies, one of the limitations is the diversity of equations used to estimate body fat percentage. Other studies have used equations such as Sloan and Siri [47], Yuhasz [10], Jackson and Pollock [48], Kerr [38], Durnin [41], $\Sigma 6$ skinfolds [37,42], Withers [39], and Eston 2005 [43].

Table 5. Descriptive statistics at baseline and at the end of pre-season and variation of kg of FM calculated with different equations adapted for female athletes: mean and standard deviations (mean $\pm$ SD).

Variable	Position	Baseline	Final	Δ Final— Baseline	p-Value
Fat (kg) (Withers)	MF	10.74 $\pm$ 3.53	9.53 $\pm$ 3.62	−1.21 $\pm$ 0.58 *	0.015 *
	DF	9.48 $\pm$ 1.99	9.24 $\pm$ 1.94	−0.24 $\pm$ 0.82	
	FW	9.93 $\pm$ 2.58	9.46 $\pm$ 2.67	−0.47 $\pm$ 0.26	
	GK	12.60 $\pm$ 5.96	12.43 $\pm$ 4.91	−0.17 $\pm$ 1.06	
	Total	10.20 $\pm$ 2.99	9.66 $\pm$ 2.90	−0.54 $\pm$ 0.73	
Fat (kg) (Slaughter)	MF	13.13 $\pm$ 4.57	11.58 $\pm$ 3.88	−1.56 $\pm$ 1.27 *	0.015 *
	DF	10.86 $\pm$ 2.18	10.66 $\pm$ 2.29	−0.20 $\pm$ 0.61	
	FW	11.49 $\pm$ 3.12	11.00 $\pm$ 2.97	−0.49 $\pm$ 0.41	
	GK	15.14 $\pm$ 7.90	14.45 $\pm$ 6.21	−0.69 $\pm$ 1.69	
	Total	11.98 $\pm$ 3.81	11.32 $\pm$ 3.32	−0.66 $\pm$ 0.98	
Fat (kg) (Carter)	MF	9.64 $\pm$ 3.15	8.45 $\pm$ 2.82	−1.19 $\pm$ 0.68 *	0.013 *
	DF	8.20 $\pm$ 1.41	8.00 $\pm$ 1.45	−0.20 $\pm$ 0.70	
	FW	8.66 $\pm$ 2.09	8.13 $\pm$ 1.99	−0.53 $\pm$ 0.27	
	GK	10.99 $\pm$ 4.93	10.51 $\pm$ 4.01	−0.48 $\pm$ 0.94	
	Total	8.93 $\pm$ 2.51	8.37 $\pm$ 2.25	−0.56 $\pm$ 0.69	
Fat (kg) (Faulkner)	MF	10.14 $\pm$ 2.54	9.32 $\pm$ 2.49	−0.81 $\pm$ 0.30 *	0.021 *
	DF	9.43 $\pm$ 1.38	9.20 $\pm$ 1.32	−0.23 $\pm$ 0.69	
	FW	9.89 $\pm$ 2.04	9.45 $\pm$ 1.95	−0.44 $\pm$ 0.31	
	GK	11.51 $\pm$ 4.32	11.65 $\pm$ 4.02	0.15 $\pm$ 0.31	
	Total	9.93 $\pm$ 2.17	9.53 $\pm$ 2.12	−0.40 $\pm$ 0.54	
Fat (kg) (Durnin)	MF	13.86 $\pm$ 3.82	12.68 $\pm$ 3.99	−1.18 $\pm$ 0.61	0.036 *
	DF	12.68 $\pm$ 2.25	12.41 $\pm$ 2.03	−0.27 $\pm$ 1.02	
	FW	13.31 $\pm$ 2.88	12.80 $\pm$ 2.96	−0.51 $\pm$ 0.51	
	GK	16.00 $\pm$ 7.13	16.24 $\pm$ 6.16	0.25 $\pm$ 0.99	
	Total	13.45 $\pm$ 3.35	12.94 $\pm$ 3.29	−0.52 $\pm$ 0.86	
Fat (kg) (Jackson and Pollock)	MF	12.68 $\pm$ 4.44	10.94 $\pm$ 3.95	−1.74 $\pm$ 1.00 *	0.018 *
	DF	10.69 $\pm$ 1.96	10.32 $\pm$ 1.94	−0.37 $\pm$ 1.06	
	FW	11.36 $\pm$ 2.93	10.46 $\pm$ 2.68	−0.90 $\pm$ 0.46	
	GK	14.33 $\pm$ 6.68	13.72 $\pm$ 5.64	−0.61 $\pm$ 1.09	
	Total	11.69 $\pm$ 3.47	10.81 $\pm$ 3.09	−0.88 $\pm$ 1.00	

MF: midfielders; DF: defenders; FW: forwards; GK: goalkeepers. * Significant differences between positions in pre-season variation ($p < 0.05$).

Table 6. Descriptive statistics at baseline and at the end of pre-season and variation of % of FM calculated with different equations adapted for female athletes: mean and standard deviations (mean $\pm$ SD).

Variable	Position	Baseline	Final	Δ Final— Baseline	p-Value
%Fat (Withers)	MF	18.27 $\pm$ 4.65	16.31 $\pm$ 4.90	−1.96 $\pm$ 0.76 *	0.003 *
	DF	16.62 $\pm$ 2.96	16.14 $\pm$ 2.80	−0.48 $\pm$ 1.12	
	FW	16.72 $\pm$ 3.07	15.97 $\pm$ 3.28	−0.75 $\pm$ 0.43	
	GK	18.91 $\pm$ 4.86	18.60 $\pm$ 3.84	−0.31 $\pm$ 1.03	
	Total	17.242 $\pm$ 3.5	16.34 $\pm$ 3.52	−0.90 $\pm$ 1.02	
%Fat (Slaughter)	MF	22.38 $\pm$ 6.26	19.93 $\pm$ 5.09	−2.46 $\pm$ 1.78 *	0.004 *
	DF	19.10 $\pm$ 3.47	18.66 $\pm$ 3.57	−0.43 $\pm$ 0.69	
	FW	19.37 $\pm$ 4.14	18.62 $\pm$ 3.79	−0.75 $\pm$ 0.76	
	GK	22.59 $\pm$ 6.90	21.55 $\pm$ 5.24	−1.04 $\pm$ 1.66	
	Total	20.26 $\pm$ 4.77	19.20 $\pm$ 4.08	−1.06 $\pm$ 1.35	
%Fat (Carter)	MF	16.40 $\pm$ 4.06	14.53 $\pm$ 3.57	−1.87 $\pm$ 0.88 *	0.002 *
	DF	14.38 $\pm$ 1.90	13.97 $\pm$ 1.90	−0.41 $\pm$ 0.90	
	FW	14.61 $\pm$ 2.38	13.77 $\pm$ 2.24	−0.85 $\pm$ 0.28	
	GK	16.54 $\pm$ 3.85	15.74 $\pm$ 3.01	−0.80 $\pm$ 0.89	
	Total	15.12 $\pm$ 2.86	14.19 $\pm$ 2.51	−0.93 $\pm$ 0.91	
%Fat (Faulkner)	MF	17.32 $\pm$ 2.86	16.09 $\pm$ 2.73	−1.23 $\pm$ 0.40 *	0.011 *
	DF	16.54 $\pm$ 1.57	16.08 $\pm$ 1.36	−0.46 $\pm$ 0.85	
	FW	16.71 $\pm$ 1.91	16.03 $\pm$ 1.80	−0.68 $\pm$ 0.36	
	GK	17.46 $\pm$ 2.65	17.51 $\pm$ 2.63	0.06 $\pm$ 0.13	
	Total	16.86 $\pm$ 2.05	16.19 $\pm$ 1.94	−0.67 $\pm$ 0.67	
% Fat (Durnin)	MF	23.65 $\pm$ 4.59	21.81 $\pm$ 4.91	−1.84 $\pm$ 0.76 *	0.020 *
	DF	22.22 $\pm$ 2.97	21.67 $\pm$ 2.49	−0.54 $\pm$ 1.35	
	FW	22.48 $\pm$ 2.96	21.70 $\pm$ 3.26	−0.79 $\pm$ 0.89	
	GK	24.06 $\pm$ 5.35	24.32 $\pm$ 4.50	0.27 $\pm$ 0.88	
	Total	22.80 $\pm$ 3.50	21.95 $\pm$ 3.49	−0.86 $\pm$ 1.18	
%Fat (Jackson and Pollock)	MF	21.54 $\pm$ 5.84	18.76 $\pm$ 5.15	−2.78 $\pm$ 1.34 *	0.006 *
	DF	18.73 $\pm$ 2.63	18.01 $\pm$ 2.53	−0.71 $\pm$ 1.46	
	FW	19.13 $\pm$ 3.35	17.68 $\pm$ 2.95	−1.45 $\pm$ 0.61	
	GK	21.52 $\pm$ 5.30	20.48 $\pm$ 4.45	−1.04 $\pm$ 1.07	
	Total	19.77 $\pm$ 4.03	18.30 $\pm$ 3.50	−1.47 $\pm$ 1.38	

MF: midfielders; DF: defenders; FW: forwards; GK: goalkeepers. * Significant differences between positions in pre-season variation ($p < 0.05$).

Moreover, Tables 7 and 8 show the descriptive statistics at the beginning and end of pre-season as well as their evolution for absolute and relative values of MM, respectively. Focusing on the kg of muscle mass and playing position (Table 7), Poortmans' equation shows statistically significant differences ($p < 0.05$) when comparing goalkeepers with defenders ($p = 0.021$) and forwards ($p = 0.031$). In contrast, Table 8 shows statistically significant differences in % muscle between midfielders versus defenders ($p < 0.05$) with Lee's equation ($p = 0.017$). In summary, the only advantage observed for goalkeepers compared to other positions was an increase in muscle mass (using Poortmans' equation) compared to defenders and forwards.

Table 7. Descriptive statistics at baseline and at the end of pre-season and variation of kg of MM calculated with different equations adapted for female athletes: mean and standard deviations (mean $\pm$ SD).

Variable	Position	Baseline	Final	Δ Final— Baseline	p-Value
Muscle (kg) (Poortmans)	MF	23.00 $\pm$ 2.10	23.56 $\pm$ 2.24	0.55 $\pm$ 0.60	0.024 *
	DF	22.93 $\pm$ 2.54	23.19 $\pm$ 2.51	0.26 $\pm$ 0.46 *	
	FW	23.94 $\pm$ 3.09	24.24 $\pm$ 3.08	0.30 $\pm$ 0.41 *	
	GK	23.25 $\pm$ 2.70	24.48 $\pm$ 2.47	1.24 $\pm$ 0.58	
	Total	23.30 $\pm$ 2.58	23.73 $\pm$ 2.58	0.43 $\pm$ 0.54	
Muscle (kg) (Lee)	MF	22.77 $\pm$ 1.90	23.21 $\pm$ 1.98	0.44 $\pm$ 0.56	0.110
	DF	22.59 $\pm$ 2.00	22.69 $\pm$ 1.94	0.11 $\pm$ 0.54	
	FW	23.60 $\pm$ 1.84	23.81 $\pm$ 1.83	0.20 $\pm$ 0.40	
	GK	23.91 $\pm$ 2.60	24.76 $\pm$ 2.36	0.85 $\pm$ 0.40	
	Total	23.08 $\pm$ 1.95	23.36 $\pm$ 1.96	0.28 $\pm$ 0.52	

MF: midfielders; DF: defenders; FW: forwards; GK: goalkeepers. * Significant differences between positions in pre-season variation ($p < 0.05$).

Table 8. Descriptive statistics at baseline and at the end of pre-season and variation of % of MM calculated with different equations adapted for female athletes: mean and standard deviations (mean $\pm$ SD).

Variable	Position	Baseline	Final	Δ Final— Baseline	p-Value
%Muscle (Poortmans)	MF	39.94 $\pm$ 3.58	41.48 $\pm$ 4.14	1.55 $\pm$ 1.66	0.083
	DF	40.33 $\pm$ 2.97	40.68 $\pm$ 3.39	0.35 $\pm$ 0.63	
	FW	40.87 $\pm$ 4.23	41.58 $\pm$ 4.16	0.71 $\pm$ 0.72	
	GK	36.57 $\pm$ 4.48	37.86 $\pm$ 3.44	1.29 $\pm$ 1.18	
	Total	40.08 $\pm$ 3.69	40.91 $\pm$ 3.81	0.83 $\pm$ 1.09	
%Muscle (Lee)	MF	39.51 $\pm$ 3.00	40.81 $\pm$ 2.78	1.30 $\pm$ 1.16 *	0.030 *
	DF	39.76 $\pm$ 2.10	39.82 $\pm$ 2.22	0.06 $\pm$ 0.86	
	FW	40.41 $\pm$ 3.43	40.94 $\pm$ 3.33	0.54 $\pm$ 0.54	
	GK	37.59 $\pm$ 3.95	38.30 $\pm$ 3.28	0.71 $\pm$ 0.80	
	Total	39.72 $\pm$ 2.91	40.28 $\pm$ 2.82	0.56 $\pm$ 0.94	

MF: midfielders; DF: defenders; FW: forwards; GK: goalkeepers. * Significant differences between positions in pre-season variation ($p < 0.05$).

Table 9 shows the descriptive statistics at the beginning and end of pre-season as well as their evolution for somatotype. In turn, Figure 1 shows the somatotype profiles of female players by position at the baseline and the end of pre-season. From the 13 categories proposed by Heath and Carter's method [33], two categories were observed. Initially, midfielders and forwards were classified as endomorphic mesomorphs (mesomorphy is dominant and endomorphy is greater than ectomorphy), while defenders and goalkeepers were mesomorph–endomorph (endomorphism and mesomorphy are equal or do not differ by more than 0.5, and ectomorphy is smaller). By the end of pre-season, it was observed that midfielders and forwards maintained their endomorphic mesomorph classification, while defenders changed from mesomorph–endomorph to endomorphic mesomorph and goalkeepers maintained their mesomorph–endomorph classification throughout pre-season. It should be noted that significant changes were observed mainly in the endomorphic component; specifically, the position that most decreased in this component were midfielders and significant decreases were observed versus defenders ($p = 0.034$) and goalkeepers ($p = 0.023$). In this research, it has been observed that midfielders, defenders, and forwards have a greater mesomorphy component than endomorphy, which differs from previous studies. Regarding midfielders, other studies have observed balanced endomorph [10,39], mesomorphic endomorph [10], and mesomorph–endomorph [36,38] somatotypes. For defenders in other studies, balanced endomorph [10], mesomorphic endomorph [10,38,39], and central [36] somatotypes have been observed. In regards to forwards, in other studies, balanced endomorph [39], mesomorph–endomorph [10,38], and central [36] somatotypes

have been observed. As for goalkeepers, the somatotype observed in other studies is mesomorphic endomorph [10,38,39], which contrasts with the somatotype observed in the goalkeepers in this research study, whose endomorphy and mesomorphy are equal.

Table 9. Descriptive statistics at baseline and at the end of pre-season and variation of somatotype values: mean and standard deviations (mean $\pm$ SD).

Variable	Position	Baseline	Final	Δ Final— Baseline	p-Value
Endomorphy	MF	3.87 $\pm$ 1.00	3.44 $\pm$ 0.97	−0.43 $\pm$ 0.15 *	0.012 *
	DF	3.53 $\pm$ 0.66	3.39 $\pm$ 0.53	−0.14 $\pm$ 0.31	
	FW	3.55 $\pm$ 0.62	3.39 $\pm$ 0.65	−0.18 $\pm$ 0.14	
	GK	3.70 $\pm$ 1.00	3.72 $\pm$ 0.82	0.02 $\pm$ 0.17	
	Total	3.63 $\pm$ 0.75	3.43 $\pm$ 0.69	−0.21 $\pm$ 0.25	
Mesomorphy	MF	4.84 $\pm$ 0.98	4.74 $\pm$ 0.95	−0.11 $\pm$ 0.15	0.479
	DF	4.04 $\pm$ 0.68	4.03 $\pm$ 0.75	−0.004 $\pm$ 0.18	
	FW	4.31 $\pm$ 0.97	4.32 $\pm$ 0.86	0.01 $\pm$ 0.18	
	GK	4.01 $\pm$ 0.77	4.05 $\pm$ 0.47	0.04 $\pm$ 0.30	
	Total	4.31 $\pm$ 0.89	4.29 $\pm$ 0.83	−0.02 $\pm$ 0.18	
Ectomorphy	MF	2.13 $\pm$ 0.87	2.26 $\pm$ 0.93	0.14 $\pm$ 0.26	0.159
	DF	2.71 $\pm$ 1.00	2.66 $\pm$ 0.99	−0.05 $\pm$ 0.24	
	FW	2.31 $\pm$ 0.81	2.36 $\pm$ 0.87	0.05 $\pm$ 0.17	
	GK	2.89 $\pm$ 0.64	2.72 $\pm$ 0.35	−0.17 $\pm$ 0.29	
	Total	2.46 $\pm$ 0.89	2.48 $\pm$ 0.88	0.02 $\pm$ 0.23	

MF: midfielders; DF: defenders; FW: forwards; GK: goalkeepers. * Significant differences between positions in pre-season variation ($p < 0.05$).

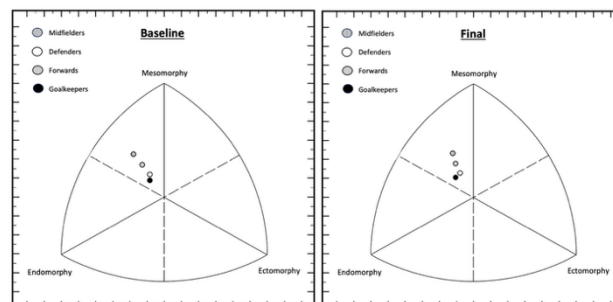


Figure 1. Somatotype of female players by position at the baseline and the end of pre-season.

Table 10 shows the descriptive statistics at baseline and at the end of pre-season and the variation of BIA values. There were observed statistically significant differences ($p < 0.05$) in only three variables (relative and absolute values of FM and relative values of water). Regarding the values of FM, as well as the values obtained by anthropometry, a decrease in FM is observed in the entire population. Observing the evolution of each playing position, midfielders are the group with the greatest decrease in FM (both absolute and relative) compared with defenders and goalkeepers who increased FM. Considering the relative values between positions, there were differences between midfielders and defenders ($p = 0.025$) and between defenders and forwards ($p = 0.040$). In turn, absolute values showed a significant difference between midfielders and defenders ($p = 0.017$). Finally, the % of water significantly varied between midfielders and defenders ($p = 0.009$) and between defenders and forwards ($p = 0.009$).

In order to complete the approach of the body composition analysis, BIA analysis was used to assess different parameters of body composition, including bone mass. It is

worth mentioning that although BIA results refer to the term “bone”, this can be misleading as it suggests the weight of the entire skeleton, whereas it actually estimates the bone mineral content (BMC) [51]. The BMC is the amount of minerals (calcium and phosphorus) expressed in grams. It should be emphasized that the BMC is not the same as the weight of the entire skeleton, which contains bone marrow, bone cells, connective tissue, cartilage tissue, water, blood vessels, and nerves, in addition to the mineral elements. The human skeleton represents 9–14% of the body weight of a thin person and the BMC is on average 40% of the skeletal weight [51]. Therefore, both fat-free mass (muscle, bone, tissue, water, and all other fat free mass in the body) and muscle mass (bone-free lean tissue mass) may be overestimated because skeletal weight is underestimated by assessing BMC rather than the actual skeletal weight.

Table 10. Descriptive statistics at baseline and at the end of pre-season and variation of bioimpedance values: mean and standard deviations (mean $\pm$ SD).

Variable	Position	Baseline	Final	Δ Final— Baseline	p-Value
Fat mass %	MF	22.58 $\pm$ 6.09	21.03 $\pm$ 6.46	−1.55 $\pm$ 1.44 *	0.010 *
	DF	21.74 $\pm$ 4.04	22.32 $\pm$ 3.61	0.58 $\pm$ 1.70 *	
	FW	23.29 $\pm$ 4.72	22.06 $\pm$ 5.29	−1.24 $\pm$ 1.50	
	GK	25.27 $\pm$ 6.86	25.87 $\pm$ 5.73	0.60 $\pm$ 1.15	
	Total	22.75 $\pm$ 4.90	22.24 $\pm$ 5.02	−0.51 $\pm$ 1.77	
Fat mass (kg)	MF	13.39 $\pm$ 4.81	12.33 $\pm$ 4.87	−1.06 $\pm$ 0.92 *	0.008 *
	DF	12.48 $\pm$ 3.18	12.86 $\pm$ 3.00	0.38 $\pm$ 1.07	
	FW	13.95 $\pm$ 4.28	13.29 $\pm$ 4.54	−0.73 $\pm$ 0.90	
	GK	16.93 $\pm$ 8.25	17.37 $\pm$ 7.10	0.43 $\pm$ 1.16	
	Total	13.56 $\pm$ 4.41	13.25 $\pm$ 4.37	−0.32 $\pm$ 1.14	
Fat-free mass (kg)	MF	44.53 $\pm$ 2.85	44.70 $\pm$ 2.75	0.18 $\pm$ 0.97	0.501
	DF	44.34 $\pm$ 1.68	44.18 $\pm$ 1.80	−0.17 $\pm$ 1.01	
	FW	44.84 $\pm$ 2.64	45.28 $\pm$ 2.42	0.45 $\pm$ 1.01	
	GK	47.60 $\pm$ 5.72	47.97 $\pm$ 5.14	0.37 $\pm$ 0.61	
	Total	44.83 $\pm$ 2.75	44.99 $\pm$ 2.68	0.16 $\pm$ 0.97	
Muscle mass (bone-free lean tissue mass) (kg)	MF	42.26 $\pm$ 2.71	42.43 $\pm$ 2.62	0.16 $\pm$ 0.91	0.490
	DF	42.09 $\pm$ 1.60	41.93 $\pm$ 1.71	−0.17 $\pm$ 0.96	
	FW	42.56 $\pm$ 2.51	42.98 $\pm$ 2.30	0.43 $\pm$ 0.98	
	GK	45.20 $\pm$ 5.46	45.53 $\pm$ 4.91	0.33 $\pm$ 0.57	
	Total	42.56 $\pm$ 2.62	42.70 $\pm$ 2.55	0.15 $\pm$ 0.92	
Water (kg)	MF	31.15 $\pm$ 2.25	31.20 $\pm$ 2.20	0.05 $\pm$ 0.74	0.360
	DF	31.11 $\pm$ 1.33	30.89 $\pm$ 1.45	−0.22 $\pm$ 0.80	
	FW	31.81 $\pm$ 2.11	32.13 $\pm$ 1.93	0.32 $\pm$ 0.73	
	GK	33.90 $\pm$ 5.03	34.20 $\pm$ 4.59	0.30 $\pm$ 0.46	
	Total	31.59 $\pm$ 2.28	31.66 $\pm$ 2.25	0.07 $\pm$ 0.74	
Water %	MF	54.09 $\pm$ 3.76	55.08 $\pm$ 3.88	0.99 $\pm$ 0.97 *	0.003 *
	DF	54.90 $\pm$ 2.67	54.30 $\pm$ 2.24	−0.60 $\pm$ 1.09 *	
	FW	54.40 $\pm$ 3.21	55.26 $\pm$ 3.46	0.86 $\pm$ 1.00	
	GK	53.03 $\pm$ 3.55	52.70 $\pm$ 2.91	−0.33 $\pm$ 0.72	
	Total	54.38 $\pm$ 3.09	54.65 $\pm$ 3.09	0.27 $\pm$ 1.22	
Bone mineral content (kg)	MF	2.26 $\pm$ 0.15	2.28 $\pm$ 0.14	0.01 $\pm$ 0.06	0.781
	DF	2.26 $\pm$ 0.08	2.25 $\pm$ 0.09	0.00 $\pm$ 0.06	
	FW	2.29 $\pm$ 0.16	2.313 $\pm$ 0.14	0.03 $\pm$ 0.05	
	GK	2.25 $\pm$ 0.07	2.30 $\pm$ 0.00	0.05 $\pm$ 0.07	
	Total	2.27 $\pm$ 0.12	2.28 $\pm$ 0.11	0.09 $\pm$ 0.40	
Basal metabolic rate (kcal)	MF	1356.38 $\pm$ 90.04	1356.88 $\pm$ 91.77	0.50 $\pm$ 25.80	0.768
	DF	1356.09 $\pm$ 55.90	1351.83 $\pm$ 60.40	−5.09 $\pm$ 26.68	
	FW	1380.63 $\pm$ 95.73	1389.75 $\pm$ 95.89	9.13 $\pm$ 27.30	
	GK	1354.00 $\pm$ 2.83	1376.00 $\pm$ 4.24	22.00 $\pm$ 7.07	
	Total	1362.79 $\pm$ 74.57	1364.90 $\pm$ 76.82	47.53 $\pm$ 249.38	

MF: midfielders; DF: defenders; FW: forwards; GK: goalkeepers. * Significant differences between positions in pre-season variation ($p < 0.05$).

Table 11 presents the correlation coefficients between the BIA analysis (fat mass %, fat mass (kg), fat-free mass (kg), and muscle mass (bone-free lean tissue mass) (kg)) and those

obtained through anthropometry. Regarding fat mass %, all correlations were statistically significant ($p < 0.001$). The highest correlation was observed with the Faulkner's equation ($r = 0.817$; "very high" range of correlation). Subsequently, "very high" correlations were also observed with other equations, such as Durnin ($r = 0.758$), Jackson and Pollock ($r = 0.746$), and Carter ($r = 0.709$). Similarly, fat mass (kg) showed a strong ($p < 0.001$) correlation with all the equations, especially with the Faulkner's equation ($r = 0.953$; "almost perfect" range of correlation) and the Durnin's equation ($r = 0.920$; "almost perfect" range of correlation). Figure 2 shows the highest correlations between % and kg fat and BIA. The comparative analysis between anthropometric equations and BIA reveals that, although Faulkner's equation demonstrates the strongest correlation, Durnin's equation shows a greater similarity in the results obtained at both the beginning and the end of the study. This finding suggests that, despite the Faulkner equation potentially being influenced by various factors such as sample size and measurement precision, the consistency in the similarity of values between BIA and anthropometric equations, particularly with Durnin's equation, is notable. It is important to note that the highest correlation can be observed with the two equations that do not consider lower body skinfolds. The finding about the high correlation between Faulkner and BIA is concordant with a previous study in professional male athletes [52]. For instance, previous research has demonstrated that anthropometric equations can be a viable alternative to BIA in estimating body composition in athletes [53–55], provided the specificities of the studied population are considered. However, it is important to note that most previous studies have been conducted on male or mixed populations, leaving a gap in the literature regarding the correlation of these equations in female populations, especially in specific sports like women's soccer. Therefore, future studies should focus on validating these equations in diverse populations of female athletes and comparing them with more precise reference methods to strengthen the applicability of these tools in sports practice. Finally for fat-free mass (kg) and muscle mass (bone-free lean tissue mass) (kg), all correlations were statistically significant ($p < 0.001$). Lee's equation had the highest correlation coefficients ($r = 0.813$ and $r = 0.812$, respectively; "very high" level of correlation in both cases). Since BIA measures BMC instead of bone mass directly, the values of fat-free mass and muscle mass (bone-free lean tissue mass) are overestimated. Due to this limitation, the results should be interpreted with caution.

Table 11. Correlation between bioimpedance and anthropometry.

Bioimpedance vs. Anthropometry	Pearson's r	Lower 95% CI	Upper 95% CI	p -Value
% Fat Mass				
% Withers	0.698	0.551	0.803	<0.001
% Slaughter	0.583	0.400	0.721	<0.001
% Carter	0.709	0.567	0.811	<0.001
% Faulkner	0.817	0.718	0.883	<0.001
% Durnin	0.758	0.635	0.844	<0.001
% Jackson and Pollock	0.746	0.618	0.836	<0.001
Fat Mass (kg)				
Kg Withers	0.871	0.798	0.919	<0.001
Kg Slaughter	0.807	0.704	0.877	<0.001
Kg Carter	0.885	0.820	0.928	<0.001
Kg Faulkner	0.953	0.924	0.971	<0.001
Kg Durnin	0.920	0.873	0.950	<0.001
Kg Jackson and Pollock	0.895	0.835	0.934	<0.001
Fat-Free Mass (kg)				
Kg Poortmans	0.657	0.497	0.774	<0.001
Kg Lee	0.813	0.712	0.880	<0.001
Muscle Mass (Bone-free lean tissue mass) (kg)				
Kg Poortmans	0.656	0.495	0.774	<0.001
Kg Lee	0.812	0.711	0.880	<0.001

$r = 0.0$ – 0.09 were considered trivial, $r = 0.10$ – 0.29 small, $r = 0.30$ – 0.49 moderate, $r = 0.50$ – 0.69 high, $r = 0.70$ – 0.89 very high, $r = 0.90$ – 0.99 almost perfect, and $r = 1$ perfect correlation [56].

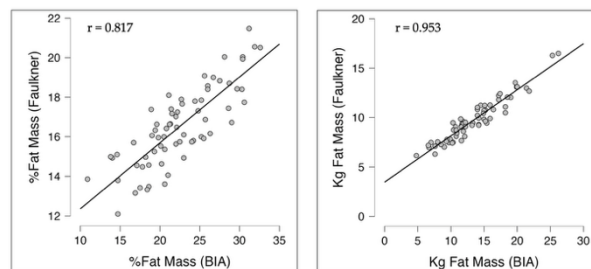


Figure 2. Graphs representing the correlation between the fat mass values obtained with the Faulkner anthropometric equation and the values obtained with BIA.

4. Limitations

In this research, the analysis was limited to a sample of only 34 female players, which is a clear limitation. Therefore, further research with larger sample sizes is needed to confirm these findings. Another limitation was the number of goalkeepers, which means that perhaps the data from this population do not have more statistical power with respect to the rest of the positions, although it is true that as there were two teams, this problem was difficult to solve as there are only two players for this position in each team.

5. Conclusions

This study evaluated anthropometric and body composition changes during pre-season among Spanish professional female soccer players according to their playing positions. The results revealed that midfielders showed the most significant improvements in body composition, including reductions in skinfolds and fat mass. In contrast, defenders exhibited the least improvement, starting with lower baseline values and demonstrating minimal changes. Goalkeepers had distinctive characteristics, such as a larger wingspan and smaller ankle diameter, with minimal changes in upper body skinfolds, leading to no significant decrease in body fat percentage for the equations that include upper body measurements. Importantly, the study also demonstrated a high correlation between anthropometric measurements and BIA for estimating body composition, with the Faulkner and Durnin equations showing the strongest correlations. This reinforces the reliability of using both methods interchangeably in monitoring and assessing body composition changes. Overall, these findings highlight the positional differences in body composition changes during pre-season and suggest that tailored training and nutrition programs could optimize performance for each position.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data are contained within the article.

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4.2. ARTÍCULO 2

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Article

Relationship Between Anthropometric Profile, Body Composition, and Physical Performance in Spanish Professional Female Soccer Players at Pre-Season Onset: A Cross-Sectional Study

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Abstract: **Background:** Body composition plays a key role in both anaerobic and aerobic performance in professional soccer. However, its relationship with physical performance in female players remains underexplored. Assessing these parameters at the beginning of the pre-season provides valuable insights for optimizing player readiness. This study investigates the correlation between anthropometric profile, body composition, and physical performance in professional female soccer players at pre-season onset. Additionally, it analyzes positional differences and their relation to the specific physical demands of women's soccer. **Methods:** A cross-sectional study of thirty-four female soccer players (age: 23.06 ± 4.29 years, height: 164.15 ± 5.84 cm, weight: 58.39 ± 6.62 kg, and $\Sigma 6$ skinfolds: 74.57 ± 18.48 mm) who performed anthropometric measurements, the Countermovement Jump (CMJ), Wingate Anaerobic Test (WAnT), and Yo-Yo Intermittent Recovery Level 1 (Yo-Yo IR1) on the first day of the pre-season. One-way ANOVA with Tukey's post hoc test assessed positional differences, while Pearson's or Spearman's correlation coefficients evaluated relationships between anthropometric variables and performance outcomes. **Results:** Very high positive correlations were found between body and muscle mass with the power variables in CMJ and WAnT ($r = 0.70\text{--}0.89$; $p < 0.001$). An almost perfect correlation was observed between body mass and peak power in WAnT ($r = 0.904$; $p < 0.001$). In contrast, fat mass showed moderate negative correlations with jump height and aerobic performance (Yo-Yo IR1 distance) ($r = 0.30\text{--}0.49$; $p < 0.04$). Significant differences were observed in the mean (757.60 ± 95.59 W) and peak power (773.59 ± 101.88 W) of CMJ pre-WAnT, with goalkeepers showing higher values compared to defenders and midfielders ($p < 0.05$). **Conclusions:** Body composition significantly influences athletic performance, showing positive correlations of muscle and body mass with anaerobic power and capacity. In contrast, fat mass showed negative correlations with explosive strength, recovery, and aerobic capacity.

Keywords: anthropometry; physical activity; athletic training; sports performance; morphology

1. Introduction

The development of women's soccer, both on and off the pitch, is progressing at an unprecedented rate. Key drivers of this growth include recent reforms in competition structures, the modernization of player development programs, and the continued professionalization of the women's game [1]. These factors collectively contribute to enhancing performance, increasing visibility, and promoting gender equity within the sport [2]. However, research on women's soccer remains comparatively limited, particularly compared with male players [3].

Soccer is a multidirectional, high-intensity intermittent sport in which success depends on the physical, technical, tactical, and body composition aspects of players [4–8]. Anthropometry provides a systematic method for measuring, quantifying, and characterization of the morphological composition of the human body [9]. Morphological characteristics and body composition have become critical factors, as they significantly influence performance in soccer [8,10,11].

From a physiological perspective, soccer can be characterized as predominantly aerobic exercise combined with frequent, intermittent, short, intense actions with a high rate of anaerobic energy turnover [12]. Players' recovery capacity during repeated high-intensity efforts is strongly related to the development of aerobic capacity [13,14], often assessed using the Yo-Yo IR1 [15]. On the other hand, explosive-type efforts, such as sprints or jumps, are important factors for soccer performance [16]. These efforts depend on the anaerobic capacity of the neuromuscular system, specifically on the explosive strength and anaerobic power of lower limbs [17], commonly evaluated using tests such as the CMJ [18–20] and the WAnT [21].

The relationship between anthropometric characteristics and physical performance can assist in evaluating the specificity of pre-season training programs. Moreover, it is essential to consider that in high-performance sports contexts such as soccer, the pre-season is often relatively short compared to the competitive period. This makes it increasingly important to assess and monitor body composition and physical fitness parameters before the competitive period begins in order to have a more complete assessment of the players' initial state [22]. However, information on physical performance metrics at the beginning of the pre-season, especially right after the off-season, is also lacking. To our knowledge, this is the first study to explore the relationship between anthropometric profiles, body composition, and performance using Yo-Yo IR1, CMJ, and WAnT on the first days of the preseason.

The primary aim of this study is to examine the correlation between anthropometric profile, body composition, and physical performance in professional female soccer players at the onset of the pre-season. A secondary aim is to examine how positional differences relate to the physical demands specific to women's soccer.

We hypothesize that anthropometric profile and body composition play a significant role in the physical performance of female soccer players at the onset of the pre-season, with muscle mass having a positive impact on anaerobic performance and fat mass being negatively associated with key performance metrics.

2. Materials and Methods

2.1. Study Design

A cross-sectional study was carried out at the beginning of the pre-season. Recruitment and initial contact with the teams were conducted two months before the study, with player recruitment finalized one month prior to data collection. All determinations were performed on all players on the first day of the pre-season. On this day, anthropometric measurements, body composition measurements, and physical performance tests, including

the CMJ and WAnT, were conducted at the facilities of the Catholic University San Antonio of Murcia (UCAM). Two days later, the Yo-Yo IR1 was performed at the respective training fields of the participating teams. Prior to starting the intervention, the protocol was approved by the Institutional Review Committee of the UCAM (code CE052204), following the Standards of Good Clinical Practice, and was conducted according to the Declaration of Helsinki. The clinical trial was registered at www.clinicaltrials.gov (accessed on 9 August 2022) (identifier NCT05525871). Current European legislation on the protection of personal data (Regulation (EU)2016/679) was complied with.

2.2. Participants

Research was conducted on a sample of 34 professional female players (age: 23.06 ± 4.29 years) from two soccer teams in the first and second Spanish professional Women's Football League.

The inclusion criteria for participant recruitment were as follows: (a) healthy subject with medical authorization for the practice of federated sport; (b) belongs to a team of the first or second division of the Spanish Women's League. The exclusion criteria for the study were: (a) changing teams during pre-season; (b) history of drug, alcohol, or other substance abuse or other factors limiting their ability to cooperate during the study. All players were previously informed of the objectives and method of the research, signing informed consent forms before starting the research. In the case of minor players, it was their parents who signed the consent.

2.3. Anthropometric Measurements

All anthropometric measurements, including basic measurements, eight skinfolds, six breadths, and six perimeters, were performed twice to minimize errors. These measurements were carried out by a level 2 anthropometrist, accredited by the International Society for the Advancement of Kinanthropometry (ISAK) [23], following the procedures established by that organization. The methodology and equipment used are detailed in a previous publication [24].

2.4. Countermovement Jump

Explosive strength, anaerobic power, and neuromuscular fatigue in the lower limbs were measured and analyzed using the CMJ [18–20] performed on a contact platform (Chronojump Boscosystem, Barcelona, Spain). The CMJ analysis was performed using Chronojump software, version 2.2.1. For correct execution, subjects placed their hands on their hips, bent their knees to a 90-degree angle, and jumped to maximum height in a single motion. The knee's right angle was monitored in the sagittal plane using real-time video digitization software. The system included an A2-sized contact platform with two isolated electrical contacts acting as a pressure-dependent switch. This switch closed as the player stood on the platform. A PC connected to the platform calculated flight time with a temporal resolution of 1 m/s. The change in center of gravity during flight (jump height, h) was calculated using the measured flight time (t) with the standardized Equation (1):

$$h \text{ (cm)} = (t^2 \times g) / 8 \quad (1)$$

where g is the acceleration due to gravity (9.81 m/s^2). A total of three jumps were performed, with the average height recorded. This same protocol was applied for the CMJs performed before and after the WAnT (see Figure 1). From each CMJ test, jump height (cm), mean ($\text{CMJ}_{\text{Pmean}}$), and peak power (W) produced ($\text{CMJ}_{\text{Ppeak}}$) were extracted, as indicators of explosive strength, anaerobic power, and neuromuscular fatigue [25].

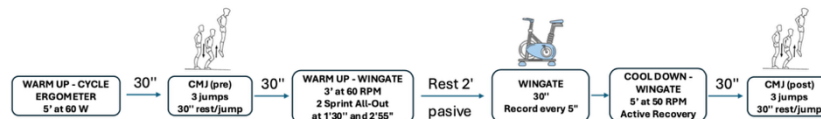


Figure 1. Timeline CMJ-WAnT.

2.5. Wingate Anaerobic Test

Anaerobic power and anaerobic capacity were analyzed using the WAnT [21]. For the execution of this test, the optimal seat height on a Monark 894E weight ergometer (Monark, Vansbro, Sweden) was adjusted for the players. The seat height was set so that knee flexion was no more than 5 degrees with the leg fully extended. Each subject underwent a 3-min warm-up period at 60 revolutions per minute. Two all-out sprints of five seconds were included during the warm-up: the first sprint at one minute and thirty seconds, and the second sprint at two minutes and fifty-five seconds. This was done to familiarize them with the test. After the warm-up, subjects rested passively for two minutes before beginning the WAnT. The resistance load was set at 7.5% of the subject's body weight [26]. The test procedure consisted of a 10-s countdown, followed by a 30-s all-out pedaling phase and an active recovery phase. During the first 5 s of the countdown, subjects remained stationary, with the dominant leg positioned forward in preparation for the start and the pedals slightly offset from being fully horizontal. At the end of the countdown, the resistance load was released, and subjects began pedaling. All subjects were verbally encouraged to continue pedaling as fast as they could for the 30 s. Power for the following 30 s was recorded using a video camera, and later, data as peak power (P_{peak}), time (s) to reach P_{peak} (TP_{peak}), mean power during the 30 s sprint (P_{mean}), and minimal power (P_{min}) were manually transcribed. The Fatigue Index (%) (FI) was calculated. In addition to P_{mean} during the entire sprint, mean power was also calculated every 10 s and 15 s of the sprint ($Split_{0-10S}$, $Split_{10-20S}$, $Split_{20-30S}$, $Split_{0-15S}$, $Split_{15-30S}$).

2.6. Yo-Yo Intermittent Recovery (Level 1) Test

Aerobic capacity was analyzed, and maximal oxygen uptake (VO_{2max}) was estimated using the Yo-Yo IR1 [15]. This test consists of 2×20 m shuttle runs at increasing speeds, interspersed with a 10-s period of active recovery (controlled by audio signals from a CD player). An individual is running until he/she is not able to maintain the speed. Finally, at the end of the test, the speed level, speed (km/h), time (s), and distance (m) were recorded. Additionally, VO_{2max} values of the players were estimated by using the following Equation (2) [15]:

$$VO_{2max} \text{ (mL/min/Kg)} = (\text{Yo-Yo IR1 distance (m)} \times 0.0084 + 36.4) \quad (2)$$

2.7. Statistical Analyses

SPSS Statistics 27 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Descriptive statistics were calculated and used to describe the physical performance variables of the players.

The statistics were reported as mean $\pm$ standard deviation (SD). The Shapiro–Wilk test was used to check the normal distribution of data. One-way analysis of variance (ANOVA) was used to determine differences between physical performance variables among the four playing positions. Tukey's post hoc test was used to determine which variables differed significantly. The level of statistical significance was set at $p \leq 0.05$. Pearson's

or Spearman's correlation coefficient (r) was used to determine possible the correlation between anthropometric profile and body composition and physical performance variables.

3. Results and Discussion

The demographic characteristics of the players can be seen in Table 1. The anthropometric measurements and body composition of the players are available in detail in a previous publication by our research team [24].

Table 1. Demographic data of the players: mean and standard deviations (mean $\pm$ SD).

	Mean $\pm$ SD
n	34
Age	23.06 $\pm$ 4.29
Height (cm)	164.15 $\pm$ 5.84
Weight (kg)	58.39 $\pm$ 6.62
BMI (kg/m ²)	21.64 $\pm$ 21.59
Σ 6 Skinfolds (mm)	74.57 $\pm$ 18.48

BMI: Body Mass Index; Σ 6 Skinfolds (triceps, subscapular, supraspinal, abdominal, front thigh, and medial calf).

The descriptive statistics for CMJ pre-WAnT are available in Table 2, including jump height, P_{mean} , and P_{peak} . Regarding jump height, goalkeepers showed the highest values. That fact agrees with the observations reported on Tunisian first-division players [27]. However, other studies reported higher jump heights for forwards [28–31] or defenders [32,33]. The greater jump height observed in goalkeepers may be partially attributed to their role-specific demands, as they regularly perform vertical and lateral jumps during both training and matches [27].

Table 2. Descriptive statistics of CMJ before WAnT: mean and standard deviations (mean $\pm$ SD).

Variable	MF	DF	FW	GK	Total	p-Value
Height ₁	24.46 $\pm$ 2.78	25.04 $\pm$ 3.31	26.97 $\pm$ 5.55	30.22 $\pm$ 5.71	26.01 $\pm$ 4.42	0.199
CMJ _{Pmean1}	617.91 $\pm$ 54.43	622.56 $\pm$ 60.62	667.11 $\pm$ 97.78	757.60 $\pm$ 95.59	648.80 $\pm$ 83.97	0.046 *
CMJ _{Ppeak1}	631.11 $\pm$ 54.34	633.38 $\pm$ 64.99	681.38 $\pm$ 101.27	773.59 $\pm$ 101.88	661.85 $\pm$ 87.31	0.049 *

MF: Midfielders; DF: Defenders; FW: Forwards; GK: Goalkeepers; Height: Jump height (cm); P_{mean} : Mean power (W); P_{peak} : Power peak (W). * Significant differences between positions ($p < 0.05$), as determined by ANOVA.

Despite these descriptive observations, no statistically significant differences were found between playing positions for jump height, confirming the findings in other studies [28–30,32,33]. However, some research has reported positional differences. For example, a study on Tunisian first-division players [27] reported that goalkeepers and forwards exhibited higher jump heights. Similarly, a study on the Chilean national team [31] found that defenders and forwards had higher values compared to other positions but did not include goalkeepers. It is important to highlight that there is a limited number of studies reporting the jump height of female soccer players in different playing positions, especially those including goalkeepers [27,28,30,32,33].

Following on from jump height and analyzing the lowest values, midfielders showed the lowest jump height. This finding is concordant with observations in Tunisian [27], Norwegian [33], and Serbian [29] first-division players and the national team of Chile [30,31]. Other studies reported the lower jump height in semi-professional South African goalkeepers [28] and Montenegrin national team forwards [32].

The jump height reported in the present research for all players (26.01 ± 4.42) is comparable to that observed in a previous study on Spanish first-division players in 2009 that measured the players during the season (26.10 ± 4.8) [16]. Since the measurements were obtained on the first day of the pre-season, this limits the comparison with other studies due to possible seasonal variations.

This research shows higher jump heights compared to pre-season data for lower-level Spanish players [34–36] and elite Serbian players [37]. However, these values are lower than those recorded in pre-season for Norwegian first-division players (32.6 ± 4.1) [33] and the Finnish national team (32.3 ± 3.4) [38]. Considering measurements obtained at different times of the season, the jump height observed was higher than those at the end of the season for the Montenegrin national team [32]. In turn, it was lower than those found in Danish [39], Spanish [40], Brazilian [8], Swedish [41], English [42], and Norwegian [41,43] first-division players, as well as the Italian [44] and Norwegian [43] national teams.

As observed in jump height, goalkeepers showed the highest CMJ_{Pmean} and CMJ_{Ppeak} values. However, in contrast to jump height, both variables were statistically different between positions (CMJ_{Pmean} (η^2 partial = 0.252, $p = 0.046$) and CMJ_{Ppeak} (η^2 partial = 0.249, $p = 0.048$). Specifically, goalkeepers had higher CMJ_{Pmean} compared to defenders ($p = 0.056$) and midfielders ($p = 0.055$) and higher CMJ_{Ppeak} compared to defenders ($p = 0.058$).

Following the examination of positional differences, the focus then shifts to understanding the correlations between CMJ performance variables and anthropometric and body composition measurements (Table 3). Initially, only those that were high, very high, or almost perfect—defined as having a correlation coefficient value greater than or equal to 0.5 [45] and a $p < 0.001$ —were included. However, this criterion excluded moderate correlations between anthropometric variables and CMJ height, as well as Yo-Yo IR1 variables such as distance and $\text{VO}_{2\text{max}}$. Thus, moderate correlations for these specific variables were included to provide a more comprehensive analysis.

Moreover, jump height, used as a measure of explosive strength, showed a moderate negative correlation with anthropometric variables related to fat mass, such as biceps skinfold, percentage, and kilograms of fat mass calculated using different equations, sum of six and eight skinfolds, and endomorphy. Additionally, a moderate positive correlation was found with muscle mass percentage (using Poortmans' equation). These findings show a negative correlation between jump height and fat mass and a positive correlation with muscle mass. Those differences were consistent with studies on Serbian elite [37] and adolescent [46] players and the Chilean national team [30].

In turn, CMJ_{Pmean} and CMJ_{Ppeak} are used for the measurement of anaerobic power. They both showed a very high positive correlation with body mass and kilograms of muscle mass. This can be explained by the fact that power is the product of force and velocity ($\text{Power} = \text{Force} \times \text{Velocity}$) and force is directly proportional to mass and acceleration ($\text{Force} = \text{Mass} \times \text{Acceleration}$). As a result, players with greater body and muscle mass generate more force, which leads to higher power output.

Similarly, both variables also had high positive correlations with body dimensions, kilograms of bone mass, and lower limb perimeters. Moreover, peak power also showed a high positive correlation with kilograms of fat using Faulkner's equation. It is important to note that, in both variables, there were no negative correlations with anthropometric variables related to fat in contrast to jump height.

Table 3. Correlation between anthropometry and CMJ before WAnT.

Anthropometry vs. CMJ Pre-WAnT	r	Magnitude	Lower 95% IC	Upper 95% IC	p-Value
Height					
Biceps (mm)	−0.448	Moderate	−0.692	−0.111	0.011
Muscle (%) (Poortmans)	0.444	Moderate	0.107	0.690	0.012
Fat (%) (Carter)	−0.429	Moderate	−0.680	−0.089	0.016
Σ6 skinfolds	−0.429	Moderate	−0.680	−0.089	0.016
Fat (%) (Slaughter)	−0.415	Moderate	−0.671	−0.071	0.020
Σ8 skinfolds	−0.410	Moderate	−0.667	−0.065	0.023
Fat (%) (Withers)	−0.398	Moderate	−0.659	−0.051	0.027
Fat (%) (Jackson & Pollock)	−0.390	Moderate	−0.654	−0.042	0.030
Endomorphy	−0.380	Moderate	−0.647	−0.029	0.035
Fat (kg) (Slaughter)	−0.370	Moderate	−0.641	−0.018	0.040
Fat (kg) (Withers)	−0.365	Moderate	−0.637	−0.012	0.045
CMJ _{Pmean}					
Body Mass (kg)	0.772	Very High	0.575	0.884	<0.001
Muscle (kg) (Lee)	0.729	Very High	0.506	0.861	<0.001
Muscle (kg) (Poortmans)	0.704	Very High	0.466	0.847	<0.001
Wrist (bistylloid) (cm)	0.692	High	0.447	0.840	<0.001
Wingspan (cm)	0.679	High	0.427	0.833	<0.001
Bone Mass (kg) (Rocha)	0.674	High	0.420	0.830	<0.001
Height (cm)	0.649	High	0.383	0.816	<0.001
Calf (cm)	0.616	High	0.334	0.796	<0.001
Sitting Height (cm)	0.613	High	0.331	0.795	<0.001
CMJ _{Ppeak}					
Body Mass (kg)	0.779	Very High	0.586	0.888	<0.001
Muscle (kg) (Lee)	0.733	Very High	0.511	0.863	<0.001
Muscle (kg) (Poortmans)	0.702	Very High	0.463	0.846	<0.001
Wrist (bistylloid) (cm)	0.683	High	0.434	0.835	<0.001
Wingspan (cm)	0.672	High	0.417	0.829	<0.001
Bone Mass (kg) (Rocha)	0.655	High	0.391	0.819	<0.001
Height (cm)	0.643	High	0.374	0.812	<0.001
Calf (cm)	0.634	High	0.361	0.807	<0.001
Fat (kg) (Faulkner)	0.596	High	0.306	0.785	<0.001
Sitting Height (cm)	0.594	High	0.304	0.784	<0.001
Humerus (cm)	0.575	High	0.277	0.772	<0.001

$r = 0.0\text{--}0.09$ were considered trivial, $r = 0.10\text{--}0.29$ small, $r = 0.30\text{--}0.49$ moderate, $r = 0.50\text{--}0.69$ high, $r = 0.70\text{--}0.89$ very high, $r = 0.90\text{--}0.99$ almost perfect, and $r = 1$ perfect correlation [45].

Tables 4–6 show the descriptive statistics for CMJ post-WAnT, as well as the variation for absolute and relative values, providing insights into neuromuscular fatigue. Although no statistically significant differences were observed between positions, goalkeepers consistently showed the highest values across all variables, followed by forwards, defenders, and midfielders. This pattern aligns with CMJ pre-WAnT measurements.

Table 4. Descriptive statistics of CMJ after WAnT: mean and standard deviations (mean $\pm$ SD).

Variable	MF	DF	FW	GK	Total	p-Value
Height ₂	23.01 $\pm$ 3.20	23.50 $\pm$ 3.03	24.10 $\pm$ 5.74	26.47 $\pm$ 4.79	23.85 $\pm$ 4.18	0.680
CMJ _{Pmean2}	598.40 $\pm$ 50.88	603.03 $\pm$ 58.26	630.68 $\pm$ 104.75	710.72 $\pm$ 102.19	621.17 $\pm$ 81.79	0.184
CMJ _{Ppeak2}	609.75 $\pm$ 51.73	613.84 $\pm$ 56.66	645.71 $\pm$ 114.22	719.69 $\pm$ 106.51	633.31 $\pm$ 85.61	0.227

MF: Midfielders; DF: Defenders; FW: Forwards; GK: Goalkeepers; Height: Jump height (cm); P_{mean}: Mean power (W); P_{peak}: Power peak (W).

Table 5. Variation of CMJ: mean and standard deviations (mean $\pm$ SD).

Variable	MF	DF	FW	GK	Total	p-Value
Δ Height	-1.45 ± 1.40	-1.54 ± 1.82	-2.87 ± 1.78	-3.76 ± 1.37	-2.16 ± 1.79	0.086
Δ CMJ _{Pmean}	-19.51 ± 19.53	-19.53 ± 23.21	-36.44 ± 22.56	-46.87 ± 7.27	-27.63 ± 22.57	0.104
Δ CMJ _{Ppeak}	-21.36 ± 23.87	-19.54 ± 32.17	-35.66 ± 26.53	-53.90 ± 4.64	-28.54 ± 27.89	0.196

MF: Midfielders; DF: Defenders; FW: Forwards; GK: Goalkeepers; P_{mean}: Mean power (W); P_{peak}: Power peak (W).**Table 6.** Variation of CMJ %: mean and standard deviations (mean $\pm$ SD).

Variable	MF	DF	FW	GK	Total	p-Value
Δ Height	-6.78 ± 7	-6.84 ± 7.90	-12.80 ± 8.45	-14.10 ± 4.39	-9.45 ± 7.93	0.188
Δ CMJ _{Pmean}	-3.27 ± 3.35	-3.30 ± 3.82	-6.15 ± 3.98	-6.78 ± 2.05	-4.55 ± 3.79	0.185
Δ CMJ _{Ppeak}	-3.54 ± 3.96	-3.21 ± 5.00	-6.05 ± 4.48	-7.67 ± 1.81	-4.65 ± 4.45	0.283

MF: Midfielders; DF: Defenders; FW: Forwards; GK: Goalkeepers; P_{mean}: Mean power (W); P_{peak}: Power peak (W).

In terms of variation, goalkeepers experienced the largest reductions in both jump height and power, which could indicate greater neuromuscular fatigue compared to other positions. Forwards also exhibited notable decreases, while defenders and midfielders showed smaller declines. Notably, midfielders experienced a more pronounced drop in peak power than jump height, suggesting that although they maintained jump height relatively well, their ability to produce peak power was more affected. This may be related to the specific demands of their position, which could emphasize repeated moderate-intensity actions over maximal power output.

These differences in the extent of the reductions may be linked to the neuromuscular recovery capacity of each position. However, the larger decreases observed in goalkeepers and forwards could also be attributed to their higher initial values of CMJ pre-WAnT, which might suggest a greater capacity for decline. Overall, the interaction between neuromuscular fatigue and initial performance levels seems to play a key role in the observed CMJ post-WAnT performance variations.

Regarding the correlation between anthropometric variables and CMJ post-WAnT data (Table 7), similar results to those observed in CMJ pre-WAnT. For jump height, there were moderate negative correlations with anthropometric variables related to fat, but in this case, a high negative correlation was specifically found with the suprailiac skinfold. For CMJ_{Pmean} and CMJ_{Ppeak}, as with CMJ pre-WAnT, there was a very high positive correlation with body mass and kilograms of muscle, but only with Lee's equation. Additionally, after WAnT, there was now a very high positive correlation with wingspan. CMJ_{Pmean} also showed a very high positive correlation with kilograms of bone mass post-WAnT. Other anthropometric variables with high positive correlations included body dimensions, bone diameters, and lower limb perimeters.

Table 8 shows the descriptive statistics for WAnT. Although no significant differences were found between positions for any of the variables, some trends are noteworthy. Goalkeepers consistently show the highest values across most variables, followed by forwards. An exception was found in P_{min} and forwards showed slightly higher values than goalkeepers. The TP_{peak} is similar across all positions.

Table 7. Correlation between anthropometry and CMJ after WAnT.

Anthropometry vs. CMJ Post-WAnT	r	Magnitude	Lower 95% IC	Upper 95% IC	p-Value
Height					
Supraspinal (mm)	−0.502	High	−0.727	−0.180	0.004
Fat (kg) (Slaughter)	−0.483	Moderate	−0.715	−0.155	0.006
Fat (%) (Carter)	−0.484	Moderate	−0.716	−0.157	0.006
Σ6 skinfolds	−0.484	Moderate	−0.716	−0.157	0.006
Fat (kg) (Withers)	−0.473	Moderate	−0.709	−0.143	0.008
Σ8 skinfolds	−0.474	Moderate	−0.709	−0.144	0.008
Fat (%) (Slaughter)	−0.464	Moderate	−0.703	−0.131	0.009
Fat (%) (Withers)	−0.455	Moderate	−0.697	−0.120	0.010
Biceps (mm)	−0.452	Moderate	−0.695	−0.116	0.011
Fat (%) (Jackson & Pollock)	−0.449	Moderate	−0.693	−0.112	0.011
Fat (kg) (Carter)	−0.451	Moderate	−0.694	−0.115	0.012
Endomorphy	−0.439	Moderate	−0.687	−0.100	0.013
Muscle (%) (Poortmans)	0.429	Moderate	0.088	0.680	0.016
Medial Calf (mm)	−0.426	Moderate	−0.678	−0.084	0.017
Front thigh (mm)	−0.400	Moderate	−0.661	−0.054	0.026
Fat (%) (Durnin)	−0.390	Moderate	−0.654	−0.041	0.030
Fat (kg) (Durnin)	−0.390	Moderate	−0.654	−0.041	0.031
Fat (kg) (Jackson & Pollock)	−0.385	Moderate	−0.651	−0.036	0.033
Fat (%) (Faulkner)	−0.374	Moderate	−0.643	−0.023	0.038
CMJ _{Pmean}					
Body Mass (kg)	0.760	Very High	0.554	0.878	<0.001
Muscle (kg) (Lee)	0.729	Very High	0.505	0.861	<0.001
Wingspan (cm)	0.711	Very High	0.476	0.851	<0.001
Bone Mass (kg) (Rocha)	0.706	Very High	0.469	0.848	<0.001
Muscle (kg) (Poortmans)	0.699	High	0.458	0.844	<0.001
Wrist (bistyloid) (cm)	0.687	High	0.440	0.838	<0.001
Height (cm)	0.675	High	0.421	0.831	<0.001
Sitting Height (cm)	0.652	High	0.388	0.818	<0.001
Calf (cm)	0.592	High	0.301	0.782	<0.001
CMJ _{Ppeak}					
Body Mass (kg)	0.740	Very High	0.523	0.867	<0.001
Muscle (kg) (Lee)	0.722	Very High	0.494	0.857	<0.001
Wingspan (cm)	0.700	Very High	0.459	0.845	<0.001
Muscle (kg) (Poortmans)	0.697	High	0.456	0.843	<0.001
Bone Mass (kg) (Rocha)	0.686	High	0.439	0.837	<0.001
Wrist (bistyloid) (cm)	0.677	High	0.425	0.832	<0.001
Height (cm)	0.659	High	0.397	0.821	<0.001
Sitting Height (cm)	0.626	High	0.348	0.802	<0.001
Calf (cm)	0.588	High	0.295	0.780	<0.001

r = 0.0–0.09 were considered trivial, r = 0.10–0.29 small, r = 0.30–0.49 moderate, r = 0.50–0.69 high, r = 0.70–0.89 very high, r = 0.90–0.99 almost perfect, and r = 1 perfect correlation [45].

Comparing midfielders and defenders, despite having similar overall values, midfielders have a higher P_{peak} and P_{min} . In turn, defenders show a slightly higher P_{mean} in Split_{20–30S} and Split_{15–30S}.

The high values of P_{peak} and P_{mean} observed for goalkeepers are consistent with observations from a study on Greek first-division players during the pre-season [21]. However, forwards have the second highest P_{peak} and P_{mean} values, whereas in the Greek study, midfielders held these values. Regarding FI, defenders and goalkeepers show the highest values, followed by forwards and midfielders, who have nearly identical

values. Comparing the values between both studies, the data observed in this research are higher than those reported for Greek players. To the best of our knowledge, the study by Nikolaidis [21] is the only one to conduct a WAnT on professional female soccer players and differentiate by playing position.

Table 8. Descriptive statistics of WAnT: mean and standard deviations (mean $\pm$ SD).

Variable	MF	DF	FW	GK	Total	p-Value
P _{peak}	600.25 $\pm$ 81.27	592.50 $\pm$ 64.95	633.64 $\pm$ 84.34	680.00 $\pm$ 145.59	616.78 $\pm$ 84.42	0.368
TP _{peak}	7.00 $\pm$ 1.60	6.40 $\pm$ 1.35	6.64 $\pm$ 1.75	6.67 $\pm$ 3.22	6.66 $\pm$ 1.68	0.912
P _{min}	385.25 $\pm$ 39.43	377.60 $\pm$ 37.89	399.64 $\pm$ 59.64	392.00 $\pm$ 35.55	388.44 $\pm$ 45.64	0.752
FI (%)	35.21 $\pm$ 7.56	35.91 $\pm$ 6.41	36.49 $\pm$ 9.58	41.29 $\pm$ 8.08	36.44 $\pm$ 7.83	0.727
P _{mean}	491.99 $\pm$ 57.24	491.27 $\pm$ 41.69	520.87 $\pm$ 63.23	538.92 $\pm$ 61.53	506.09 $\pm$ 55.52	0.398
Split _{0-10S}	550.44 $\pm$ 81.74	545.59 $\pm$ 53.99	585.60 $\pm$ 83.35	626.60 $\pm$ 130.63	568.15 $\pm$ 79.88	0.358
Split _{10-20S}	517.77 $\pm$ 59.64	515.74 $\pm$ 53.49	546.68 $\pm$ 68.69	562.67 $\pm$ 41.53	531.28 $\pm$ 59.76	0.468
Split _{20-30S}	418.11 $\pm$ 45.02	422.21 $\pm$ 40.24	441.22 $\pm$ 58.67	440.12 $\pm$ 27.14	429.40 $\pm$ 46.86	0.690
Split _{0-15S}	547.87 $\pm$ 73.93	542.37 $\pm$ 50.28	580.95 $\pm$ 77.23	614.04 $\pm$ 101.34	563.72 $\pm$ 71.53	0.345
Split _{15-30S}	441.20 $\pm$ 48.92	445.47 $\pm$ 44.51	466.14 $\pm$ 60.49	469.94 $\pm$ 28.87	453.80 $\pm$ 49.92	0.642

MF: Midfielders; DF: Defenders; FW: Forwards; GK: Goalkeepers; P_{peak}: Peak power (W); TP_{peak}: Time to P_{peak} (s); P_{min}: Minimum power (W); FI (%): Fatigue index; P_{mean}: Mean power (W); Split: P_{mean} calculated every 10 and 15 s.

It is important to note the lack of scientific literature conducting WAnT on female soccer players. Comparing the values from this research for all players, P_{peak} is lower (616.78 $\pm$ 84.42) than that observed in American collegiate players during the pre-season (716.87 $\pm$ 137.18) [47] but higher than that in Turkish (439.7 $\pm$ 61.1) [48] and Greek first-division players (578 $\pm$ 72) [49]. P_{mean} is greater (506.09 $\pm$ 55.52) compared to both American collegiate (356.61 $\pm$ 68.89) [47], Turkish [48] (316.1 $\pm$ 34.4), and Greek first-division players (431 $\pm$ 50) [49]. Additionally, FI is significantly lower than the values reported for the American (82.08 $\pm$ 11.86) [47], Turkish (55.7 $\pm$ 3.75) [48], and Greek players (45.9 $\pm$ 8.3) [49].

After examining the descriptive statistics by position, the focus shifts to understanding how WAnT performance variables correlate with anthropometric and body composition measurements (Table 9). P_{peak}, defined as the highest mechanical strength achieved during any 5-s time period during the test, and an indicator of anaerobic power [48], shows an almost perfect positive correlation with body mass, and very high positive correlations with muscle mass (Lee's equation), fat mass (Faulkner and Durnin equations), and perimeters such as thigh, contracted and relaxed arm, waist, and calf.

Table 9. Correlation between anthropometry and WAnT.

Anthropometry vs. WAnT	r	Magnitude	Lower 95% IC	Upper 95% IC	p-Value
		P _{peak}			
Body Mass (kg)	0.904	Almost perfect	0.810	0.952	<0.001
Muscle (kg) (Lee)	0.796	Very High	0.619	0.896	<0.001
Fat (kg) (Faulkner)	0.761	Very High	0.561	0.877	<0.001
Thigh (cm)	0.733	Very High	0.517	0.862	<0.001
Humerus (cm)	0.730	Very High	0.512	0.860	<0.001
Fat (kg) (Durnin)	0.730	Very High	0.512	0.860	<0.001
Relaxed arm (cm)	0.726	Very High	0.505	0.858	<0.001
Calf (cm)	0.726	Very High	0.505	0.857	<0.001
Contracted arm (cm)	0.713	Very High	0.485	0.850	<0.001
Waist (cm)	0.700	Very High	0.464	0.843	<0.001

Table 9. Cont.

Anthropometry vs. WAnT	r	Magnitude	Lower 95% IC	Upper 95% IC	p-Value
Fat (kg) (Jackson & Pollock)	0.695	High	0.457	0.840	<0.001
Muscle (kg) (Poortmans)	0.680	High	0.435	0.832	<0.001
Wrist (bistylod) (cm)	0.677	High	0.430	0.830	<0.001
Hip (cm)	0.666	High	0.414	0.824	<0.001
Sitting Height (cm)	0.654	High	0.395	0.816	<0.001
Bone Mass (kg) (Rocha)	0.642	High	0.378	0.810	<0.001
Fat (kg) (Carter)	0.631	High	0.362	0.803	<0.001
Height (cm)	0.629	High	0.359	0.802	<0.001
Hip (cm)	0.629	High	0.358	0.802	<0.001
Fat (kg) (Withers)	0.609	High	0.218	0.739	<0.001
Subscapular (mm)	0.567	High	0.273	0.765	<0.001
Wingspan (cm)	0.564	High	0.268	0.763	<0.001
P _{mean}					
Body Mass (kg)	0.799	Very High	0.624	0.898	<0.001
Muscle (kg) (Lee)	0.763	Very High	0.565	0.878	<0.001
Wrist (bistylod) (cm)	0.706	Very High	0.474	0.846	<0.001
Muscle (kg) (Poortmans)	0.677	High	0.430	0.830	<0.001
Bone Mass (kg) (Rocha)	0.667	High	0.415	0.824	<0.001
Sitting Height (cm)	0.659	High	0.403	0.820	<0.001
Height (cm)	0.649	High	0.388	0.813	<0.001
Humerus (cm)	0.625	High	0.353	0.799	<0.001
Fat (kg) (Faulkner)	0.619	High	0.345	0.796	<0.001
Wingspan (cm)	0.600	High	0.318	0.785	<0.001
Thigh (cm)	0.580	High	0.290	0.773	<0.001
Calf (cm)	0.580	High	0.290	0.772	<0.001
Split _{0-10S}					
Body Mass (kg)	0.889	Very High	0.782	0.945	<0.001
Muscle (kg) (Lee)	0.796	Very High	0.619	0.896	<0.001
Fat (kg) (Faulkner)	0.777	Very High	0.587	0.886	<0.001
Fat (kg) (Durnin)	0.757	Very High	0.555	0.875	<0.001
Calf (cm)	0.751	Very High	0.546	0.872	<0.001
Thigh (cm)	0.747	Very High	0.538	0.869	<0.001
Humerus (cm)	0.724	Very High	0.503	0.857	<0.001
Fat (kg) (Jackson & Pollock)	0.711	Very High	0.482	0.849	<0.001
Relaxed arm (cm)	0.710	Very High	0.480	0.849	<0.001
Contracted arm (cm)	0.702	Very High	0.467	0.844	<0.001
Waist (cm)	0.686	High	0.443	0.835	<0.001
Muscle (kg) (Poortmans)	0.681	High	0.436	0.832	<0.001
Hip (cm)	0.669	High	0.418	0.825	<0.001
BMI (kg/m ²)	0.662	High	0.408	0.821	<0.001
Fat (kg) (Carter)	0.645	High	0.382	0.811	<0.001
Fat (kg) (Withers)	0.630	High	0.360	0.802	<0.001
Wrist (bistylod) (cm)	0.624	High	0.352	0.799	<0.001
Height (cm)	0.611	High	0.333	0.791	<0.001
Sitting Height (cm)	0.605	High	0.325	0.788	<0.001
Subscapular (mm)	0.589	High	0.303	0.778	<0.001
Bone Mass (kg) (Rocha)	0.575	High	0.283	0.770	<0.001
Split _{10-20S}					
Body Mass (kg)	0.688	High	0.447	0.836	<0.001
Muscle (kg) (Lee)	0.679	High	0.433	0.831	<0.001
Wrist (bistylod) (cm)	0.665	High	0.412	0.823	<0.001
Muscle (kg) (Poortmans)	0.633	High	0.364	0.804	<0.001
Bone Mass (kg) (Rocha)	0.589	High	0.303	0.778	<0.001
Humerus (cm)	0.583	High	0.293	0.774	<0.001

Table 9. Cont.

Anthropometry vs. WAnT	r	Magnitude	Lower 95% IC	Upper 95% IC	p-Value
Fat (kg) (Faulkner)	0.570	High	0.277	0.767	<0.001
Height (cm)	0.564	High	0.269	0.763	<0.001
Sitting Height (cm)	0.560	High	0.263	0.760	<0.001
Split _{20–30S}					
Bone Mass (kg) (Rocha)	0.631	High	0.362	0.803	<0.001
Wrist (bistylloid) (cm)	0.619	High	0.344	0.796	<0.001
Sitting Height (cm)	0.607	High	0.328	0.789	<0.001
Height (cm)	0.558	High	0.260	0.759	<0.001
Split _{0–15S}					
Body Mass (kg)	0.877	Very High	0.762	0.939	<0.001
Muscle (kg) (Lee)	0.803	Very High	0.632	0.900	<0.001
Fat (kg) (Faulkner)	0.758	Very High	0.556	0.875	<0.001
Fat (kg) (Durnin)	0.722	Very High	0.498	0.855	<0.001
Thigh (cm)	0.719	Very High	0.494	0.853	<0.001
Calf (cm)	0.713	Very High	0.485	0.850	<0.001
Muscle (kg) (Poortmans)	0.703	Very High	0.469	0.845	<0.001
Humerus (cm)	0.699	High	0.463	0.842	<0.001
Relaxed arm (cm)	0.684	High	0.441	0.834	<0.001
Contracted arm (cm)	0.672	High	0.422	0.827	<0.001
Fat (kg) (Jackson & Pollock)	0.665	High	0.411	0.823	<0.001
Wrist (bistylloid) (cm)	0.663	High	0.408	0.821	<0.001
BMI (kg/m ²)	0.646	High	0.383	0.812	<0.001
Waist (cm)	0.642	High	0.378	0.810	<0.001
Height (cm)	0.617	High	0.342	0.795	<0.001
Sitting Height (cm)	0.612	High	0.335	0.792	<0.001
Fat (kg) (Carter)	0.607	High	0.328	0.789	<0.001
Bone Mass (kg) (Rocha)	0.604	High	0.324	0.787	<0.001
Hip (cm)	0.603	High	0.322	0.786	<0.001
Fat (kg) (Withers)	0.587	High	0.300	0.777	<0.001
Wingspan (cm)	0.566	High	0.271	0.764	<0.001
Subscapular (mm)	0.560	High	0.262	0.760	<0.001
Split _{15–30S}					
Wrist (bistylloid) (cm)	0.627	High	0.357	0.801	<0.001
Bone Mass (kg) (Rocha)	0.619	High	0.344	0.796	<0.001
Sitting Height (cm)	0.590	High	0.304	0.778	<0.001
Height (cm)	0.564	High	0.268	0.763	<0.001
Muscle (kg) (Lee)	0.563	High	0.266	0.762	<0.001

r = 0.0–0.09 were considered trivial, r = 0.10–0.29 small, r = 0.30–0.49 moderate, r = 0.50–0.69 high, r = 0.70–0.89 very high, r = 0.90–0.99 almost perfect, and r = 1 perfect correlation [45].

Moreover, P_{mean} is defined as the average strength produced during the test and an indicator of anaerobic capacity [48]. It shows a very high positive correlation with body mass, muscle mass (Lee's equation), and wrist diameter.

To our knowledge, only one study correlates WAnT performance with anthropometric variables in female soccer players. This study on Greek first-division players [49] observed a moderate correlation of P_{peak} with body fat percentage, a high positive correlation with kilograms of fat mass, and a very high correlation with fat-free mass. For P_{mean} , the Greek study found no significant correlation with body fat percentage, but there was a moderate positive correlation with kilograms of fat mass and a very high correlation with fat-free mass.

The descriptive statistics for Yo-Yo IR1 are presented in Table 10. All variables are included, though the most used measures for assessing aerobic capacity are the distance covered and the estimated $\text{VO}_{2\text{max}}$ [15].

Table 10. Descriptive statistics of test Yo-Yo IR1: mean and standard deviations (mean $\pm$ SD).

Variable	MF	DF	FW	GK	Total	p-Value
Speed Level	16.75 $\pm$ 1.12	16.11 $\pm$ 1.15	15.72 $\pm$ 0.65	14.30 $\pm$ 1.41	16.03 $\pm$ 1.20	0.068
Speed (km/h)	14.84 $\pm$ 0.45	14.63 $\pm$ 0.40	14.54 $\pm$ 0.20	14.06 $\pm$ 0.48	14.62 $\pm$ 0.42	0.137
Distance (m)	1366.67 $\pm$ 355.45	1155.00 $\pm$ 376.11	1016.00 $\pm$ 221.99	640.00 $\pm$ 339.41	1133.33 $\pm$ 375.52	0.086
Time (s)	685.33 $\pm$ 174.13	580.75 $\pm$ 184.84	512.60 $\pm$ 110.31	323.50 $\pm$ 171.83	569.91 $\pm$ 185.43	0.083
VO _{2max}	47.88 $\pm$ 2.99	46.10 $\pm$ 3.16	44.93 $\pm$ 1.87	41.78 $\pm$ 2.85	45.92 $\pm$ 3.15	0.086

MF: Midfielders; DF: Defenders; FW: Forwards; GK: Goalkeepers.

Regarding distance covered, goalkeepers covered the least distance. That fact agrees with the observations reported for the national teams of Montenegro [32], Serbia [50], and Chile [30], as well as Tunisian first-division [27] and South African semi-professional players [28]. As expected, goalkeepers recorded lower values in the distance covered by Yo-Yo IR1. This result aligns with their typical game performance, covering approximately 4000 m compared to field players who average around 10,000 m [51].

Following with distance covered, but analyzing the highest values, midfielders covered the most. This finding is concordant with observations in Tunisian first-division players [27] and the national team of Chile [30]. Other studies reported the most distance covered by defenders from South Africa [28] and the national team of Montenegro [32].

No significant differences were found between positions for the distance covered, aligning with observations in semi-professional South African players [28] and the Serbian national team [50]. Conversely, other studies have reported significant positional differences. For example, the Montenegro national team showed differences between defenders and both goalkeepers and forwards [32]. Similarly, the Chilean national team found differences between midfielders and all other field positions [30]. Furthermore, significant differences were observed between goalkeepers and the rest positions in Tunisian first-division players [27].

The distance covered reported in the present research for all players (1133 $\pm$ 375.52) is comparable to that observed in a 2013 study on German second-division players (1102 $\pm$ 316). However, the timing of this test within the season is not specified. As mentioned earlier, since the measurements were taken on the first day of the pre-season, this introduces a limitation in comparing these results with other studies due to seasonal variations in performance. This research shows a higher distance covered compared to Turkish first-division players (676.3 $\pm$ 156.4) [48] and the Montenegro national team at the end of the season (801.42 $\pm$ 253.09) [32]. However, these values are lower than those found in Danish first-division players (1379), Spanish first-division players during the season (1224 $\pm$ 255), and Brazilian first-division players at the end of the pre-season (1556.4 $\pm$ 470.23).

Regarding VO_{2max}, there were no significant differences between positions. Midfielders had the highest VO_{2max} values, while goalkeepers had the lowest. These results are in line with those of the Chilean study [30], although the Chilean study found significant differences between these positions. The VO_{2max} values in this study are lower than those observed in the Chilean national team during the season [30]. When comparing total VO_{2max} values, the results of this research are similar to those reported for German second-division players [52]. In addition, these values are higher than those observed in Turkish first-division players [48] but lower than those observed in Spanish semi-professional players during the season [53].

These findings align with the physiological demands of each playing position. Midfielders typically cover the greatest distances during matches, requiring superior aerobic capacity to sustain both offensive and defensive contributions. Their ability to maintain

high-intensity efforts throughout the game may explain their higher Yo-Yo IR1 performance compared to other positions.

After examining the descriptive statistics by position, the focus shifts to understanding how Yo-Yo IR1 performance variables correlate with anthropometric and body composition measurements (Table 11).

Table 11. Correlation between anthropometry and Yo-Yo IR1.

Anthropometry vs. Yo-Yo IR1	r	Magnitude	Lower 95% IC	Upper 95% IC	p-Value
Distance					
Supraspinal (mm)	−0.452	Moderate	−0.739	−0.025	0.040
VO _{2max}					
Supraspinal (mm)	−0.452	Moderate	−0.739	−0.025	0.040
Wingspan (cm)	−0.454	Moderate	−0.740	−0.027	0.039

$r = 0.0\text{--}0.09$ were considered trivial, $r = 0.10\text{--}0.29$ small, $r = 0.30\text{--}0.49$ moderate, $r = 0.50\text{--}0.69$ high, $r = 0.70\text{--}0.89$ very high, $r = 0.90\text{--}0.99$ almost perfect, and $r = 1$ perfect correlation [45].

Regarding the distance covered, a moderate negative correlation was observed with the supraspinal skinfold. Other studies have also reported negative correlations with fat-related variables. For instance, a study on the Chilean national team players [30] found a moderate negative correlation between the distance covered and both body fat percentage and the sum of six skinfolds. Similarly, a study on Brazilian first-division players [8] reported a high negative correlation between the distance covered and the sum of six skinfolds.

For estimated VO_{2max}, there was a moderate negative correlation with the supraspinal skinfold and wingspan. As with the distance covered, there is a lack of studies for comparison. Current literature indicates only one study has correlated anthropometric variables with estimated VO_{2max} based on distance covered by the Yo-Yo IR1. This study, conducted on semi-professional Spanish players [53], found a moderate negative correlation between BMI and estimated VO_{2max}.

Limitations

One limitation of this study was the relatively small sample size of 34 female players. Additionally, not all players were able to complete every test due to injuries or discomfort, which further reduced the number of participants in certain analyses. This impacted the statistical power of the findings, particularly for positions such as goalkeeper, with only two players per team available. However, it is important to note that access to professional soccer teams is inherently difficult, and these limitations are common in studies involving elite athletes. Future research with larger and more consistent samples is necessary to confirm and expand upon these results. Additionally, the lack of dual-energy X-ray absorptiometry (DEXA) assessment may have limited the precision of body composition measurements. However, its high cost and limited accessibility make it impractical for field-based studies in team sports. Instead, ISAK-standardized anthropometry was used, a validated and widely applied method in sports science. Future research integrating different assessment techniques could provide further insights.

4. Conclusions

This study highlights the significant influence of body composition on the physical performance of female soccer players at the onset of the pre-season immediately following the off-season. A strong positive correlation was observed between muscle and body

mass with anaerobic power and capacity. On the other hand, fat mass was moderately negatively correlated with explosive strength, recovery capacity, and aerobic performance. Regarding positional differences, goalkeepers demonstrated the highest values in anaerobic power, as well as in explosive strength. In contrast, midfielders excelled in aerobic capacity. These findings emphasize the need for individualized training programs that focus on increasing muscle mass and managing fat levels to optimize both anaerobic and aerobic performance. In summary, muscle mass and body mass are strongly related to better anaerobic performance, reinforcing the importance of strength training in female players, while fat mass negatively impacts explosive strength, recovery capacity, and aerobic performance, emphasizing the need for an optimal body composition to enhance athletic performance. These insights are crucial for coaches and nutritionists in developing training strategies and personalized nutrition plans tailored to the needs of each position as players return from the off-season, aimed at enhancing the specific attributes that can optimize performance.

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4.3. ARTÍCULO 3

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nutrients



Article

Impact of Chronic Nitrate and Citrulline Malate Supplementation on Performance and Recovery in Spanish Professional Female Soccer Players: A Randomized Controlled Trial

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Abstract

Background: Pre-season training is critical for developing tolerance to high physical demands in professional soccer, and nitric oxide (NO) precursors such as dietary nitrate (NO_3^-) and citrulline malate (CM) can support performance and recovery during this demanding phase. This study aimed to examine the effects of a four-week supplementation protocol combining 500 mg of NO_3^- from amaranth extract and 8 g of CM (NIT + CM) on external training load and post-match recovery in professional female soccer players during pre-season. **Methods:** A randomized, double-blind, placebo-controlled trial was conducted with 34 female soccer players who received either the NIT + CM product or a placebo for four weeks during pre-season. Global positioning system (GPS)-derived external load was recorded throughout the intervention. Performance tests—a countermovement jump (CMJ) test and the Wingate anaerobic test (WAnT)—and blood sampling for plasma NO_3^- and nitrite (NO_2^-) concentrations were conducted at baseline and the day after a competitive match. **Results:** The supplementation with NIT + CM increased maximal speed (V_{max}) throughout training and match play. During post-match testing, the NIT + CM group exhibited a significantly smaller decline in mean (P_{mean}) and minimum (P_{min}) power during the WAnT, along with reduced power loss in both the first (0–15 s) and second (15–30 s) intervals. Plasma NO_3^- concentrations significantly increased from baseline in the NIT + CM group and remained elevated 24 h after the final dose, confirming sustained systemic exposure. **Conclusions:** Chronic NIT + CM supplementation may enhance V_{max} and help preserve anaerobic performance the day after a match. These effects could reflect improved tolerance to high training loads and sustained NO_3^- availability during recovery.

Keywords: ergogenic aids; nitric oxide booster; performance; recovery; female soccer



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1. Introduction

Soccer is an intermittent sport involving repeated high-intensity efforts interspersed with low-intensity activities [1,2]. Recent developments in women's soccer, including

its increased visibility and professionalization, have led to greater physical demands on players [3–5]. Consequently, the pre-season preparation period has become essential in developing the fitness required to sustain high training loads and match intensities throughout the competitive season and during tournaments [6–8].

Athletes, including female soccer players, frequently use dietary supplements to support training adaptations and competitive performance, with NO precursors ranking among the most widely marketed and accessible supplement groups [9]. NO is a signaling molecule involved in numerous physiological functions, including vasodilation, mitochondrial respiration and biogenesis, angiogenesis, muscle glucose uptake, and sarcoplasmic reticulum calcium handling [10–12]. Through these mechanisms, NO has been linked to enhanced muscle efficiency [13,14], reduced oxygen demand during exercise, and improved neuromuscular recovery [15], making it a potential target for performance optimization.

The human body generates NO through two complementary pathways [16]. The first is the citrulline-arginine-NO pathway, in which NO is synthesized endogenously from L-arginine and oxygen through nitric oxide synthase (NOS) enzymes, producing L-citrulline as a byproduct [17]. This process is commonly referred to as the NOS-dependent pathway.

The second pathway is partially exogenous, as it relies on NO_3^- and NO_2^- obtained from both endogenous NO oxidation [18] and dietary sources rich in NO_3^- [16] such as spinach, beets, and arugula [19]. A portion of the ingested NO_3^- accumulates in saliva and is then reduced to NO_2^- by bacteria in the oral cavity [20–22]. Subsequent ingestion of this NO_2^- results in the reduction of a portion of it to NO within the acidic environment of the stomach [23], with the remainder entering the systemic circulation. This NO_2^- is rapidly distributed in blood and other tissues, including peripheral vasculature and skeletal muscle [18], and can readily undergo a one-electron reduction to yield NO [9]. This reaction is enhanced under conditions of hypoxia [24] and acidity [25] such as those observed in skeletal muscle during contraction [26]. This route is known as the NO_3^- - NO_2^- -NO pathway or the NOS-independent pathway.

Given these pathways, it has been suggested that combining different NO precursors, such as dietary NO_3^- and citrulline, may further enhance its bioavailability [27]. L-citrulline could support the NOS-dependent pathway by increasing L-arginine availability [28,29], while dietary NO_3^- could contribute to the NOS-independent route by providing additional substrate for NO synthesis [18,30].

In this context, L-citrulline is commonly supplemented in the form of CM [31], a compound that consists of L-citrulline in combination with malic acid, an intermediate of the tricarboxylic acid cycle [32,33]. In addition to supporting NO synthesis, CM may contribute to enhanced ATP production and improved ammonia clearance, which could be particularly beneficial during repeated high-intensity efforts [34,35]. These mechanisms are particularly relevant in team sports such as soccer, which involve frequent explosive actions interspersed with short recovery periods [36] and constant alternation between anaerobic [37,38] and aerobic [39,40] energy systems.

Dietary NO_3^- supplementation has been associated with performance improvements in endurance, muscular endurance, and high-intensity power output, as reported in a recent umbrella review summarizing 20 systematic reviews and meta-analyses [41].

These effects are thought to be mediated by NO-related physiological adaptations [10–14]. However, methodological variability and limited representation of female athletes highlight the need for further research in applied sport contexts [41–44].

In recent years, it has been suggested that type II muscle fibers—primarily recruited during explosive and high-intensity efforts [26]—may serve as NO_3^- reservoirs, promoting localized NO production under hypoxic and acidic conditions and contributing to intra-

muscular buffering [45,46]. These observations support the hypothesis that skeletal muscle functions as a physiological NO_3^- reservoir sensitive to dietary intake. Animal studies have shown that NO_3^- depletion reduces intramuscular stores, while reintroduction leads to rapid recovery and even supraphysiological accumulation, suggesting a potential adaptive mechanism that may reinforce the rationale for chronic supplementation strategies [47]. Regarding the mode of administration, beetroot juice has been the most extensively studied source of inorganic NO_3^- [48]. However, alternative dietary sources, such as amaranth, have gained attention in recent years as potential alternatives, although research on them remains limited [49–51].

Due to the physiological complementarity of CM and dietary NO_3^- , their combined use may offer a practical strategy to support performance and recovery by targeting distinct NO synthesis pathways. Although several studies have examined their effects separately [31,33,35,41,52,53], to our knowledge, no study has investigated their co-supplementation. This is the first study to evaluate the combined effects of CM and dietary NO_3^- under a chronic supplementation protocol in professional soccer players. Exploring this strategy during pre-season may provide valuable insight into approaches aimed at attenuating fatigue, sustaining performance, and optimizing recovery in applied sport-specific settings.

The primary aim of this study is to examine whether chronic supplementation with 500 mg of NO_3^- from amaranth extract and 8 g of CM over four weeks during pre-season influences external load and post-match recovery in professional female soccer players. We hypothesize that combined supplementation with CM and NO_3^- may help attenuate performance decline and support recovery during pre-season.

2. Materials and Methods

2.1. Study Design

This study was a randomized, double-blind, team-stratified, and placebo-controlled trial designed to evaluate the effects of a four-week supplementation regimen during pre-season with NO_3^- and CM (NIT + CM) on GPS-derived performance metrics and recovery one day after a match in professional female soccer players. On the first day of pre-season, CMJ, WAnT, and blood sampling were conducted. Three days later, supplementation began. GPS data were recorded daily during training sessions and matches over the 30-day period. On the final day of supplementation, both teams played a match, and all tests from the first day were repeated the following day. Prior to starting the intervention, the protocol was approved by the Institutional Review Committee of the Catholic University San Antonio of Murcia (UCAM) (code CE052204), following the Standards of Good Clinical Practice and was conducted according to the Declaration of Helsinki. The clinical trial was registered at www.clinicaltrials.gov (accessed on 9 August 2022) (identifier NCT05525871). Current European legislation on the protection of personal data (Regulation (EU)2016/679) was complied with.

2.2. Participants

Research was conducted on a sample of 34 professional female players (age: 23.06 ± 4.29 years) from two soccer teams in the first and second Spanish professional women's football leagues.

The inclusion criteria for participant recruitment were as follows: (a) being a healthy subject with medical authorization for the practice of federated sport, and (b) belonging to a team of the first or second division of the Spanish Women's League. The exclusion criteria for the study were as follows: (a) changing teams during pre-season, and (b) a history of drug, alcohol, or other substance abuse or other factors limiting the ability to cooperate

during the study. All players were previously informed of the objectives and method of the research, signing informed consent forms before starting the research; in the case of minors, it was their parents who signed the consent. An outside researcher carried out the randomization using a computed generator (Epidat v4.1 Epi-dat, Spain), assigning the subjects to one of the groups (NIT + CM or PLA). Each product was assigned a unique code, ensuring there were as many codes as participants. A master sheet was prepared, linking each participant's code with the corresponding product code. This allocation sheet was signed and dated to confirm the randomization process. The group assignments remained confidential and were disclosed only at the end of the study, identifying which codes corresponded to the NIT + CM and which to the PLA.

Player codes and group assignments were provided to the manufacturing company. Based on these codes, the company prepared individual bags containing the precise number of doses required for each player throughout the study. Each bag was labeled with the corresponding player code, ensuring that the correct product was assigned according to the randomization process. The company managed the separation of active supplement and placebo, maintaining blinding throughout the study.

2.3. Supplementation

The NIT + CM and PLA developed by Nutripeople (Reel & Innovation S.L., Murcia, Spain) was supplied in flexible 100 g pouches. Regarding the NO precursors, the CM used was L-citrulline-DL-malate (2:1), sourced from HSN Store (Harrison Sport Nutrition S.L., Oviedo, Spain) (Sanitary Registration: 26.11001/GR). The NO_3^- source was Oxystorm®, a high-content standardized extract of amaranth derived from *Amaranthus hypochondriacus* leaves with 9% NO_3^- concentration, provided by Efficient Science (Black Fenix S.L., Cádiz, Spain) and distributed by EMFit (EMFit Nutrition S.L., Cádiz, Spain) (Sanitary Registration: 26.021287/CA).

The NIT + CM was formulated with the following ingredients: 60% strawberry purée, 22.15% apple purée, 10% grape concentrate, 4% CM, 3.65% Oxystorm® (extract of amaranth, NO_3^- , and potassium), and 0.2% ascorbic acid. In contrast, the PLA contained 66.8% strawberry purée, 22% apple purée, 10% grape concentrate, 1% lemon concentrate, and 0.2% ascorbic acid.

Beetroot, a primary dietary source of NO_3^- , contains vitamin C (ascorbic acid) and phenolic compounds such as flavonoids, which help reduce oxidative stress and protect NO, enhancing its biological activity [54,55]. Amaranth, used as the NO_3^- source in this study, naturally contains the antioxidant amaranthine [56]. To strengthen the antioxidant capacity and provide additional protection for NO against oxidative degradation, ascorbic acid was included to improve its stability and bioavailability.

Both the NIT + CM and PLA were designed to be nutritionally equivalent. Each 100 g pouch provided 207 kJ (49 kcal), 0.3 g of total fat (0.03 g saturated), 10.2 g of carbohydrates (8.2 g sugars), 1.5 g of fiber, 0.6 g of protein, 0.001 g of salt, and 21.5 mg of vitamin C.

Participants consumed two pouches daily, resulting in a total intake of 200 g per day, which provided 500 mg of NO_3^- (8 mmol) and 8 g of CM.

To prevent any attenuation in the reduction of NO_3^- to NO_2^- by commensal bacteria in the oral cavity during the intervention, participants were instructed to refrain from using antibacterial mouthwash or toothpaste [21] and to ensure sufficient time elapsed between brushing their teeth and consuming the supplement. Additionally, to ensure the accuracy of the study results, participants were asked to refrain from using other ergogenic aids.

2.4. Blood Collection and Plasma Nitrate/Nitrite Analysis

Venous blood samples were collected from the antecubital vein using K₃EDTA blood collection tubes (3 mL; BD Vacutainer®, Becton Dickinson, Franklin Lakes, NJ, USA). Samples were immediately centrifuged at 1000× *g* for 5 min at 4 °C. Plasma aliquots were snap-frozen in dry ice and stored at −80 °C until analysis at an external laboratory (Laboratorios MUNERA S.L., Murcia, Spain).

Before analysis, plasma samples were thawed at room temperature and deproteinized using 10 kDa molecular weight cut-off filters (ultrafiltration), as recommended by the assay manufacturer. Total plasma NO₃[−] and NO₂[−] concentrations were quantified using a commercial colorimetric assay kit (Nitrite/Nitrate Assay Kit, Bioquochem®, KB03010; Oviedo, Spain), based on the Griess reaction. This colorimetric method has been widely used in plasma for the determination of NO_x species [57].

The method involves enzymatic reduction of NO₃[−] to NO₂[−], followed by reaction with Griess reagents to form an azo dye measurable at 540 nm. Native NO₂[−] was quantified in parallel by omitting the enzymatic reduction step. NO₃[−] concentration was calculated by subtracting native NO₂[−] from total NO₂[−] values.

All samples and standards were assayed in duplicate, and standard calibration curves (0–200 μM) were generated using sodium nitrite solutions, following the manufacturer's instructions. Absorbance was measured at 540 nm using a microplate reader.

2.5. Training and Match Data

External load monitoring in team sports is commonly performed using GPS devices [58]. For this study, a lower-limb wearable GPS tracker (calf-worn) developed by Oliver Sports (Sport Data Innovation SL, Barcelona, Spain) was utilized to monitor player performance metrics during pre-season training sessions and matches. The device integrates inertial measurement unit (IMU) sensors with a sampling rate of 50 Hz and GPS technology with a sampling rate of 25 Hz, enabling precise measurement of variables such as time, maximal speed, total distance covered (TD), and distance per minute.

This system is currently included in the FIFA Innovation Programme [59], which aims to explore emerging electronic performance and tracking systems (EPTS) [60] to improve data accuracy and player performance insights.

The wearable tracker incorporates AI-supported software that provides actionable insights for players and coaching staff, enhancing individual and team performance. The software automatically categorized movement into four predefined speed thresholds: walking (0 to 7.1 km/h), jogging (7.1 to 14 km/h), high-intensity running (HIR) (14 to 22.3 km/h), and maximal-intensity running (MIR) (22.3 to 36 km/h). These thresholds were fixed by the system and could not be adjusted. GPS data from training sessions and matches during pre-season were obtained from the tracking software and exported into Excel for further processing and analysis. Goalkeepers did not wear GPS devices and were therefore excluded from this part of the study.

2.6. Countermovement Jump and Wingate Anaerobic Tests

Explosive strength, anaerobic power, and neuromuscular fatigue in the lower limbs were measured and analyzed using the CMJ test [61–63], while anaerobic power and anaerobic capacity were analyzed using the WAnT [64]. The methodology, equipment, and variables used are detailed in a previous publication [65].

2.7. Statistical Analyses

SPSS Statistics 27 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Descriptive statistics were calculated and used to describe the GPS-derived training and

match data, physical performance variables of CMJ and WAnT, and plasma NO_2^- and NO_3^- concentration.

The statistics were reported as mean $\pm$ standard deviation (SD). The Shapiro–Wilk and Levene tests were used to verify the normality and homoscedasticity of the data, respectively. Depending on the distribution of the variables, parametric (Student's *t*-test) or non-parametric (Mann–Whitney U test) analyses were applied.

Additionally, a two-way independent measures ANOVA was performed to examine the main effects of week (Week 1 to Week 4) and group (NIT + CM vs. placebo), as well as their interaction on performance and physiological outcomes. In case of non-normally distributed dependent variables, the Kruskal–Wallis test was used as a non-parametric alternative. Bonferroni post hoc tests were applied if the homogeneity of variance assumption was met; otherwise, Games–Howell post hoc tests were used. In case of a significant interaction, simple main effects were explored using Bonferroni correction to account for multiple comparisons. Effect sizes were estimated using partial eta squared (η^2p) and classified as small ($0.01 \leq \eta^2p < 0.06$), medium ($0.06 \leq \eta^2p < 0.14$), or large ($\eta^2p \geq 0.14$) [66]. The level of statistical significance was set at $p \leq 0.05$.

3. Results and Discussion

A total of 42 professional female soccer players were enrolled in the study. All participants met the inclusion criteria and agreed to participate, so no exclusions were necessary. Players were randomly assigned to two equal groups (NIT + CM and placebo, $n = 21$ each). During the intervention period, five players in the NIT + CM group and three in the placebo group discontinued participation. In total, 34 players completed the study and were included in the final analysis (NIT + CM: $n = 16$; PLA: $n = 18$). A flow chart illustrating participant progression is presented in Figure 1.

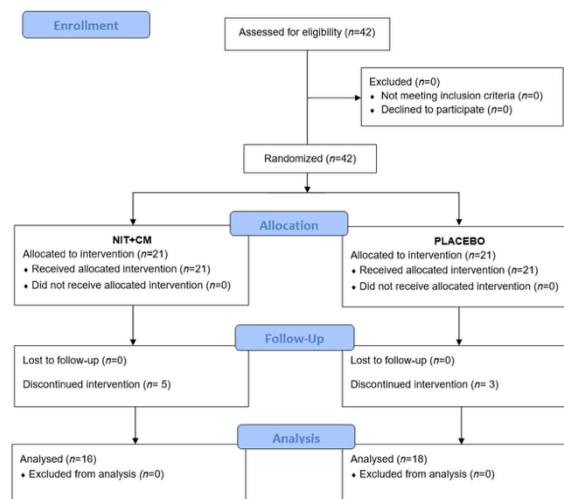


Figure 1. Flow diagram.

The demographic characteristics of the players are displayed in Table 1. The anthropometric measurements and body composition of the players are available in detail in a previous publication by our research team [67].

Table 1. Demographic data of the players: mean and standard deviations (mean $\pm$ SD).

	Mean $\pm$ SD
<i>n</i>	34
Age	23.06 $\pm$ 4.29
Height (cm)	164.15 $\pm$ 5.84
Weight (kg)	58.39 $\pm$ 6.62
BMI (kg/m ²)	21.64 $\pm$ 21.59
Σ 6 Skinfolds (mm)	74.57 $\pm$ 18.48

BMI: body mass index; Σ 6 Skinfolds (triceps, subscapular, supraspinal, abdominal, front thigh, and medial calf).

To the best of our knowledge, this is the first study to assess the chronic effects of combined CM and NO₃[−] supplementation in professional soccer players. As this combination has been largely unexplored in the literature, investigating its potential effects under real-world training conditions may provide relevant insight for performance and recovery strategies.

GPS-derived data from training sessions were used to assess player performance, evaluate changes throughout pre-season, and explore potential differences between the NIT + CM supplementation group and the placebo group. The interaction between the type of product (NIT + CM vs. PLA) and time (the different weeks of the study) was not statistically significant for any of the variables analyzed. This indicates that the changes observed over the course of the weeks were similar in both groups, with no differences attributable to the product administered. Table 2 summarizes ANOVA results, while Table 3 presents weekly descriptive values by group and the entire sample.

Vmax showed significant main effects for both product and week. The product factor had a large effect size ($p < 0.001$; $\eta^2 p = 0.351$), with post hoc analysis confirming that values were consistently higher in the NIT + CM group compared to placebo throughout pre-season period. The week factor also had a large effect size ($p < 0.001$; $\eta^2 p = 0.160$), although smaller compared to the product factor. Post hoc tests for the week factor indicated progressive improvements from week 1 to weeks 3 and 4, and from week 2 to weeks 3 and 4 (all $p < 0.001$), peaking in week 4. Weekly comparisons using independent *t*-tests revealed that the NIT + CM group consistently reached higher maximum speeds than the placebo group throughout the pre-season period (all $p < 0.001$), indicating a stable between-group difference across the measured weeks.

Although previous studies on the chronic effects of NO precursor supplementation on Vmax in elite female soccer players are limited, some findings from team-sport athletes in sprint-related protocols provide indirect support. Improvements in cycling sprint performance have been reported following 5 to 7 days of beetroot juice supplementation (8.2–12.8 mmol NO₃[−]/day), with increases in mean power [68] and total work [69]. Enhanced sprint times have also been observed in running-based tests under similar protocols [70]. These data suggest a potential benefit of NO₃[−] for explosive efforts, particularly following repeated-day administration [71].

From a physiological perspective, these effects may be mediated by the increased NO availability. Elevated NO levels have been associated with enhanced calcium handling, improved excitation–contraction coupling, and increased recruitment of type II muscle fibers [9,16], all of which are closely involved in generating Vmax in soccer [72,73]. High-speed running demands rapid activation of the posterior chain and strong neuromuscular coordination under fatigue [74,75], which could benefit from improved contractile effi-

ciency and reduced ATP cost. Furthermore, the NO-induced increases in muscle perfusion and mitochondrial efficiency [76] could support the maintenance of sprinting capacity during consecutive sessions, particularly under high training loads. The malate component may further contribute by enhancing the rate of ATP production during high-intensity efforts [35].

Although our intervention combined NO_3^- and CM, previous evidence suggests that CM alone may contribute to sprint performance. In a study, three days of CM supplementation (8 g/day) improved repeated sprint performance in male team sport athletes, with faster sprint times and reduced fatigue [77].

Taken together, the consistently higher V_{max} values in the NIT + CM group, along with a larger effect size for the product than for the training week, may reflect a beneficial neuromuscular adaptation in response to NO precursor supplementation. Nevertheless, given the observational nature of the study and the multifactorial determinants of sprint performance, these findings should be interpreted with caution.

Regarding baseline performance, the mean V_{max} recorded during the pre-season training period was 26.79 ± 4.45 km/h. Although direct comparisons must be interpreted cautiously due to methodological differences and seasonal context, it is worth noting that this value exceeds the average reported in Spanish professional female players during a four-week in-season training period (23.55 ± 1.30) [78]. This difference may be partially explained by the fact that pre-season training sessions are typically more demanding and focused on accumulating higher training loads compared to in-season microcycles, which prioritize recovery and match preparation [79].

As for distance covered per minute, this variable showed a significant main effect for product ($p < 0.001$), with a small effect size ($\eta^2 p = 0.035$). Post hoc analyses revealed consistently higher overall values in the NIT + CM group. The effect of the training weeks was close to statistical significance ($p = 0.053$) and showed a small effect size ($\eta^2 p = 0.025$). Post hoc analysis for the week factor showed a significant improvement from week 1 to week 3 ($p = 0.009$). Independent weekly *t*-tests revealed significantly higher values in the NIT + CM group only in weeks 1 ($p < 0.001$) and 3 ($p = 0.029$).

Considering all findings, it may indicate a modest but consistent advantage for the NIT + CM group in sustaining work rate during training. This effect is likely to involve mechanisms similar to those proposed for V_{max} , such as enhanced muscle efficiency, improved blood flow, and increased ATP production through higher NO availability. Further studies under controlled conditions are needed to clarify the physiological relevance of this finding and its potential impact on training outcomes.

The overall mean distance per minute was 70.66 ± 22.72 m/min. Similar to the findings for V_{max} , this value is also higher than those reported in Spanish professional players during the in-season period (60.12 ± 10.31) [78].

In addition, TD and walking distance showed significant main effects for the product. TD showed a moderate effect size ($p < 0.001$; $\eta^2 p = 0.113$), with the NIT + CM group presenting higher overall values compared to placebo. Additionally, a significant main effect was found for the week factor, although with a small effect size ($p = 0.003$; $\eta^2 p = 0.029$), with post hoc tests revealing significantly higher values in week 3 compared to week 2 ($p = 0.006$). Independent weekly *t*-tests confirmed consistently higher TD covered by the NIT + CM group compared to the placebo across all weeks (all $p < 0.001$).

Although a direct comparison cannot be made with distance covered during training, previous studies have reported increased TD in team-sport athletes during the Yo-Yo IR1 test following chronic NO_3^- supplementation. These effects have been observed with protocols ranging from 2 to 6 days and doses between 4.1 and 12.9 mmol/day of NO_3^- [70,80,81]. While those findings come from controlled performance tests and did not involve CM, the

present results appear to follow a similar trend, suggesting a potential improvement in locomotor output during physically demanding periods such as pre-season.

The overall mean TD was 5281.13 ± 1272.78 m across the pre-season period. In line with previous findings for Vmax and distance per minute, this value was also higher than that reported in Spanish professional players from the same reference study (4883.5 ± 780.49) [78] but lower than the value reported in a study of Australian National League players during pre-season training (6646 ± 111) [79].

Walking distance showed a moderate product effect size ($p < 0.001$; $\eta^2p = 0.133$), consistently favoring the NIT + CM group throughout pre-season. There was no significant week effect, yet independent weekly *t*-tests indicated significantly higher walking distances in the NIT + CM group at weeks 1, 2, and 4 ($p < 0.001$) and week 3 ($p = 0.004$).

Therefore, the consistent advantage observed in both total and walking distances among players in the NIT + CM group may reflect greater fatigue tolerance and an enhanced capacity to remain active for extended periods during training. Rather than indicating reduced intensity, the accumulation of walking distance may suggest a more effective active recovery process, allowing players to continue moving without the need for complete passive rest [82–84]. Considering the moderate effect sizes observed for the product factor and the consistent differences between groups, these findings support the hypothesis that NIT + CM supplementation could have contributed to a more favorable physiological response under sustained pre-season training demands. Nevertheless, these interpretations should be approached with caution, as the observational nature of the study precludes any inference of causality.

Physiologically, increased NO availability may have enhanced peripheral blood flow during training [85], improving oxygen and nutrient delivery while facilitating the clearance of metabolic byproducts [86]. Moreover, the malate component has been proposed to reduce lactate production by promoting aerobic utilization of pyruvate, especially under high blood flow conditions [35]. This combination may reflect a synergistic effect that supports sustained low-intensity activity and delays fatigue accumulation, potentially contributing to the greater total and walking distances observed in the NIT + CM group.

Moreover, jogging, HIR, and MIR distances exhibited significant main effects only for week, with no significant product effects. Specially, jogging distance presented a small effect size ($p = 0.001$; $\eta^2p = 0.034$), with post hoc analysis showing fluctuations across weeks (higher in week 1 vs. week 2, $p = 0.003$; week 2 vs. week 3, $p = 0.029$). HIR distance displayed a moderate effect size ($p < 0.001$; $\eta^2p = 0.061$), with a significant decrease from week 1 to week 2 ($p < 0.001$), followed by significant increases from week 2 to weeks 3 and 4 (both $p \leq 0.001$). MIR distance showed a small effect size ($p < 0.001$; $\eta^2 = 0.059$), with a progressive increase and significant increments from weeks 1 and 2 to weeks 3 and 4 ($p \leq 0.037$).

Comparisons with previous studies are inherently limited by methodological differences, such as the velocity thresholds used in this analysis, which were defined by the GPS system and may not match those in other research. The variations observed in jogging, HIR, and MIR distances across the pre-season period likely reflect differences in the physical and tactical emphasis of each microcycle. Small effect sizes for jogging and MIR distances suggest minor week-to-week adjustments, while the moderate effect size for HIR may indicate a progressive increase in exposure to high-intensity efforts. These patterns are consistent with the natural variability of training content in professional soccer and the specific objectives set by the coaching staff each week [87].

Finally, training duration showed a small but statistically significant product effect ($p = 0.001$; $\eta^2p = 0.013$). Despite reaching statistical significance, the small effect size limits its practical interpretation and calls for cautious analysis.

Table 2. Two-way ANOVA used to examine the effect of the product (NIT + CM vs. PLA) and the week on GPS-derived performance variables during pre-season training.

Variable	Product		Week		Product × Week	
	<i>p</i> -Value	η^2p	<i>p</i> -Value	η^2p	<i>p</i> -Value	η^2p
Time (min)	0.001 *	0.013	0.098	0.009	0.668	0.003
Vmax (km/h)	<0.001 *	0.351	<0.001 *	0.160	0.995	0.000
TD (m)	<0.001 *	0.113	0.003 *	0.029	0.694	0.003
Distance × minute	<0.001 *	0.035	0.053 *	0.025	0.789	0.002
Walking (m)	<0.001 *	0.133	0.902	0.003	0.480	0.005
Jogging (m)	0.194	0.007	0.001 *	0.034	0.763	0.002
HIR (m)	0.507	0.000	<0.001 *	0.061	0.515	0.005
MIR (m)	0.082	0.003	<0.001 *	0.059	0.645	0.004

Vmax: maximal speed; TD: total distance covered; Distance × minute: distance per minute; HIR: high-intensity running; MIR: maximal-intensity running. * Statistically significant ($p < 0.05$).

Table 3. Descriptive statistics of GPS-derived training performance variables by group and week: mean and standard deviations (mean ± SD).

Variable	Group	Total	Week 1	Week 2	Week 3	Week 4
Time (min)	NIT + CM	81.71 ± 21.12	84.63 ± 20.67	82.00 ± 25.10	77.17 ± 19.41	82.64 ± 16.62
	Placebo	76.62 ± 19.99	79.22 ± 22.01	74.28 ± 24.54	75.92 ± 16.74	77.85 ± 13.17
	All players	78.82 ± 20.62	81.5 ± 21.52	77.77 ± 25.01	76.41 ± 17.75	80.05 ± 14.97
Vmax (km/h)	NIT + CM	29.63 ± 4.04	28.48 ± 3.21 *	28.19 ± 4.33 *	31.18 ± 4.06 *	31.27 ± 3.23 *
	Placebo	24.62 ± 3.40	23.39 ± 2.83	23.06 ± 3.50	26.11 ± 2.89	26.35 ± 2.87
	All players	26.79 ± 4.45	25.54 ± 3.91	25.38 ± 4.65	28.08 ± 4.19	28.61 ± 3.9
TD (m)	NIT + CM	5761.54 ± 1315.70	6001.50 ± 1580.04 *	5521.58 ± 1336.87 *	5961.62 ± 1175.81 *	5686.26 ± 1090.62 *
	Placebo	4915.52 ± 1109.51	4949.58 ± 1297.76	4594.22 ± 1022.62	5213.75 ± 983.84	4971.47 ± 1058.21
	All players	5281.13 ± 1272.78	5393.51 ± 1509.73	5014.03 ± 1259.41	5504.96 ± 1199.21	5293.35 ± 1265.53
Distance × minute	NIT + CM	75.26 ± 27.37	72.48 ± 15.20 *	74.06 ± 30.13	83.51 ± 30.92 *	72.19 ± 28.22
	Placebo	67.15 ± 17.68	64.02 ± 12.65	67.49 ± 21.58	71.47 ± 18.48	64.99 ± 14.45
	All players	70.66 ± 22.72	67.59 ± 14.35	70.46 ± 25.92	76.16 ± 24.68	68.29 ± 22.06
Walking (m)	NIT + CM	3735.42 ± 1068.13	3727.05 ± 1143.61 *	3840.14 ± 1125.00 *	3690.77 ± 1052.16 *	3642.09 ± 945.23 *
	Placebo	2994.69 ± 788.09	2901.70 ± 828.61	2957.38 ± 837.43	3149.77 ± 777.51	2963.85 ± 677.06
	All players	3314.80 ± 989.76	3250.01 ± 1052.04	3570.01 ± 1069.71	3604.43 ± 928.58	3274.97 ± 875.68
Jogging (m)	NIT + CM	1560.69 ± 780.93	1720.05 ± 700.95	1351.38 ± 826.37	1715.61 ± 863.55	1558.22 ± 658.11
	Placebo	1462.65 ± 614.45	1580.24 ± 632.83	1317.36 ± 657.53	1505.78 ± 620.94	1486.10 ± 490.20
	All players	1505.02 ± 692.27	1639.24 ± 662.9	1332.76 ± 736.33	1587.48 ± 728.67	1519.18 ± 571.71
HIR (m)	NIT + CM	435.17 ± 374.52	535.58 ± 372.81	313.75 ± 334.79	516.34 ± 434.05	434.07 ± 331.95
	Placebo	433.16 ± 331.86	448.10 ± 290.91	304.37 ± 256.09	527.87 ± 407.23	483.25 ± 320.32
	All players	434.03 ± 350.55	485.02 ± 329.21	308.62 ± 293.32	523.38 ± 415.99	460.69 ± 325.12
MIR (m)	NIT + CM	30.26 ± 58.93	18.83 ± 26.03	16.31 ± 37.62	38.90 ± 60.29	51.87 ± 89.14
	Placebo	25.02 ± 38.75	19.54 ± 23.16	15.11 ± 24.83	30.33 ± 44.01	38.27 ± 54.22
	All players	27.28 ± 48.52	19.24 ± 24.3	15.65 ± 31.17	33.67 ± 50.88	44.51 ± 72.32

Vmax: maximal speed; TD: total distance covered; Distance × minute: distance per minute; HIR: high-intensity running; MIR: maximal-intensity running. * Statistically significant ($p < 0.05$).

GPS-derived data from match play were analyzed to evaluate performance changes throughout pre-season and to identify potential differences between the NIT + CM supplementation group and the placebo group. Table 4 summarizes the ANOVA results, while Table 5 presents weekly descriptive values by group and for the entire sample.

Vmax showed a significant interaction among product and training week ($p = 0.022$; $\eta^2p = 0.079$, moderate effect size). Post hoc analysis within the NIT + CM group revealed progressive increases from week 1 to weeks 3 and 4 (both $p < 0.001$), as well as from week 2 to weeks 3 ($p = 0.006$) and 4 ($p = 0.028$). Additionally, significant main effects were observed for both product ($p < 0.001$; $\eta^2p = 0.413$, large effect size) and week ($p < 0.001$; $\eta^2p = 0.253$, large effect size). Post hoc analysis for the product factor indicated consistently

higher overall Vmax values in the NIT + CM group compared to placebo. For the week factor, post hoc tests showed clear and significant increases from week 1 to weeks 3 and 4 (both $p < 0.001$), and from week 2 to weeks 3 ($p = 0.021$) and 4 ($p = 0.003$). Independent weekly *t*-tests further confirmed significantly higher Vmax values for the NIT + CM group in weeks 1, 3, and 4 ($p < 0.001$) and week 2 ($p = 0.012$).

From a physiological perspective, the influence of both the product and the training week on this variable appears to be substantial, with the effect of the product being particularly pronounced. In addition, a significant interaction between product and training week suggests that differences between the NIT + CM and placebo groups evolved over time, potentially reflecting an enhanced physiological response in the supplemented group as pre-season progressed. This hypothesis was further supported by consistently higher Vmax values in the NIT + CM group across all weeks, as confirmed by independent *t*-tests. While causality cannot be established due to the observational design, the magnitude and stability of the group differences, along with the significant interaction, are compatible with a potential neuromuscular benefit of NIT + CM supplementation.

Due to the physiological mechanisms of NO_3^- and CM discussed earlier, the product-related advantage observed here may reflect improved sprint capacity under competitive load. However, interpreting these findings requires consideration of the complex and variable nature of match demands. In elite women's soccer, performance metrics such as Vmax are influenced by several factors including physical readiness, tactical role, opponent characteristics [88], and contextual factors such as match status or competition phase [89]. While these variables introduce a degree of variability, Vmax appears to be relatively stable. According to a study on match load variability in elite female players, this metric displays low inter-match variability ($\text{CV} \approx 4.5\%$) compared to other high-intensity measures [90], suggesting that changes are more likely to reflect genuine physiological adaptations rather than contextual fluctuations.

Moreover, the ability to express peak velocity in matches may be partially conditioned by the neuromuscular capacities developed during training. The consistent advantage observed in both training and match Vmax values among players in the NIT + CM group raises the hypothesis that enhanced high-speed performance during training could have facilitated a more robust translation of this ability into match contexts. Still, this interpretation must remain speculative and subject to the limitations inherent to non-experimental designs.

Descriptively, the overall mean Vmax observed during matches was 29.52 ± 4.26 km/h, which aligns with previous reports indicating that elite female players can reach peak speeds of approximately 30–32 km/h during match play [91,92]. In comparison, this value is slightly lower than the average Vmax reported in male professional players throughout the season (≈ 30.7 km/h), although most players tend to reach peak velocities between 32 and 33.9 km/h [93]. Finally, compared to the average reported in Spanish female players during a four-week match period in the competitive season (25.3 ± 0.3), the value observed in the present study appears notably higher [78]. Considering that all teams followed their usual training routines, this difference may reflect a potential influence of the supplementation strategy implemented.

Distance per minute showed a marginally significant interaction between product and match week ($p = 0.052$; $\eta^2 p = 0.064$, moderate effect size). Within-group analysis showed a significant increase from week 1 to week 4 in the NIT + CM group ($p < 0.001$). No significant main effect was found for the product factor; however, a significant main effect was observed for week ($p < 0.001$; $\eta^2 p = 0.117$, moderate effect size). Post hoc tests confirmed a clear improvement from week 1 to week 4 ($p < 0.001$). Independent *t*-tests revealed significantly higher values in the NIT + CM group at week 4 ($p = 0.004$).

The moderate main effect of training week on distance per minute, with significant increases from week 1 to week 4 across all players, suggests that the final pre-season match was played at a higher overall intensity. Although no main effect was found for the product factor, the presence of an almost significant interaction between product and match week, combined with the descriptively progressive increase observed in the NIT + CM group, may point to a more favorable adjustment in this group over time. The fact that this culminated in significantly higher values for the supplemented group in week 4 may reflect a more favorable adjustment to competitive demands. This pattern aligns with the physiological mechanisms previously discussed and warrants further exploration in controlled settings.

Descriptively, the overall mean distance per minute observed during matches was 93.30 ± 44.39 m/min, which is slightly lower but comparable to the average reported in Spanish professional female players during a four-week in-season period (96.3 ± 8.8) [78]. TD exhibited no significant effects for the product factor or the Product $\times$ Week interaction. However, a significant main effect for week was detected ($p = 0.033$; $\eta^2 p = 0.079$, moderate effect size), with increased values from week 1 to week 4 ($p = 0.007$). Moreover, the overall mean TD covered during matches was 7545.4 ± 4653.78 m, which falls slightly below the average reported in a recent systematic review of professional female players, which indicated typical match demands ranging from 8800 to 10,800 m, with an observed range between 7800 and 11,200 m depending on competition level, position, and measurement protocols [94,95].

Although TD increased from week 1 to week 4, this pattern should be interpreted with caution. As seen with distance per minute, the last match of pre-season appears to have been the most intense, which may partially explain the observed increase. TD is also highly influenced by contextual factors such as tactical role, match dynamics, and playing position [88], which are known to affect locomotor output during competition. The high standard deviation in this sample further highlights the large inter-individual and match-to-match variability, suggesting that the weekly differences may be more reflective of situational demands than systematic adaptations or product-related effects.

Regarding the distances covered at different velocity thresholds, no significant effects were found for the product factor or the Product $\times$ Week interaction in any of the four categories. However, independent *t*-tests revealed significantly higher walking distance in the NIT + CM group during week 4 ($p = 0.026$), which may reflect the elevated overall match intensity observed that week rather than a direct effect of the supplementation. Jogging distance displayed a significant main effect for week ($p = 0.011$; $\eta^2 p = 0.095$, moderate effect size), with post hoc tests revealing differences between week 1 and all subsequent weeks. Similarly, HIR showed a significant week effect ($p = 0.002$; $\eta^2 p = 0.143$, large effect size), again driven by differences between the first and later matches. MIR also followed this pattern, with a significant week effect ($p = 0.021$; $\eta^2 p = 0.103$, moderate effect size) and a trend toward higher values in week 3 compared to week 1 ($p = 0.056$).

Overall, the changes observed across matches suggest lower physical demands in the first game, followed by a relatively stable load in the remaining weeks. One isolated difference was found in HIR during week 1, with higher values in the placebo group ($p = 0.006$). However, the absence of consistent group effects and the high variability across players and contexts suggest that match-related situational factors were the main drivers of these fluctuations.

Table 4. Two-way ANOVA used to examine the effect of the product (NIT + CM vs. PLA) and the week on GPS-derived performance variables during pre-season matches.

Variable	Product		Week		Product × Week	
	p-Value	η ² p	p-Value	η ² p	p-Value	η ² p
Time (min)	0.749	0.005	0.742	0.006	0.921	0.004
Vmax (km/h)	<0.001 *	0.413	<0.001 *	0.253	0.022 *	0.079
TD (m)	0.325	0.023	0.033 *	0.079	0.238	0.036
Distance × minute	0.160	0.002	<0.001 *	0.117	0.052 *	0.064
Walking (m)	0.134	0.036	0.184	0.042	0.420	0.024
Jogging (m)	0.541	0.005	0.011 *	0.095	0.279	0.032
HIR (m)	0.081	0.000	0.002 *	0.143	0.277	0.033
MIR (m)	0.962	0.018	0.021 *	0.103	0.501	0.020

Vmax: maximal speed; TD: total distance covered; Distance × minute: distance per minute; HIR: high-intensity running; MIR: maximal-intensity running. * Statistically significant at ($p < 0.05$).

Table 5. Descriptive statistics of GPS-derived match performance variables by group and week: mean and standard deviations (mean ±SD).

Variable	Group	Total	Week 1	Week 2	Week 3	Week 4
Time (min)	NIT + CM	82.15 ± 31.30	80.73 ± 32.47	87.04 ± 32.16	76.21 ± 33.65	84.19 ± 29.48
	Placebo	77.75 ± 30.29	75.20 ± 30.75	78.70 ± 31.38	78.45 ± 26.07	78.61 ± 35.99
	All players	79.70 ± 30.70	77.69 ± 31.13	82.34 ± 31.49	77.54 ± 28.88	81.3 ± 32.55
Vmax (km/h)	NIT + CM	32.28 ± 4.26	29.22 ± 2.50 *	30.65 ± 4.40 *	35.03 ± 4.29 *	34.42 ± 2.67 *
	Placebo	27.33 ± 2.73	25.69 ± 2.34	27.32 ± 2.60	27.44 ± 2.81	29.04 ± 2.27
	All players	29.52 ± 4.26	27.29 ± 2.97	28.78 ± 3.83	30.52 ± 5.11	31.64 ± 3.66
TD (m)	NIT + CM	8296.85 ± 6216.69	5361.70 ± 3157.51	7895.66 ± 6448.85	8709.23 ± 7525.30	11,250.29 ± 6139.06
	Placebo	6946.41 ± 2768.35	5985.29 ± 1852.95	7572.10 ± 2991.31	6879.60 ± 3240.47	7369.49 ± 2666.64
	All players	7545.4 ± 4653.78	5703.67 ± 2500.19	7713.65 ± 4730.07	7622.89 ± 5371.41	9242.98 ± 4994.85
Distance × minute	NIT + CM	95.54 ± 62.48	64.63 ± 35.04	84.69 ± 59.72	98.79 ± 74.74	134.25 ± 58.98 *
	Placebo	91.51 ± 21.37	83.49 ± 14.72	97.39 ± 11.01	87.60 ± 31.49	98.52 ± 18.75
	All players	93.30 ± 44.39	74.97 ± 27.18	91.83 ± 40.04	92.14 ± 52.62	115.79 ± 46.06
Walking (m)	NIT + CM	4864.74 ± 3825.86	3780.37 ± 2622.78	4536.51 ± 4256.96	4568.32 ± 4380.76	6552.60 ± 3680.61 *
	Placebo	3768.59 ± 1791.96	3609.23 ± 1628.64	3896.78 ± 1799.28	3592.53 ± 1939.08	4018.39 ± 1907.63
	All players	4254.79 ± 2915.52	3686.52 ± 2098.34	4176.66 ± 3078.76	3988.95 ± 3138.33	5241.8 ± 3125.71
Jogging (m)	NIT + CM	2676.28 ± 2523.16	1282.38 ± 1091.75	2734.77 ± 2654.58	3169.10 ± 3001.26	3554.06 ± 2592.83
	Placebo	2442.78 ± 1248.90	1821.92 ± 545.27	2957.51 ± 1459.65	2523.50 ± 1425.19	2426.49 ± 1104.39
	All players	2546.35 ± 1915.95	1578.26 ± 865.78	2860.06 ± 2033.74	2785.78 ± 2184.02	2970.83 ± 2014.93
HIR (m)	NIT + CM	677.85 ± 680.38	271.70 ± 203.65	574.29 ± 401.43	856.78 ± 843.89	1021.42 ± 845.33
	Placebo	680.40 ± 372.76	510.05 ± 235.67 *	691.76 ± 294.60	685.19 ± 386.85	853.78 ± 494.13
	All players	679.27 ± 529.0	402.41 ± 249.26	640.37 ± 344.49	754.9 ± 608.19	934.71 ± 679.06
MIR (m)	NIT + CM	77.98 ± 124.37	27.25 ± 31.02	50.09 ± 51.07	115.04 ± 155.63	122.20 ± 176.75
	Placebo	54.64 ± 59.35	44.09 ± 38.02	26.05 ± 25.68	78.37 ± 84.35	70.84 ± 57.22
	All players	64.99 ± 94.2	36.48 ± 35.5	36.57 ± 40.03	93.27 ± 117.65	95.64 ± 129.71

Vmax: maximal speed; TD: total distance covered; Distance × minute: distance per minute; HIR: high-intensity running; MIR: maximal-intensity running. * Statistically significant at ($p < 0.05$).

To assess post-match recovery, performance outcomes from the CMJ test and WAnT conducted on the first day of pre-season were compared with those obtained the day after a pre-season match. This second assessment took place after a four-week supplementation period, with the final dose consumed on match day. The aim was to determine which group

showed better maintenance of performance—or, conversely, a smaller decline—under conditions of accumulated fatigue.

The CMJ pre-WAnT was used to assess anaerobic power and explosive strength under post-match conditions (Table 6). None of the variables showed statistically significant differences between groups. However, the NIT + CM group showed slightly more stable values in jump height, CMJ_{Pmean} and CMJ_{Ppeak}, while the placebo group presented a small decline in both power metrics.

Table 6. Descriptive statistics at baseline and at the end of pre-season, and variation of CMJ before WAnT: mean and standard deviations (mean $\pm$ SD).

Variable	Group	Baseline	Final	Δ Final – Baseline	p-Value
Height ₁	NIT + CM	24.93 $\pm$ 4.30	25.57 $\pm$ 3.85	0.64 $\pm$ 0.91	0.437
	Placebo	26.33 $\pm$ 4.77	26.54 $\pm$ 4.90	0.21 $\pm$ 1.54	
CMJ _{Pmean1}	NIT + CM	648.00 $\pm$ 66.36	654.20 $\pm$ 72.70	6.20 $\pm$ 24.99	0.424
	Placebo	673.43 $\pm$ 100.60	670.16 $\pm$ 91.56	−3.27 $\pm$ 30.67	
CMJ _{Ppeak1}	NIT + CM	659.07 $\pm$ 74.00	661.67 $\pm$ 74.74	2.60 $\pm$ 26.48	0.478
	Placebo	708.20 $\pm$ 109.47	703.31 $\pm$ 108.06	−4.89 $\pm$ 24.31	

Height: jump height (cm); P_{mean}: mean power (W); P_{peak}: power peak (W).

Although the differences were small and not significant, this pattern may suggest that the NIT + CM group recovered slightly better in terms of anaerobic performance.

The CMJ post-WAnT was used to evaluate neuromuscular fatigue and recovery capacity the day after the match. None of the variables showed statistically significant differences between groups (Table 7).

Table 7. Descriptive statistics at baseline and at the end of pre-season, and variation of CMJ after WAnT: mean and standard deviations (mean $\pm$ SD).

Variable	Product	Baseline	Final	Δ Final – Baseline	p-Value
Height ₂	NIT + CM	22.76 $\pm$ 2.78	23.70 $\pm$ 2.77	0.95 $\pm$ 1.06	0.931
	Placebo	24.49 $\pm$ 4.39	25.55 $\pm$ 4.65	1.06 $\pm$ 1.06	
CMJ _{Pmean2}	NIT + CM	614.15 $\pm$ 56.17	632.04 $\pm$ 62.62	17.89 $\pm$ 21.65	0.563
	Placebo	664.81 $\pm$ 99.19	677.43 $\pm$ 83.02	12.62 $\pm$ 22.32	
CMJ _{Ppeak2}	NIT + CM	623.99 $\pm$ 57.90	643.64 $\pm$ 62.76	19.65 $\pm$ 18.77	0.285
	Placebo	679.66 $\pm$ 103.49	689.49 $\pm$ 85.50	9.83 $\pm$ 22.99	

Height: jump height (cm); P_{mean}: mean power (W); P_{peak}: power peak (W).

Jump height increased slightly in both groups, with very similar values in the change from baseline to the final test. This suggests that explosive strength was not markedly affected by the intervention in either condition.

In contrast, CMJ_{Pmean} and CMJ_{Ppeak} values showed a slightly more favorable evolution in the NIT + CM group. Both groups improved from baseline, but the NIT + CM group showed a greater increase in power compared to the placebo group. Although these differences were not significant, the trend may point toward a better recovery of neuromuscular performance following high-intensity effort in the NIT + CM group.

As with the CMJ pre-WAnT, these results do not confirm a clear effect of the product but suggest a consistent pattern that warrants further exploration under similar post-match conditions.

The WAnT was used to evaluate anaerobic power and capacity under post-match conditions (Table 8). Several variables showed significant differences between groups, indicating a better preservation of performance in the NIT + CM group. P_{mean} and P_{min} decreased in both groups, but the reduction was significantly smaller in the NIT + CM group. The decline in power across the 30 s effort was also consistently lower in the NIT + CM group, with significant differences in both the first and second half of the test.

Table 8. Descriptive statistics at baseline and at the end of pre-season, and variation of WAnT: mean and standard deviations (mean $\pm$ SD).

Variable	Group	Baseline	Final	Δ Final – Baseline	p-Value
P_{peak}	NIT + CM	635.09 $\pm$ 89.93	625.91 $\pm$ 81.80	−9.18 $\pm$ 25.30	0.070
	Placebo	631.71 $\pm$ 113.53	596.64 $\pm$ 108.63	−35.07 $\pm$ 39.14	
TP_{peak}	NIT + CM	6.36 $\pm$ 1.21	5.91 $\pm$ 1.81	−0.46 $\pm$ 1.64	0.469
	Placebo	6.64 $\pm$ 2.27	5.57 $\pm$ 2.62	−1.07 $\pm$ 2.37	
P_{min}	NIT + CM	378.82 $\pm$ 43.49	349.73 $\pm$ 45.33	−29.09 $\pm$ 28.04	0.036 *
	Placebo	404.79 $\pm$ 48.21	345.21 $\pm$ 24.79	−59.57 $\pm$ 37.95	
FI (%)	NIT + CM	39.70 $\pm$ 8.05	42.12 $\pm$ 7.95	2.49 $\pm$ 2.58	0.087
	Placebo	38.01 $\pm$ 6.02	42.89 $\pm$ 9.17	4.88 $\pm$ 3.95	
P_{mean}	NIT + CM	517.94 $\pm$ 58.80	497.49 $\pm$ 51.74	−20.46 $\pm$ 21.06	0.004 *
	Placebo	520.27 $\pm$ 73.52	468.31 $\pm$ 57.52	−51.96 $\pm$ 23.38	
Split _{10–10S}	NIT + CM	591.53 $\pm$ 88.83	585.95 $\pm$ 78.96	−5.58 $\pm$ 23.40	0.046 *
	Placebo	586.14 $\pm$ 111.71	553.59 $\pm$ 97.79	−32.55 $\pm$ 36.23	
Split _{10–20S}	NIT + CM	541.87 $\pm$ 57.09	511.13 $\pm$ 55.72	−30.73 $\pm$ 33.16	0.019 *
	Placebo	543.39 $\pm$ 71.94	483.30 $\pm$ 65.55	−60.09 $\pm$ 23.44	
Split _{20–30S}	NIT + CM	431.28 $\pm$ 40.27	398.87 $\pm$ 37.02	−32.41 $\pm$ 23.94	0.021 *
	Placebo	443.85 $\pm$ 51.27	386.36 $\pm$ 41.52	−57.48 $\pm$ 25.08	
Split _{0–15S}	NIT + CM	583.25 $\pm$ 78.48	571.01 $\pm$ 70.53	−12.23 $\pm$ 23.64	0.012 *
	Placebo	579.49 $\pm$ 97.27	537.91 $\pm$ 85.21	−41.58 $\pm$ 28.16	
Split _{15–30S}	NIT + CM	457.00 $\pm$ 43.76	425.35 $\pm$ 40.73	−31.65 $\pm$ 23.32	0.011 *
	Placebo	467.56 $\pm$ 55.74	408.97 $\pm$ 46.03	−58.59 $\pm$ 23.88	

P_{peak} : peak power (W); TP_{peak} : time to P_{peak} (s); P_{min} : minimum power (W); FI (%): fatigue index; P_{mean} : mean power (W); Split: P_{mean} calculated every 10 and 15 s. * Statistically significant at ($p < 0.05$).

Although P_{peak} and FI (%) did not reach statistical significance, both followed the same pattern observed in other variables. TP_{peak} showed no relevant differences between groups.

These findings suggest that the NIT + CM product may have contributed to preserving anaerobic capacity under conditions of accumulated fatigue. In the context of female soccer, this finding becomes especially relevant, as a systematic review and meta-analysis [96] has shown that high-intensity anaerobic performance, including sprint capacity, remains impaired for up to 48 h following a match. Supporting the ability to maintain anaerobic output the day after competition may therefore have practical implications for performance management during congested schedules or periods of high training load.

To complement the performance data, plasma concentrations of NO_3^- and NO_2^- were analyzed at baseline and again the day after the match (Table 9). The NIT + CM group showed significant increases from baseline in both NO_3^- ($p < 0.001$) and NO_2^- ($p = 0.008$). At the final time point, NO_3^- was significantly higher in the NIT + CM group compared to placebo ($p < 0.001$), while no significant between-group difference was observed for NO_2^- .

Table 9. Descriptive statistics at baseline and at the end of pre-season, and variation of NO_2^- and NO_3^- plasma concentration: mean and standard deviations (mean $\pm$ SD).

Variable	Group	Baseline	Final	Δ Final – Baseline	p-Value
NO_2^-	NIT + CM	4.43 $\pm$ 2.59	13.20 $\pm$ 14.35	8.78 $\pm$ 13.19 *	0.008 *
	Placebo	5.02 $\pm$ 2.33	7.12 $\pm$ 3.55	1.92 $\pm$ 2.50	
NO_3^-	NIT + CM	12.32 $\pm$ 4.20	86.10 $\pm$ 40.18 *	73.78 $\pm$ 40.74 *	<0.001 *
	Placebo	12.85 $\pm$ 5.66	26.56 $\pm$ 15.05	14.82 $\pm$ 14.41	

* Statistically significant at ($p < 0.05$). Concentrations were expressed in micromoles per liter ($\mu\text{mol/L}$).

Importantly, plasma NO_3^- concentrations in the NIT + CM group remained elevated the day after the final dose. This aligns with previous research showing that chronic supplementation leads to progressive increases in plasma NO_3^- , and that concentrations remain above baseline even 24 h after the last intake. For instance, in a study with 19.5 mmol/day over 8 days, values rose from $37 \pm 15 \mu\text{M}$ to $270 \pm 182 \mu\text{M}$ one day after the final dose, indicating prolonged systemic exposure [97]. Although absolute values are not directly comparable due to population and dose differences, this finding is consistent with the present observations using 8 mmol/day over four weeks.

Moreover, previous studies have shown that multi-day NO_3^- intake results in higher plasma NO_3^- compared to single bolus ingestion [45], and that NO_2^- responses are more closely associated with total NO_3^- intake across the protocol than with daily intake alone [12]. In the present analysis, NO_2^- was the only biomarker that did not show a significant between-group difference at the final measurement, despite a significant increase from baseline in the NIT + CM group. This finding may be related to the markedly shorter plasma half-life of NO_2^- compared to NO_3^- [98], which limits its persistence in circulation during the recovery phase. In addition, NO_2^- may be buffered or stored within skeletal muscle tissue [45], potentially reducing its detectability in plasma while still contributing to local NO availability. These mechanisms could help explain the absence of a significant difference between groups, even under conditions of elevated systemic NO_3^- availability.

4. Limitations

This study has some limitations that should be acknowledged to contextualize the findings. The sample size, while adequate for a pilot investigation in elite athletes, may limit the generalizability of the results to female soccer. However, it is important to note that access to professional soccer teams is inherently difficult, and these limitations are common in studies involving elite athletes. Although certain external variables were controlled, such as the use of ergogenic aids and mouthwash, detailed monitoring of dietary intake was not performed, which could have influenced NO_3^- availability. The study was conducted exclusively during the pre-season, a period with specific training demands, so caution is needed when extrapolating to other phases of the season. Additionally, while relevant performance and recovery metrics were included, no biochemical markers of muscle damage were assessed, due to limitations from the teams. The sample included players from two different professional teams, which may have introduced variability in training methods, coaching decisions, and recovery protocols. This reflects a broader limitation of studies in team sports, as standardization is often constrained by factors that lie outside the control of the research team. Finally, although the supplemented group showed consistent positive trends, not all differences reached statistical significance, suggesting that further research is warranted to confirm these preliminary findings.

5. Conclusions

This study evaluated the effects of chronic supplementation with NO_3^- and CM during pre-season in professional female soccer players. Players who received the NIT + CM product consistently achieved higher V_{max} values during both training sessions and matches, along with greater total and walking distances and a higher distance covered per minute in training. These results suggest a greater tolerance to high training loads during pre-season. Most importantly, the NIT + CM group showed better preservation of anaerobic capacity the day after a competitive match, with significantly smaller reductions in mean and minimum power during the WAnT. These findings were supported by elevated plasma NO_3^- concentrations measured 24 h after the final dose, indicating sustained systemic exposure during the recovery phase. Overall, these results highlight the potential utility of NIT + CM supplementation to support neuromuscular performance and attenuate post-match fatigue during pre-season, a period characterized by high physical demands and limited recovery windows. This strategy may offer practical benefits for nutritionists aiming to optimize player readiness and recovery during pre-season, as well as to support performance across other high-demand phases of the competitive season.

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Abbreviations

The following abbreviations are used in this manuscript:

NO	Nitric oxide
NO_3^-	Nitrate
NO_2^-	Nitrite
CM	Citrulline malate
GPS	Global positioning system
CMJ	Countermovement jump
WAnT	Wingate anaerobic test
NOS	Nitric oxide synthase
IMU	Inertial measurement unit
EPTS	Electronic performance and tracking systems
V_{max}	Maximal speed
TD	Total distance covered

HIR	High-intensity running
MIR	Maximal-intensity running
BMI	Body mass index
P _{mean}	Mean power
P _{peak}	Power peak
TP _{peak}	Time to P _{peak}
P _{min}	Minimum power
FI	Fatigue index

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4.4. ARTÍCULO 4

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Article

External Load Across Menstrual Cycle Phases in Spanish Professional Female Soccer Players: An Observational Study Using App-Based Cycle Tracking

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Abstract

Background: Monitoring training load in professional soccer should account for female-specific physiological rhythms, including menstrual cycle fluctuations. Although app-based tracking tools provide a non-invasive solution for estimating cycle phases, their relationship with performance metrics remains unclear. This study investigates whether external training load varies across menstrual cycle phases estimated via app-based tracking in professional female soccer players during pre-season and early competition. **Methods:** A prospective, longitudinal, and observational study was conducted with 31 professional female soccer players (age: 23.29 ± 4.27 years) from two Spanish teams. Global Positioning System (GPS)-derived external load was recorded daily during all training sessions over five weeks of pre-season and early competition. Menstrual cycle phases were estimated using the FitWoman app and classified into four physiological phases. Phase-based differences were analyzed using one-way ANOVA with post hoc tests. **Results:** The follicular to ovulation phase consistently showed the highest values in total distance (TD), maximum velocity (Vmax), and walking distance compared to the other phases (all $p \leq 0.002$). No significant effects were observed in the remaining GPS-derived variables. **Conclusions:** Menstrual cycle phase may influence specific aspects of training load, and app-based monitoring may offer a feasible strategy to contextualize performance data in elite environments.

Keywords: menstrual cycle phases; performance; external load; tracking apps; female soccer

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1. Introduction

Monitoring player status is a key component in designing and evaluating training performance in elite soccer [1]. In professional women's soccer, this monitoring should also account for physiological rhythms unique to female athletes, such as the menstrual

cycle [2]. This cycle is regulated by the hypothalamic–pituitary–ovarian axis [3] and is characterized by fluctuations in the secretion of sex hormones, primarily estrogen and progesterone [4]. Scientific literature has described how these hormones influence cardiovascular function, substrate utilization and thermoregulation [5–7]. Among eumenorrheic women, menstrual cycle length typically ranges from 21 to 35 days, with an average of approximately 28 days [8]. Traditionally, the cycle is divided into two main phases: a follicular phase (days 1–14), characterized by high estrogen and low progesterone concentrations, and a luteal phase (days 15–28), characterized by low estrogen and high progesterone levels [7,9,10]. Although more detailed classifications exist [11,12], to date, no consensus has been reached within sport and exercise science regarding the nomenclature or the subdivision of menstrual cycle phases based on hormonal profiles [9].

Interest in the menstrual cycle has increased substantially in sport science, leading to a growing emphasis on methodological rigor in the estimation of menstrual phases. Recent position statements have recommended the use of hormonal verification to improve accuracy and standardization in research protocols [13]. While this level of control is valuable for advancing the understanding of endocrine responses, its implementation in elite team sports is often limited by logistical and financial restrictions [14]. In this context, app-based tracking tools have emerged as a feasible and non-invasive alternative for longitudinal monitoring in real training environments [14–16]. However, it remains unclear whether the phases estimated through these tools correspond to meaningful differences in physical performance.

A recent on-field study highlighted the absence of standardized guidelines for menstrual monitoring in sport and emphasized the value of individual tracking as a first step toward understanding cycle-related patterns in athlete behavior [14].

The relevance of this approach has recently been acknowledged at an institutional level. In 2023, FIFA launched its Women's Health Project, emphasizing the importance of understanding how female-specific physiology, including the menstrual cycle, can influence health and performance in soccer [17]. According to the report, 95% of players experience menstrual-related symptoms, 66% believe their performance is affected, 90% of players do not communicate menstrual cycle issues with coaches, and 85% identify a general lack of knowledge surrounding this issue. These figures underscore the demand for applied research capable of capturing the physiological complexity of female athletes in authentic training contexts. Although adapting training content to the menstrual phase may not be feasible in team sports due to asynchronous cycles and practical limitations [18], monitoring menstrual status over time could provide valuable context for interpreting training responses, managing fatigue, and supporting individualized follow-up.

The primary aim of this study is to examine whether external training load differs across menstrual cycle phases estimated using the FitrWoman app in professional female soccer players. Monitoring was conducted over a five-week period covering pre-season and early competition. We hypothesize that external training load may vary across phases, potentially reflecting the influence of cyclical hormonal fluctuations.

2. Materials and Methods

2.1. Study Design

This was a prospective, longitudinal, and observational study designed to examine potential differences in training performance across distinct phases of the menstrual cycle in professional female soccer players. The study lasted five weeks, including four weeks of pre-season and one week at the beginning of the competitive period. GPS-derived performance metrics were collected daily during all training sessions. Menstrual cycle phase was estimated using a mobile application specifically designed for female athletes. This

observational study was conducted as part of a larger intervention study. Prior to starting the intervention, the protocol was approved by the Institutional Review Committee of the UCAM (code CE052204), following the Standards of Good Clinical Practice, and was conducted according to the Declaration of Helsinki. The clinical trial was registered at www.clinicaltrials.gov (accessed on 9 August 2022) (identifier NCT05525871). Current European legislation on the protection of personal data (Regulation (EU)2016/679) was complied with.

2.2. Participants

Research was conducted on a sample of 31 professional female players (age: 23.29 ± 4.27 years) from two soccer teams in the first and second Spanish professional Women's Football League.

The inclusion criteria for participant recruitment were as follows: (a) healthy subject with medical authorization for the practice of federated sport; (b) belongs to a team of the first or second division of the Spanish Women's League. The exclusion criteria for the study were: (a) changing teams during pre-season; (b) history of drug, alcohol, or other substance abuse or other factors limiting their ability to cooperate during the study; (c) the use of oral contraceptives; (d) self-reported menstrual cycle irregularity, defined as an average cycle length outside the 21–35 day range. All players were previously informed of the objectives and method of the research, signing informed consent forms before starting the research. In the case of minor players, it was their parents who signed the consent.

2.3. Menstrual Cycle

Two weeks prior to the start of pre-season, all players were contacted by the research team and instructed to begin tracking their menstrual cycle using the FitrWoman mobile app [19] (Orreco Ltd., Galway, Ireland), which has been used in previous studies [1,20–23].

They were also asked to retrospectively log the onset date of their most recent cycle to ensure that at least one full menstruation had been recorded before the start of the study. Throughout the five-week observation period, participants received daily reminders to update their records, and the research team provided ongoing supervision to ensure compliance. The research team had access to FitrWoman app data in real time via a remote desktop as part of the FitrCoach platform [24] (Orreco Ltd., Galway, Ireland). This system allowed the supervision of player tracking profiles, verification of menstrual log consistency, and export of historical data for analysis.

Based on the data entered by each player, the app's proprietary algorithm classified each calendar day into one of four menstrual cycle phases: Menstruation (Early Follicular), Follicular to Ovulation, Luteal, and Premenstrual (Late Luteal). These categories and their physiological descriptions were defined by the app's internal software and were adopted without modification for the present analysis. The research team then matched each day's assigned phase with the corresponding GPS-derived training session, enabling all sessions to be categorized according to the menstrual cycle phase indicated by the application. The app assumes a eumenorrheic cycle length of 21 to 35 days, and its algorithm automatically adjusts to variations in the user's reported cycle length. A visual representation of this classification model, as displayed within the app interface, is shown in Figure 1.

According to the app, the Menstruation (Early Follicular) phase spans from the first day of bleeding until the cessation of menstrual flow, during which estrogen and progesterone levels are considered to be at their lowest. The Follicular to Ovulation phase begins once bleeding has ended and continues until the estimated day of ovulation; it is characterized by a steady rise in estrogen, while progesterone remains low. The Luteal phase

follows ovulation and extends to approximately five days before the start of the next cycle, with both estrogen and progesterone remaining elevated. Finally, the Premenstrual (Late Luteal) phase comprises the final four to five days of the cycle and is marked by a progressive decline in both hormones.

This approach was selected due to logistical constraints related to professional sports environments. Access to players was limited throughout the observation period, and invasive testing procedures could not be implemented without disrupting club routines. In amateur teams or laboratory-based studies, daily hormonal sampling may be more feasible, but such methods were not applicable in this context.

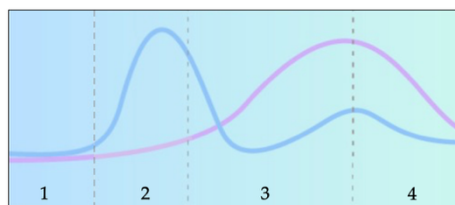


Figure 1. Phase 1: Menstruation (Early Follicular); Phase 2: Follicular to Ovulation; Phase 3: Luteal; Phase 4: Premenstrual (Late Luteal); Blue line: Estrogen; Purple line: Progesterone.

2.4. Training data

In team sports, external load is typically assessed through GPS-based tracking systems [25]. For this study, a lower-limb wearable GPS tracker (calf-worn) developed by Oliver Sports (Sport Data Innovation SL, Barcelona, Spain) was utilized to monitor player performance metrics during all training sessions throughout the five-week observation period. The device integrates Inertial Measurement Unit (IMU) sensors with a sampling rate of 50 Hz and GPS technology with a sampling rate of 25 Hz, enabling precise measurement of variables such as Vmax, TD, and distance per minute.

This system is currently included in the FIFA Innovation Programme [26] which aims to explore emerging electronic performance and tracking systems (EPTS) [27] to enhance the precision of collected data and the understanding of player performance.

The wearable tracker incorporates AI-supported software that provides actionable insights for players and coaching staff, enhancing individual and team performance. The software automatically categorized movement into four predefined speed thresholds: walking (0 to 7.1 km/h), jogging (7.1 to 14 km/h), high-intensity running (HIR) (14 to 22.3 km/h), and maximal intensity running (MIR) (22.3 to 36 km/h). These thresholds were fixed by the system and could not be adjusted. GPS data from training sessions were obtained from the tracking software and exported into Excel for further processing and analysis.

2.5. Statistical Analyses

SPSS Statistics 27 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Descriptive statistics were calculated and used to describe the GPS-derived training data. The statistics were reported as mean $\pm$ standard deviation (SD). The Shapiro-Wilk and Levene's tests were used to verify the normality and homoscedasticity of the data, respectively.

One-way ANOVA was applied to TD and Vmax, as both variables met the assumption of normality. For the remaining variables (walking, jogging, HIR, and MIR), which showed non-normal distributions, the Kruskal-Wallis test was used as a non-parametric

alternative. Post hoc comparisons were carried out using the Bonferroni test if homogeneity of variances was confirmed; otherwise, the Games–Howell test was applied. Effect sizes were estimated using partial eta squared (η^2p) and classified as small ($0.01 \leq \eta^2p < 0.06$), medium ($0.06 \leq \eta^2p < 0.14$), or large ($\eta^2p \geq 0.14$) [28]. The level of statistical significance was set at $p \leq 0.05$.

3. Results and Discussion

The demographic characteristics of the players are displayed in Table 1.

Table 1. Demographic data of the players: mean and standard deviations (mean $\pm$ SD).

	Mean $\pm$ SD
n	31
Age	23.29 $\pm$ 4.27
Height (cm)	163.41 $\pm$ 5.15
Weight (kg)	57.80 $\pm$ 8.58
BMI (kg/m ²)	21.64 $\pm$ 1.74
$\Sigma 6$ Skinfolds (mm)	73.68 $\pm$ 18.03

BMI: Body Mass Index; $\Sigma 6$ Skinfolds (triceps, subscapular, supraspinal, abdominal, front thigh, and medial calf).

Table 2 summarizes the ANOVA results and descriptive statistics for each GPS-derived variable across the four menstrual cycle phases. Vmax showed significant phase-related differences ($p < 0.001$; $\eta^2p = 0.058$), with higher values in the follicular to ovulation phase compared to luteal and premenstrual phases (both $p < 0.001$). Although no significant difference was found relative to the menstrual phase, descriptive data indicated that Vmax remained lower than in the follicular to ovulation phase and closely resembled the values observed during the luteal phase. A similar trend has been reported in professional basketball players [29], with GPS-based monitoring suggesting that the late follicular phase and ovulation may offer more favorable conditions for speed and explosive actions. Although the demands differ between sports, this phase-related pattern appears to align with the present findings.

One possible explanation for this performance pattern lies in the known physiological effects of hormonal fluctuations throughout the menstrual cycle, particularly those presumed to occur during the phase estimated by the app. Estrogen is considered to have a neuroexcitatory role, while progesterone has been linked to decreased cortical excitability [30]. These contrasting actions suggest a differential impact on neuromuscular function, with estrogen supporting and progesterone potentially limiting force generation [31,32]. It has been proposed that strength and power capacities are more likely to be optimized under hormonal profiles characterized by low progesterone and elevated estrogen, such as those occurring during the late follicular phase [33]. In contrast, increased progesterone during the luteal phase may contribute to reduced neuromuscular efficiency. The ability to produce force rapidly depends in part on the initial rate of motor unit recruitment, a key determinant of explosive performance [34]. In soccer-specific contexts, this neuromuscular activation is particularly relevant to the lower limbs, as it underpins the capacity to progressively accelerate and reach maximum velocity.

Notably, the lower Vmax values observed in the premenstrual phase may be linked to increased fatigue and changes in mood and motivation, patterns frequently reported during this stage [10]. Although no significant differences were found for the menstrual phase, descriptive values also remained below those recorded during the follicular to ovulation phase.

In addition, TD and walking distance both showed significant phase-related differences. Regarding TD ($p < 0.001$; $\eta^2p = 0.058$), values were significantly higher in the follicular to ovulation phase compared to all other phases (all $p < 0.01$), with the lowest values observed during the menstrual and premenstrual phases and intermediate values during the luteal phase. A comparable pattern was found for walking distance ($p = 0.002$; $\eta^2p = 0.038$), which peaked during the follicular to ovulation phase as well, with significantly greater values than those recorded in the menstrual ($p = 0.013$) and premenstrual ($p = 0.043$) phases, although no significant difference was observed compared to the luteal phase ($p = 0.164$).

These findings may reflect physiological differences in the ability to sustain prolonged activity across phases. Estrogen has been associated with increased reliance on lipid oxidation and reduced glycogen utilization during submaximal exercise, particularly under conditions of moderate intensity [35]. It has also been linked to improved cardiovascular regulation during exercise [36]. These physiological effects may collectively support sustained locomotor activity during the follicular to ovulation phase.

In contrast, the lower values recorded in the menstrual and premenstrual phases may be related to physical symptoms frequently reported during these stages. These phases have been commonly associated with menstrual pain, swelling, and general physical discomfort, which could influence an athlete's pacing strategy or overall movement behavior during training [37]. While the luteal phase presented intermediate results, the absence of significant differences in walking distance compared to the follicular to ovulation phase may reflect inter-individual variability in symptom expression, hormonal sensitivity, or adaptive strategies used by players across the cycle.

To our knowledge, only one previous study has examined menstrual cycle-related variations in external training load in elite female soccer players. In that analysis, total distance was significantly lower during the menstruation week compared to the post-ovulation week, while no other significant differences were detected between phases [38]. Although this partially aligns with the present results, which also showed lower values in the menstrual and premenstrual phases, the two studies reported different methodological approaches. A similar direction was observed in a controlled study involving high-level female soccer players, in which performance in a Yo-Yo Intermittent Endurance Test showed a tendency to decline during the mid luteal phase compared to the early follicular phase [7]. While statistical significance was not reached, the authors interpreted this trend as a possible reduction in aerobic performance. Differences in design, testing protocols, and phase classification limit direct comparison, but both studies point toward a potential decrease in endurance-related outputs during the luteal phase.

A recent systematic review on the effects of the menstrual cycle on performance in female soccer players [39] reported that several studies assessing aerobic and intermittent endurance outcomes did not detect significant differences between phases. While results across the literature remain mixed, the present findings provide observational, field-based data suggesting a possible increase in total and walking distance during the follicular to ovulation phase. These patterns should be interpreted with caution, and additional studies using comparable methodologies are needed to confirm their consistency.

No statistically significant differences were observed across menstrual cycle phases for distance per minute, jogging, HIR, or MIR. However, most of these variables followed a consistent directional trend, with higher average values in the follicular to ovulation phase and lower values in the premenstrual phase. Distance per minute showed an opposite pattern. These trends reflect a similar phase-related response to that observed in TD and Vmax. Although not all variables appear equally sensitive, this consistency supports the idea that menstrual cycle monitoring may help identify individual patterns in external load, providing useful context for performance assessment in elite athletes [40].

Table 2. One-way ANOVA and descriptive statistics of GPS-derived performance variables by menstrual cycle phases: mean and standard deviations (mean $\pm$ SD).

Variable	<i>p</i>	η^2p	Phase 1	Phase 2	Phase 3	Phase 4
Vmax (Km/h)	< 0.001*	0.058	24.28 $\pm$ 5.78	26.04 $\pm$ 3.24	24.07 $\pm$ 4.54	23.63 $\pm$ 3.41
TD (m)	< 0.001*	0.058	4434.11 $\pm$ 1676.30	5233.72 $\pm$ 1276.53	4617.49 $\pm$ 1442.48	4416.64 $\pm$ 1570.72
Distance x minute	0.854	0.004	66.87 $\pm$ 18.50	65.78 $\pm$ 17.57	67.35 $\pm$ 30.21	71.09 $\pm$ 34.75
Walking (m)	0.002*	0.038	2704.88 $\pm$ 1115.32	3193.46 $\pm$ 780.99	2950.77 $\pm$ 975.56	2764.17 $\pm$ 1023.44
Jogging (m)	0.407	0.009	1308.09 $\pm$ 665.70	1440.64 $\pm$ 658.84	1310.84 $\pm$ 687.94	1304.59 $\pm$ 723.02
HIR (m)	0.074	0.030	369.77 $\pm$ 261.83	459.84 $\pm$ 399.38	337.31 $\pm$ 284.73	332.79 $\pm$ 300.88
MIR (m)	0.341	0.011	18.91 $\pm$ 23.62	24.96 $\pm$ 43.54	18.56 $\pm$ 28.66	15.08 $\pm$ 28.75

Vmax: Maximal speed; TD: total distance covered; Distance x minute: Distance per minute; HIR: High-intensity running; MIR: maximal intensity running; Phase 1: Menstruation (Early Follicular); Phase 2: Follicular to Ovulation; Phase 3: Luteal; Phase 4: Premenstrual (Late Luteal). * Statistically significant ($p < 0.05$).

4. Limitations

This study has some limitations that should be acknowledged to contextualize the findings. One of them was the relatively small sample size of 31 female players, which reflects the inherent difficulty of accessing professional soccer teams. This is a common limitation in studies involving elite athletes. Menstrual cycle phases were estimated using an app-based tracking tool rather than through hormonal verification. While this approach is feasible and commonly applied in elite sport environments, it does not provide direct hormonal data, which may have affected the precision of phase classification. The observational design and the absence of control over training content reduce the capacity to attribute observed differences exclusively to menstrual phase. Moreover, the study was conducted during a four-week pre-season period followed by one week at the beginning of the competitive season, so extrapolation to other stages of the competitive calendar should be made with caution. Despite these limitations, the study offers practical insight into phase-related trends in training load under real-world conditions in professional female soccer.

5. Conclusions

This study aimed to examine whether external training load varies across menstrual cycle phases in professional female soccer players using an app-based monitoring tool (FitWoman). The results revealed significant differences in Vmax, TD, and walking distance, with higher values consistently observed during the follicular to ovulation phase. Although other performance variables did not reach statistical significance, most followed a coherent directional trend supporting a potential phase-related influence. These

findings suggest that menstrual cycle phase may have an impact on specific aspects of physical output under real training conditions. The use of a practical, non-invasive tracking tool underscores the feasibility of incorporating individualized cycle monitoring to contextualize external load data in elite environments. Future research should continue exploring how cyclical hormonal changes interact with training demands, emphasizing personalized approaches and applied methodologies suited to high-performance sport.

6. Patents

Author Contributions: Conceptualization, M.R.-M., R.A., F.J.L.-R., V.Á.-G., S.P.-P., J.C.M.-C., A.J.L.-R. and J.M.; methodology, M.R.-M., R.A., F.J.L.-R., V.Á.-G., S.P.-P., J.C.M.-C., A.J.L.-R. and J.M.; software, M.R.-M., R.A., and J.M.; validation, M.R.-M., R.A., and J.M.; formal analysis, M.R.-M., R.A., and J.M.; investigation, M.R.-M., R.A., F.J.L.-R., V.Á.-G., S.P.-P., J.C.M.-C., A.J.L.-R. and J.M.; resources, M.R.-M., R.A., and J.M.; data curation, M.R.-M., R.A., and J.M.; writing—original draft preparation, M.R.-M., R.A., and J.M.; writing—review and editing, M.R.-M., R.A., and J.M.; visualization, M.R.-M., R.A., and J.M.; supervision, M.R.-M., R.A., F.J.L.-R. and J.M.; project administration, F.J.L.-R.; funding acquisition, F.J.L.-R. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data are contained within the article.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

GPS	Global Positioning System
TD	Total distance
Vmax	Maximum velocity
EPTS	Electronic performance and tracking systems
HIR	High-intensity running
MIR	Maximal intensity running
SD	Standard deviation
BMI	Body Mass Index

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V – RESUMEN Y DISCUSIÓN DE LOS RESULTADOS

V - RESUMEN Y DISCUSIÓN DE LOS RESULTADOS

Los cuatro artículos que conforman esta tesis comparten un objetivo común: aportar evidencia científica que permita optimizar la pretemporada en fútbol femenino profesional. Para ello, se han explorado distintas dimensiones que afectan al rendimiento y la recuperación de las jugadoras, incluyendo la evolución de la composición corporal, las correlaciones con variables físicas clave, el papel de la suplementación con NO_3^- y CM, y la influencia del ciclo menstrual sobre la carga de entrenamiento.

5.1. ARTÍCULO 1

Para este artículo se analizaron los datos antropométricos y de composición corporal al inicio y al final de la pretemporada, con el objetivo principal de describir las características por posición de juego y observar posibles cambios a lo largo del periodo. Hasta ahora, los cambios de composición corporal entre posiciones en el periodo de pretemporada habían sido analizados principalmente en fútbol masculino (67, 151, 152) sin que existieran estudios equivalentes en fútbol femenino.

No se observaron diferencias significativas entre posiciones ni al inicio ni al finalizar la pretemporada. Esta similitud entre posiciones coincide con lo descrito en algunos estudios previos (153-157). Aunque también existen trabajos que sí reportan diferencias significativas entre posiciones, cabe destacar que en la mayoría de ellos las muestras no estaban formadas exclusivamente por jugadoras profesionales o de élite (127, 158-160), mientras que los estudios centrados en poblaciones de ese nivel suelen reportar únicamente diferencias mínimas entre posiciones (161-163).

Entre todas las variables analizadas, las dimensiones corporales como la estatura de pie y sentado, la envergadura, los diámetros óseos y la masa ósea se midieron únicamente al inicio de la pretemporada, al tratarse de características estructurales que no se modifican en periodos tan breves. Aunque la mayoría de las mediciones iniciales no mostró diferencias significativas entre posiciones, algunas de estas dimensiones sí presentaron valores distintos en función del rol en

el campo. En particular, se hallaron diferencias estadísticamente significativas ($p<0.05$) en la envergadura y el diámetro del tobillo entre las porteras y el resto de posiciones.

Respecto a la envergadura, las porteras mostraron los valores más altos (porteras vs. centrocampistas: $\Delta=15.30$ cm, $p=0.004$; porteras vs. defensas: $\Delta=13.11$ cm, $p=0.012$; porteras vs. delanteras: $\Delta=12.26$ cm, $p=0.021$). Estos resultados coinciden con los observados en jugadoras españolas de 16 y 18 años (164), donde también se registraron mayores valores de envergadura en porteras, aunque no se especificó si las diferencias eran estadísticamente significativas respecto al resto de posiciones. Estos datos podrían estar relacionados con la necesidad de las porteras de cubrir un mayor espacio bajo palos y detener los disparos del equipo rival. Este mismo patrón se ha descrito también en fútbol masculino, tanto en contextos profesionales como en etapas de formación, donde entrenadores y ojeadores tienden a seleccionar porteros con mayor estatura y envergadura (165). Esta tendencia se justifica, en parte, por la correlación entre ambas variables (153) y por el hecho de que los porteros suelen ser los jugadores más altos y pesados del equipo (166). En el presente estudio, aunque estas diferencias no alcanzaron significación estadística, las porteras también presentaron la mayor altura y el mayor peso medio del grupo, en línea con lo observado en otros estudios sobre fútbol femenino (153, 155, 157-162, 167-169). Aun así, los datos sobre envergadura en fútbol femenino profesional siguen siendo escasos, especialmente en el caso de las porteras, cuya menor representación por equipo dificulta su análisis específico y la comparación con otros estudios. Por tanto, este hallazgo podría contribuir a perfilar mejor las características estructurales asociadas a esta posición.

En contraste con la envergadura, el diámetro del tobillo fue menor en las porteras (5.80 ± 0.89 cm) en comparación con las centrocampistas (6.93 ± 0.38 cm), defensas (6.70 ± 0.28 cm) y delanteras (6.66 ± 0.56 cm), con un valor de $p=0.012$. Este hallazgo difiere con un estudio en jugadoras sudafricanas de nivel sub-élite (159), que reportó diámetros de tobillo de 6.4 ± 0.4 cm en delanteras, 6.4 ± 0.3 cm en centrocampistas, 6.6 ± 0.4 cm en defensas y 6.7 ± 0.3 cm en porteras ($p=0.034$). Aunque dicho estudio también encontró diferencias estadísticamente significativas, su relevancia práctica era dudosa debido a los amplios intervalos de confianza y tamaños del efecto reducidos. Las discrepancias entre ambos estudios podrían deberse a diferencias entre categorías, ya que el presente estudio se centra en

jugadoras profesionales, con demandas físicas e intensidades de entrenamiento distintas a las de jugadoras sub-élite (170). Además, factores como la etnia (171), los determinantes genéticos y ambientales (172), así como posibles variaciones en los métodos de medición, podrían contribuir a estas diferencias. En cualquier caso, el diámetro del tobillo sigue siendo una variable poco explorada en el contexto del fútbol femenino, lo que limita las posibilidades de comparación y refuerza el valor de estos resultados como referencia inicial para futuras investigaciones.

Continuando con el análisis de la estatura, pero centrándonos ahora en los valores más bajos, las centrocampistas fueron las jugadoras de menor estatura. Este hallazgo coincide con lo descrito en otros estudios (21, 159, 168). Sin embargo, otros estudios han señalado a las delanteras como las jugadoras más bajas (154, 156, 158, 160, 162, 163, 169), lo que refleja una amplia variabilidad en la distribución de la estatura según la posición. Para comprender las diferencias observadas en los distintos estudios, sería interesante considerar tanto el estilo de juego de los diferentes equipos o divisiones como los criterios de los investigadores a la hora de clasificar a una jugadora dentro de una categoría específica de posición de juego. Por ejemplo, no es lo mismo considerar a una jugadora que juega por banda (que normalmente es una jugadora de baja estatura) como centrocampista lateral o como extremo, ya que en el primer caso se la definiría como centrocampista y en el segundo, como delantera.

En cuanto a la masa corporal, no se observó en este estudio ninguna posición claramente diferenciada por presentar un menor peso. Al revisar la literatura reciente para contrastar estos resultados, se identificó un número prácticamente equivalente de estudios que señalan a las delanteras (154, 156, 158, 162, 163), las centrocampistas (21, 155, 159, 161, 167-169) o las defensas (153, 155, 157, 160, 169) como las jugadoras con menor peso corporal. Por consiguiente, la presente investigación no contradice ni respalda de forma específica los estudios existentes sobre este tema.

Dado que no se observaron diferencias significativas entre posiciones ni al inicio ni al final de la pretemporada, excepto en algunas variables estructurales como la envergadura y el diámetro del tobillo, el análisis posterior se centró en la evolución de la composición corporal durante este periodo. En la mayoría de las futbolistas se observó una mejora general, con una tendencia hacia el aumento de la masa muscular y la reducción de la masa grasa, algo esperable en una etapa como

la pretemporada. Sin embargo, la magnitud y la dirección de estos cambios no fue homogénea entre posiciones.

En la medición de pliegues cutáneos, las centrocampistas fueron las jugadoras que mostraron las reducciones más marcadas, con descensos significativos en tríceps, muslo frontal y pantorrilla medial frente a las defensas, así como en pantorrilla medial frente a las delanteras y en tríceps respecto a las porteras. Esta tendencia también se refleja en los distintos sumatorios de pliegues, donde las centrocampistas presentaron las disminuciones más pronunciadas en las tres variantes analizadas (sumatorio de 3, 6 y 8 pliegues). Las diferencias más consistentes se observaron frente a las defensas, con reducciones significativamente mayores en los sumatorios de 6 y 8 pliegues, y también frente a las delanteras en el sumatorio de 6 pliegues. Además de las diferencias encontradas entre posiciones, conviene destacar dos observaciones relevantes a nivel descriptivo. En primer lugar, los pliegues cutáneos con mayores valores se localizaron en el muslo frontal, el suprailíaco y el tríceps, mientras que los de menor grosor se registraron en el bíceps, el subescapular y el supraespinal. Este patrón coincide con lo descrito en un estudio realizado en más de 700 atletas femeninas de distintas disciplinas, que muestra que, además de tener medidas de pliegues cutáneos más altas que sus homólogos masculinos, la acumulación de grasa tiende a producirse más en las zonas inferiores del cuerpo y el tronco (173). En segundo lugar, al analizar el sumatorio de 6 pliegues, se observó que las porteras presentaron los valores más altos y las delanteras los más bajos. Esta diferencia podría explicarse por el menor volumen de desplazamiento de las porteras durante los partidos y, en contraposición, por la necesidad de las delanteras de ser más ligeras para poder cubrir mayores distancias en el terreno de juego (166).

En el análisis de la evolución del componente graso, se observó una disminución general tanto en términos absolutos (kg) como relativos (%), estimada mediante distintas ecuaciones antropométricas. Sin embargo, en el caso de las porteras, esta reducción solo se evidenció en aquellas fórmulas que consideran pliegues de todo el cuerpo, no así en las que únicamente incluyen pliegues del tren superior, como Faulkner (174) o Durnin (175). En términos absolutos, con todas las ecuaciones analizadas, las centrocampistas fueron la posición que mostró las mayores reducciones, destacando diferencias significativas frente a las defensas en la mayoría de los casos (Withers (176), Slaughter (177), Carter (178) y Jackson y

Pollock (179)), así como frente a las porteras con la ecuación de Faulkner. Este patrón se repitió también al considerar el porcentaje de grasa, con diferencias significativas frente a defensas (Withers, Slaughter, Carter, Faulkner y Jackson y Pollock), delanteras (Withers, Slaughter y Carter) y porteras (Withers, Faulkner y Durnin).

La mejora observada en las centrocampistas podría estar influida por sus valores de partida en composición corporal. En este contexto, los sumatorios de 6 y 8 pliegues mostraron que las defensas eran las jugadoras con menor componente graso al inicio de la pretemporada, seguidas por las delanteras, las centrocampistas y, por último, las porteras. Este nivel inicial más bajo en las defensas implicaría un margen de mejora más reducido, lo que podría explicar las diferencias significativas encontradas frente a las centrocampistas. Asimismo, aunque estas últimas partían de valores similares a los de las porteras, su evolución fue más favorable, probablemente debido a una mayor adaptación al entrenamiento o a las exigencias específicas de su posición. Esta misma tendencia se refleja también en otras variables analizadas, como el somatotipo o los valores estimados mediante bioimpedancia (BIA).

Continuando con el componente graso, cabe mencionar que los valores de grasa más bajos se obtuvieron utilizando la ecuación de Carter, seguida de las de Faulkner, Withers, Jackson y Pollock, y Slaughter, mientras que los valores de grasa más altos los proporcionó la ecuación de Durnin. Para interpretar los resultados es necesario comprender qué pliegues cutáneos se tienen en cuenta en las ecuaciones antropométricas que estiman la grasa corporal. Por ejemplo, Carter utiliza una ecuación que incluye el sumatorio de 6 pliegues, los cuales están ubicados en distintas regiones corporales. Por el contrario, otras ecuaciones, como las de Durnin y Faulkner, solo tienen en cuenta los pliegues cutáneos de la parte superior del cuerpo. Esto puede llevar a una sobreestimación o subestimación del porcentaje de grasa corporal, ya que no se tiene en cuenta la parte inferior del cuerpo, una zona habitual de acumulación de grasa en las mujeres deportistas (173). En comparación con otros estudios, una de las limitaciones es la diversidad de ecuaciones utilizadas para estimar el porcentaje de grasa corporal. Otros estudios han utilizado ecuaciones como las de Sloan y Siri (168), Yuhasz (157), Jackson y Pollock (169), Kerr (158), Durnin (161), sumatorio de 6 pliegues (153, 162), Withers (159) y Eston 2005 (163).

En lo que respecta a la masa muscular, se observaron diferencias por posición tanto en términos absolutos como relativos. Al analizar los valores en kilogramos mediante la ecuación de Poortmans, las porteras presentaron una mayor masa muscular en comparación con defensas y delanteras, siendo estas diferencias estadísticamente significativas. Esta superioridad en masa muscular absoluta podría estar relacionada con su mayor tamaño corporal global, como ya se observó en variables estructurales como la envergadura, la estatura o la masa corporal. En cambio, al considerar el porcentaje de masa muscular a través de la ecuación de Lee, fueron las centrocampistas quienes destacaron con valores significativamente superiores a los de las defensas. Este resultado refleja que, a pesar de no tener una masa muscular total tan elevada como las porteras, las centrocampistas presentaron una mejor proporción relativa, lo cual podría estar vinculado a su evolución favorable en composición corporal y al tipo de carga física propia de su posición.

En lo que respecta a la masa muscular, en todas las posiciones se observó un aumento a lo largo de la pretemporada, tanto en valores absolutos (kg) como relativos (%). No obstante, la magnitud de esta evolución varió según la posición y la ecuación utilizada para su estimación. Al considerar los valores absolutos mediante la ecuación de Poortmans (180), las porteras mostraron el mayor incremento, con diferencias estadísticamente significativas frente a defensas y delanteras. En cambio, al analizar el porcentaje de masa muscular con la ecuación de Lee (181), las centrocampistas fueron la única posición que registró un aumento significativamente superior al de las defensas. En resumen, la única ventaja observada para las porteras respecto al resto de posiciones fue el mayor incremento en masa muscular absoluta, estimado mediante la ecuación de Poortmans. Esta superioridad podría estar relacionada con su mayor tamaño corporal global, como ya se observó en variables estructurales como la envergadura, la estatura o la masa corporal.

Otro de los parámetros analizados fue el somatotipo. De las 13 categorías propuestas por el método de Heath y Carter (182), en este estudio se identificaron únicamente dos. Al inicio de la pretemporada, las centrocampistas y las delanteras se clasificaron como endo-mesomorfas (mesomorfia dominante y endomorfia mayor que ectomorfia) (183). En cambio, las defensas y las porteras presentaron un perfil mesomorfo-endomorfo (endomorfia y mesomorfia iguales, con una

diferencia menor a 0.5, y ectomorfia inferior) (183). Al finalizar la pretemporada, todas las posiciones conservaron su clasificación inicial, a excepción de las defensas, que pasaron de mesomorfo-endomorfos a endo-mesomorfos. En línea con lo observado previamente respecto al componente graso, los principales cambios en el somatotipo se produjeron en el componente endomórfico, con una disminución más marcada en las centrocampistas, significativamente superior a la de defensas y porteras. En esta investigación, se ha observado que las centrocampistas, defensas y delanteras presentan un mayor componente mesomórfico que endomórfico, lo que difiere de lo descrito en estudios previos (154, 157-159). En cuanto a las porteras, el somatotipo reportado en otros estudios es meso-endomorfo (157-159) (endomorfia dominante y mesomorfia mayor que ectomorfia) (183), lo que contrasta con el perfil observado en este estudio, donde la endomorfia y la mesomorfia son iguales.

Por último, además de la estimación mediante antropometría, la composición corporal también se evaluó mediante BIA. Al igual que con la antropometría, se observó una disminución general de la masa grasa durante la pretemporada, especialmente en las centrocampistas, que mostraron mayores reducciones que defensas y porteras. Como objetivo secundario, se examinó la correlación entre los valores de composición corporal estimados mediante BIA y los obtenidos a partir de diferentes ecuaciones antropométricas. En el caso de la masa grasa, tanto en porcentaje como en kilogramos, todas las ecuaciones mostraron correlaciones estadísticamente significativas con los valores de BIA, siendo las de Faulkner y Durnin las que presentaron las asociaciones más elevadas. Aunque la ecuación de Faulkner fue la que alcanzó mayor grado de correlación, la de Durnin mostró una mayor estabilidad en sus estimaciones a lo largo del estudio, replicando de forma más consistente los valores de BIA tanto al inicio como al final de la pretemporada. Esta diferencia podría estar relacionada con factores como el tamaño muestral o la sensibilidad de ciertas ecuaciones a errores en la toma de pliegues cutáneos, lo que explicaría una mayor variabilidad en las estimaciones de Faulkner. Es importante señalar que las ecuaciones con mayores correlaciones no consideraban pliegues cutáneos de la parte inferior del cuerpo. El hallazgo sobre la alta correlación entre Faulkner y BIA concuerda con lo observado en un estudio previo en atletas masculinos profesionales (184). Además, investigaciones anteriores han demostrado que las ecuaciones antropométricas pueden ser una alternativa viable

a la BIA para estimar la composición corporal en atletas (185-187), siempre que se tengan en cuenta las especificidades de la población estudiada. No obstante, la mayoría de los estudios previos se han realizado en poblaciones masculinas o mixtas, lo que deja un vacío en la literatura respecto a su aplicación en mujeres deportistas, especialmente en disciplinas específicas como el fútbol femenino. Por tanto, futuras investigaciones deberían validar estas ecuaciones en poblaciones femeninas diversas y compararlas con métodos de referencia más precisos, a fin de reforzar su aplicabilidad en contextos deportivos.

Respecto a la masa libre de grasa y la masa muscular, la ecuación de Lee fue la que mostró mayor correlación con los valores estimados mediante BIA. Sin embargo, al interpretar estos datos, es fundamental considerar las limitaciones del método. Aunque la BIA reporta una variable etiquetada como “hueso”, en realidad lo que estima es el contenido mineral óseo (CMO) (60), es decir, la cantidad de minerales presentes en el tejido óseo (principalmente calcio y fósforo), y no el peso total del esqueleto. Este matiz es relevante: el esqueleto humano representa aproximadamente entre el 9 % y el 14 % del peso corporal en sujetos delgados, mientras que el CMO supone, en promedio, solo el 40 % del peso esquelético (60). Esta diferencia implica que los valores de masa libre de grasa (músculo, hueso, tejidos, agua y el resto de componentes sin grasa) y masa muscular (tejido magro libre de hueso) podrían estar sobreestimados, ya que el cálculo de masa muscular parte de una estimación de masa magra que subestima el componente óseo real. Por tanto, estos resultados deben interpretarse con cautela. Con todo ello, los hallazgos respaldan el uso complementario de ambas herramientas en el control rutinario de la composición corporal en contextos profesionales. En función de los recursos disponibles, la antropometría puede representar una opción económica y accesible para el seguimiento longitudinal, mientras que la BIA ofrece una alternativa rápida y no invasiva para la monitorización frecuente de los cambios durante el periodo preparatorio.

5.2. ARTÍCULO 2

Este segundo artículo se centró en analizar la relación entre el perfil antropométrico y de composición corporal con el rendimiento físico de las futbolistas profesionales en el inicio de la pretemporada. A diferencia del primer

artículo, que evaluó la evolución entre el inicio y final de pretemporada, este estudio transversal permitió identificar cómo las características corporales condicionan la preparación inicial de las jugadoras. Asimismo, se comparó el rendimiento físico entre posiciones de juego.

En el CMJ pre-WAnT, las porteras registraron los valores más altos en altura de salto, seguidas por delanteras, defensas y centrocampistas, que obtuvieron los más bajos. Esta tendencia podría explicarse por las demandas específicas de su posición, que requieren saltos laterales y verticales frecuentes durante entrenamientos y partidos (161). Sin embargo, no se observaron diferencias estadísticamente significativas entre posiciones. En cambio, al analizar la potencia media y la potencia pico, sí se encontraron diferencias significativas. Las porteras volvieron a mostrar los valores más altos en ambas variables. En concreto, la potencia media fue superior en porteras respecto a defensas y centrocampistas, mientras que la potencia pico también fue mayor en comparación con las defensas.

En relación con el perfil corporal, la altura de salto, considerada una medida de fuerza explosiva, mostró correlaciones negativas moderadas con diversos indicadores de masa grasa, como el pliegue de bíceps, el porcentaje y los kilogramos de grasa estimados por distintas ecuaciones, los sumatorios de 6 y 8 pliegues y el componente endomórfico del somatotipo. Por el contrario, presentó una correlación positiva moderada con el porcentaje de masa muscular estimado mediante la ecuación de Poortmans. Estos resultados reflejan que un mayor contenido graso se asocia con una menor altura de salto, mientras que una mayor masa muscular se relaciona con un mejor rendimiento en esta variable, en línea con lo descrito en estudios previos (69, 162, 188).

Por otro lado, la potencia media y pico, consideradas indicadores del potencia anaeróbica, mostraron correlaciones positivas muy altas con la masa corporal y los kilogramos de masa muscular. Este hallazgo es coherente desde el punto de vista fisiológico, ya que la potencia depende del producto de la fuerza por la velocidad, y la fuerza está directamente relacionada con la masa. Como resultado, las jugadoras con mayor masa corporal y muscular generan más fuerza, lo que conduce a una mayor potencia. De forma adicional, la potencia pico mostró una correlación positiva alta con los kilogramos de grasa estimados mediante la ecuación de Faulkner. Es importante señalar que, en ambas variables de potencia,

no hubo correlaciones negativas con las variables antropométricas relacionadas con la grasa, en contraste con la altura del salto.

En la prueba de CMJ post-WAnT, utilizada para valorar la fatiga neuromuscular tras un esfuerzo intenso, no se observaron diferencias estadísticamente significativas entre posiciones. No obstante, el patrón de rendimiento se repitió respecto al CMJ pre-WAnT: las porteras obtuvieron los valores más altos, seguidas por delanteras, defensas y centrocampistas, que volvieron a registrar los más bajos. En cuanto a la variación, las porteras fueron las que mostraron las mayores reducciones tanto en la altura de salto como en la potencia, lo que podría indicar una mayor fatiga neuromuscular en comparación con el resto de posiciones. Las delanteras también presentaron descensos notables, mientras que las defensas y centrocampistas registraron caídas más moderadas. En el caso de las centrocampistas, la caída fue más pronunciada en la potencia pico que en la altura de salto, lo que sugiere que, aunque lograron mantener relativamente bien la altura alcanzada, su capacidad para generar altos niveles de potencia se vio más comprometida. Este patrón puede estar relacionado con las demandas específicas de su posición, que podrían favorecer esfuerzos repetidos de intensidad moderada por encima de acciones que requieran una producción máxima de potencia. Estas diferencias en la magnitud de la reducción podrían estar vinculadas con la capacidad de recuperación neuromuscular según la posición. No obstante, las mayores caídas observadas en porteras y delanteras también podrían explicarse, al menos en parte, por los valores iniciales más elevados registrados en el CMJ pre-WAnT, lo que implicaría un mayor margen de descenso tras un esfuerzo máximo. En conjunto, la interacción entre la fatiga neuromuscular y el nivel de rendimiento inicial parece desempeñar un papel clave en las variaciones observadas en el rendimiento del CMJ post-WAnT.

En cuanto a las correlaciones entre las variables antropométricas y los resultados del CMJ post-WAnT, se observó un patrón similar al del CMJ pre-WAnT. La altura de salto mostró correlaciones negativas moderadas con diversos indicadores de masa grasa, destacando en este caso una correlación negativa alta con el pliegue supraespinal. Tanto la potencia media como pico presentaron correlaciones positivas muy altas con la masa corporal y con los kilogramos de masa muscular estimados mediante la ecuación de Lee. Además, se observó una correlación positiva muy alta con la envergadura, y en el caso de la potencia media

también con los kilogramos de masa ósea. Aunque estos resultados no pueden compararse directamente con estudios previos debido a la ausencia de investigaciones similares, reflejan una continuidad en el patrón observado antes del esfuerzo.

En el WAnT, que evalúa la potencia y capacidad anaeróbica, no se encontraron diferencias estadísticamente significativas entre posiciones en ninguna de las variables analizadas. No obstante, los datos descriptivos revelaron un patrón claro: las porteras obtuvieron los valores más altos en la mayoría de las variables de potencia, seguidas por las delanteras. Estos resultados coinciden parcialmente con lo observado en un estudio previo realizado con jugadoras griegas de primera división durante la pretemporada, donde también se registraron los valores más altos de potencia pico y media en porteras (42). Sin embargo, en ese estudio los segundos valores más altos correspondieron a las centrocampistas. En relación con el índice de fatiga, los valores más elevados se observaron en defensas y porteras, seguidos por delanteras y centrocampistas, que mostraron valores muy similares entre sí. Además, al comparar los resultados entre ambos estudios, los valores registrados en esta muestra fueron más altos que los descritos previamente para las futbolistas griegas. Según nuestro conocimiento, el estudio de Nikolaidis (42) es el único que ha utilizado el WAnT en futbolistas profesionales femeninas diferenciando por posición, lo que refuerza la singularidad de los datos aquí presentados.

En cuanto a las correlaciones entre las variables antropométricas y las variables del WAnT. Esta prueba de esfuerzo máximo de treinta segundos en cicloergómetro permite valorar tanto la potencia anaeróbica como la capacidad para sostener esfuerzos intensos. En el presente estudio, la potencia pico mostró una correlación casi perfecta con la masa corporal, así como correlaciones muy altas con la masa muscular estimada mediante la ecuación de Lee, la masa grasa según las ecuaciones de Faulkner y Durnin, y distintos perímetros corporales. Asimismo, la potencia media, entendida como la potencia mantenida durante todo el test, también presentó correlaciones muy altas con la masa corporal y la masa muscular. Según la literatura disponible, solo un estudio ha analizado la relación entre el rendimiento en el WAnT y las variables antropométricas en futbolistas femeninas (68). Este estudio realizado con jugadoras de primera división griega, se observó una correlación positiva moderada entre la potencia pico y el porcentaje de grasa

corporal, una correlación positiva alta con los kilogramos de grasa, y una correlación muy alta con la masa libre de grasa. En el caso de la potencia media, no se hallaron asociaciones significativas con el porcentaje graso, aunque sí se observaron correlaciones moderadas positivas con la grasa absoluta y muy altas con la masa libre de grasa.

En la prueba Yo-Yo IR1, utilizada para estimar la capacidad aeróbica mediante la distancia recorrida y el consumo máximo de oxígeno (VO_2 máx) calculado, no se encontraron diferencias estadísticamente significativas entre posiciones. No obstante, los datos descriptivos mostraron un patrón claro: las porteras fueron las que recorrieron menos distancia, mientras que las centrocampistas registraron los valores más altos. Estos resultados coinciden con lo observado en estudios previos (155, 160-162, 189), que también reportaron un rendimiento inferior en porteras. Esta diferencia concuerda con su participación más limitada en desplazamientos durante el partido, ya que suelen recorrer en torno a 4000 metros, frente a los aproximadamente 10.000 metros que recorren de media las jugadoras de campo (21). Por otro lado, el hecho de que las centrocampistas fueran las que recorrieron mayor distancia concuerda con lo descrito en algunos estudios (161, 162), mientras que otros han identificado a las defensas como la posición con valores más altos (155, 160). De forma similar, la ausencia de diferencias significativas entre posiciones en este estudio coincide con ciertos trabajos previos (160, 189), aunque contrasta con investigaciones que sí han reportado diferencias, especialmente entre porteras y jugadoras de campo (155, 161) o entre centrocampistas y el resto (162).

En cuanto al VO_2 máx estimado, tampoco se observaron diferencias significativas entre posiciones, aunque nuevamente las centrocampistas obtuvieron los valores más altos y las porteras los más bajos. Estos resultados coinciden con el patrón descrito para la distancia recorrida y son coherentes con las exigencias fisiológicas propias de cada posición. Las centrocampistas suelen recorrer las mayores distancias durante los partidos (26, 190), lo que requiere una capacidad aeróbica superior para mantener su contribución tanto en ataque como en defensa. Su capacidad para mantener esfuerzos de alta intensidad a lo largo del partido puede explicar su mayor rendimiento en el Yo-Yo IR1 en comparación con otras posiciones.

Respecto a las correlaciones entre rendimiento en el Yo-Yo IR1 y variables antropométricas, se observó una correlación negativa moderada entre la distancia recorrida y el pliegue supraespinal, en línea con estudios previos que también reportaron asociaciones negativas entre variables de grasa corporal y el rendimiento aeróbico. Por ejemplo, en jugadoras de la selección chilena se hallaron correlaciones negativas moderadas entre la distancia recorrida y tanto el porcentaje graso como el sumatorio de 6 pliegues (162), mientras que en futbolistas brasileñas de primera división se observó una correlación negativa alta con el sumatorio de 6 pliegues (191). Para el VO_2 máx estimado, se encontró una correlación negativa moderada con el pliegue supraespinal y con la envergadura. Aunque la literatura disponible sobre este aspecto es escasa, un estudio con futbolistas españolas semiprofesionales también halló una correlación negativa moderada entre el índice de masa corporal y el VO_2 máx estimado a partir de la distancia del Yo-Yo IR1 (192).

En conjunto, los resultados de este segundo artículo evidencian que el perfil antropométrico y de composición corporal condiciona de forma significativa el rendimiento físico de las futbolistas profesionales en el inicio de la pretemporada. La masa muscular y corporal se asoció positivamente con la producción de potencia en esfuerzos explosivos, mientras que el contenido graso mostró una relación negativa con la fuerza explosiva y la capacidad aeróbica. Esta diferenciación invita a replantear el modo en que se valoran los perfiles corporales en el fútbol femenino, priorizando un enfoque funcional e individualizado frente a estándares físicos generalizados centrados únicamente en la reducción de grasa. Además, la conexión con los hallazgos del primer artículo refuerza la idea de que estas características no solo influyen en el punto de partida, sino también en la evolución durante la pretemporada, lo que justifica la necesidad de estrategias de planificación específicas según el perfil y la posición de cada jugadora.

5.3. ARTÍCULO 3

En el tercer artículo se incorporó un componente de intervención mediante la suplementación crónica con NO_3^- y CM, con el objetivo de explorar su posible efecto sobre el rendimiento y la recuperación en un entorno competitivo real. Hasta la fecha, este es el primer estudio que analiza los efectos crónicos de la suplementación combinada de estos dos precursores de NO en futbolistas

profesionales en condiciones de entrenamiento y partidos reales. Para ello, se analizaron variables de carga externa mediante GPS a lo largo de la pretemporada, así como el rendimiento en pruebas físicas realizadas en condiciones de fatiga pospartido, comparando en ambos casos al grupo que recibió el suplemento con un grupo placebo.

En cuanto a los entrenamientos, se analizó en primer lugar la interacción entre el tipo de producto administrado (NIT+CM vs. placebo) y la semana (semanas de pretemporada), con el fin de comprobar si la evolución de las variables de carga externa dependía del suplemento recibido. Esta interacción no resultó estadísticamente significativa en ninguna de las variables analizadas, lo que indica que la progresión observada a lo largo del estudio fue similar en ambos grupos. Por tanto, se procedió a analizar por separado los efectos principales del producto y del tiempo de intervención.

La $V_{\text{máx}}$ alcanzada durante los entrenamientos fue una de las variables con resultados más destacados. Se observaron efectos principales significativos tanto en función del producto como del tiempo de estudio. Las diferencias observadas comparando el producto y el placebo fueron significativas ($p < 0.001$; $\eta^2 p = 0.351$), y el análisis post hoc confirmó que los valores de $V_{\text{máx}}$ fueron más altos en las jugadoras que recibieron la suplementación en comparación con aquellas que tomaron placebo. Por su parte, el efecto de la semana también fue significativo, con un tamaño del efecto grande, aunque inferior al observado para el producto ($p < 0.001$; $\eta^2 p = 0.160$). Las pruebas post hoc revelaron mejoras progresivas desde la semana 1 hasta las semanas 3 y 4, así como desde la semana 2 hasta esas mismas semanas finales. Los valores más elevados se registraron en la semana 4, coincidiendo con el final del periodo de análisis. Además, las comparaciones semanales mediante pruebas t-Student para muestras independientes mostraron que el grupo que recibió la suplementación alcanzó sistemáticamente valores más altos de $V_{\text{máx}}$ que el grupo placebo durante toda la pretemporada (todas $p < 0.001$), lo que sugiere una diferencia estable entre grupos.

Aunque los estudios previos sobre los efectos crónicos de la suplementación con precursores de NO en jugadoras profesionales son limitados, algunos resultados obtenidos en deportistas de equipo bajo protocolos basados en *sprint* ofrecen un respaldo indirecto. Se han descrito mejoras en el rendimiento de *sprints* realizados en cicloergómetro tras cinco a siete días de suplementación con zumo

de remolacha (8.2–12.8 mmol NO_3^- /día), con aumentos en la potencia media (193) y el trabajo total (194). También se han reportado mejoras en los tiempos de *sprint* en pruebas de carrera con protocolos similares (195). Estos datos sugieren un potencial beneficio del NO_3^- en esfuerzos explosivos, especialmente cuando se administra durante varios días consecutivos (196).

Desde una perspectiva fisiológica, estos efectos podrían estar mediados por el aumento en la disponibilidad de NO. Niveles elevados de NO se han asociado con una mejora en el manejo del calcio, un acoplamiento excitación-contracción más eficiente y una mayor activación de fibras musculares tipo II (88, 96), mecanismos estrechamente relacionados con la generación de $V_{\text{máx}}$ en el fútbol (197, 198). La carrera a alta velocidad exige una rápida activación de la cadena posterior y una fuerte coordinación neuromuscular bajo fatiga (199, 200), lo que podría beneficiarse de una mayor eficiencia contráctil y un menor coste de ATP. Además, el aumento de la perfusión muscular y la mejora en la eficiencia mitocondrial inducidos por el NO (201) podrían favorecer el mantenimiento de la capacidad de *sprint* durante sesiones consecutivas, especialmente bajo cargas de entrenamiento elevadas. El malato, además, podría reforzar este mecanismo al aumentar la velocidad de producción de ATP durante esfuerzos de alta intensidad (126).

Aunque en este estudio se empleó la combinación de NO_3^- y CM, la evidencia previa sugiere que la CM por sí sola también podría contribuir al rendimiento en *sprints*. En un estudio, tres días de suplementación con CM (8 g/día) mejoraron el rendimiento en *sprints* repetidos en atletas masculinos de deportes de equipo, con tiempos de *sprint* más rápidos y menor fatiga (202).

Considerando estos resultados, el hecho de que el grupo NIT+CM presentara valores de $V_{\text{máx}}$ consistentemente superiores, junto con un mayor tamaño del efecto para el producto en comparación con la semana de entrenamiento, podría reflejar una adaptación neuromuscular favorable en respuesta a la suplementación con precursores de NO. No obstante, debido a la naturaleza observacional del presente estudio y a la complejidad multifactorial del rendimiento en *sprints*, estos hallazgos deben interpretarse con cautela.

Respecto a la distancia recorrida por minuto, se observó un efecto principal significativo del producto ($p < 0.001$), aunque con un tamaño del efecto pequeño ($\eta^2 p = 0.035$). El análisis post hoc mostró que los valores fueron más elevados en el

grupo NIT+CM. El efecto de la semana fue cercano a la significación estadística ($p=0.053$), también con un tamaño del efecto pequeño ($\eta^2p=0.025$). En este caso, las comparaciones post hoc indicaron una mejora significativa entre la semana 1 y la semana 3. Las pruebas t-Student para muestras independientes revelaron valores significativamente más altos en el grupo NIT + CM en esas mismas semanas.

Teniendo en cuenta todos los resultados, esto podría indicar una ventaja moderada pero constante para el grupo NIT + CM en el mantenimiento del ritmo de trabajo durante el entrenamiento. Es probable que este efecto implique mecanismos similares a los propuestos para la $V_{m\acute{a}x}$, como una mayor eficiencia muscular, una mejora del flujo sanguíneo y un aumento de la producción de ATP gracias a una mayor disponibilidad de NO. Se necesitan más estudios en condiciones controladas para aclarar la relevancia fisiológica de este hallazgo y su posible impacto en los resultados del entrenamiento.

Sumado a lo anterior, la TD presentó efectos principales significativos en función del producto ($p<0.001$; $\eta^2p=0.113$), con un tamaño del efecto moderado. El análisis post hoc mostró valores generales más altos en el grupo NIT+CM en comparación con el placebo. Además, se encontró un efecto principal significativo de la semana, aunque con un tamaño del efecto pequeño ($p=0.003$; $\eta^2p=0.029$), y las pruebas post hoc revelaron valores significativamente más altos en la semana 3 en comparación con la semana 2. Las pruebas t-Student para muestras independientes confirmaron que el grupo NIT + CM cubrió una TD consistentemente mayor en comparación con el placebo en todas las semanas (todas $p<0.001$).

Aunque no se puede hacer una comparación directa con la TD durante el entrenamiento, estudios anteriores han informado un aumento de la TD en atletas de deportes de equipo durante la prueba Yo-Yo IR1 tras la suplementación crónica con NO_3^- . Estos efectos se han observado con protocolos que van de 2 a 6 días y dosis entre 4.1 y 12.9 mmol/día de NO_3^- (195, 203, 204). Si bien esos hallazgos provienen de pruebas de rendimiento controladas y no incluyeron CM, los resultados actuales parecen seguir una tendencia similar, lo que sugiere una posible mejora en el rendimiento deportivo durante períodos físicamente exigentes, como la pretemporada.

Por último, en relación con los entrenamientos, Las distancias recorridas a trote, en carrera de alta intensidad (HIR) y de máxima intensidad (MIR) mostraron efectos significativos únicamente en función de la semana, sin diferencias asociadas

al producto. Estas variaciones parecen responder a la lógica del diseño semanal del entrenamiento, con ajustes planificados por el cuerpo técnico en función de los objetivos físicos y tácticos de cada microciclo. Los tamaños del efecto, en general pequeños o moderados, reflejan cambios esperables dentro de la dinámica natural del fútbol profesional (205).

Una vez analizadas las variables asociadas a los entrenamientos, el siguiente paso fue examinar el comportamiento de estas mismas variables durante los partidos disputados en pretemporada. A diferencia de lo observado en los entrenamientos, en este caso se detectó una interacción significativa entre el tipo de producto y la semana en la variable de $V_{\text{máx}}$.

En los partidos, esta variable mostró una interacción significativa ($p=0.022$; $\eta^2p=0.079$), con un tamaño del efecto moderado. El análisis post hoc dentro del grupo NIT + CM reveló incrementos progresivos desde la semana 1 y 2 hasta las semanas 3 y 4. Además, se observaron efectos principales significativos tanto en función del producto ($p<0.001$; $\eta^2p=0.413$) como de la semana ($p<0.001$; $\eta^2p=0.253$), ambos con tamaños del efecto grandes. El análisis post hoc del factor producto mostró valores de $V_{\text{máx}}$ consistentemente superiores en el grupo NIT + CM frente al placebo. En cuanto al factor semana, se observaron aumentos significativos desde las semanas 1 y 2 hacia las semanas 3 y 4. Por último, las comparaciones semanales mediante pruebas t-Student para muestras independientes confirmaron valores de $V_{\text{máx}}$ significativamente más altos en el grupo NIT + CM durante todas las semanas.

Desde un punto de vista fisiológico, la influencia tanto del producto como de la semana de entrenamiento sobre esta variable parece ser importante, siendo especialmente notable el efecto del producto. Además, la interacción significativa entre el producto y la semana sugiere que las diferencias entre el grupo NIT + CM y el grupo placebo fueron cambiando con el tiempo, lo que podría reflejar una respuesta fisiológica más favorable en las jugadoras suplementadas a medida que avanzaba la pretemporada. Esta hipótesis se ve reforzada por los valores de $V_{\text{máx}}$ consistentemente superiores en el grupo NIT + CM a lo largo de todas las semanas, tal como confirmaron las pruebas t-Student para muestras independientes. Si bien el diseño observacional impide establecer una relación causal, la magnitud y estabilidad de las diferencias entre grupos, junto con la interacción significativa,

son compatibles con un posible beneficio neuromuscular asociado a la suplementación con NIT + CM.

Debido a los mecanismos fisiológicos previamente descritos del NO_3^- y el CM, la ventaja asociada al producto observada en esta variable podría reflejar una mejora en la capacidad de *sprint* bajo carga competitiva. No obstante, la interpretación de estos resultados debe tener en cuenta la complejidad y variabilidad inherentes a las demandas de un partido. En el fútbol femenino de élite, métricas de rendimiento como la $V_{\text{máx}}$ están influenciadas por múltiples factores, entre ellos el estado físico, el rol táctico, las características del rival (206) y aspectos contextuales como el marcador o la fase de la competición (207). A pesar de esta variabilidad potencial, la $V_{\text{máx}}$ se considera una métrica relativamente estable. Según un estudio sobre la variabilidad de la carga competitiva en jugadoras profesionales, esta variable presenta una baja variabilidad entre partidos ($\text{CV} \approx 4.5\%$) en comparación con otras medidas de alta intensidad (208), lo que sugiere que los cambios observados probablemente reflejan adaptaciones fisiológicas reales más que fluctuaciones contextuales. Además, la capacidad para alcanzar la $V_{\text{máx}}$ durante los partidos podría estar influida, al menos en parte, por las adaptaciones neuromusculares desarrolladas en el entrenamiento. La ventaja constante observada en los valores de $V_{\text{máx}}$ tanto en entrenamientos como en partidos en el grupo NIT + CM respalda la hipótesis de que una mejora del rendimiento a alta velocidad durante los entrenamientos podría haber facilitado una transferencia más eficaz de esta capacidad al entorno competitivo. No obstante, esta interpretación sigue siendo especulativa y debe considerarse con cautela, dadas las limitaciones propias de un diseño no experimental.

Más allá de la $V_{\text{máx}}$, únicamente la distancia por minuto presentó una posible interacción entre el tipo producto administrado y la semana, aunque con una significación estadística marginal y sin efectos principales del producto. En esta variable, la interacción entre el producto y la semana se acercó al nivel de significación estadística ($p=0.052$; $\eta^2p=0.064$) con un tamaño del efecto moderado. El análisis intragrupal reveló un aumento significativo de la semana 1 a la semana 4 en el grupo NIT + CM ($p<0.001$). Aunque no se observaron efectos principales del producto, sí se encontró un efecto principal significativo del factor semana ($p<0.001$; $\eta^2p=0.117$) también con un tamaño del efecto moderado. Las comparaciones post hoc para este factor confirmaron una mejora clara entre la semana 1 y la semana 4.

Las pruebas t-Student para muestras independientes revelaron valores significativamente más altos en el grupo NIT + CM en la semana 4.

El efecto moderado de la semana de entrenamiento sobre la distancia por minuto, con aumentos significativos de la semana 1 a la semana 4 en todos los jugadoras, sugiere que el último partido de pretemporada se jugó con una intensidad en general más alta. Aunque no se encontró ningún efecto principal para el factor producto, la presencia de una interacción casi significativa entre el producto y la semana, combinada con el aumento descriptivamente progresivo observado en el grupo NIT + CM, puede apuntar a una adaptación más favorable en este grupo a lo largo del tiempo. El hecho de que esto culminara en valores significativamente más altos para el grupo suplementado en la semana 4 puede reflejar un acondicionamiento más favorable a las exigencias competitivas. Este patrón se ajusta a los mecanismos fisiológicos discutidos anteriormente y justifica una mayor exploración en entornos controlados.

En TD, únicamente se encontró un efecto principal significativo para el factor semana ($p=0.033$; $\eta^2p=0.079$, tamaño del efecto moderado), con un aumento significativo de la semana 1 a la semana 4. Al igual que en la distancia por minuto, es probable que el último partido de pretemporada presentara una intensidad más elevada, lo que podría explicar parcialmente este incremento. Además, se trata de una variable muy influida por factores contextuales como el rol táctico, la dinámica del juego o la posición de la jugadora (206), todos ellos conocidos por afectar el rendimiento locomotor durante la competición. La elevada desviación estándar registrada en esta muestra pone de manifiesto una gran variabilidad interindividual y entre partidos, lo que sugiere que las diferencias semanales podrían reflejar demandas situacionales más que adaptaciones progresivas o efectos atribuibles al producto.

Por último, en los partidos, las distancias cubiertas a distintas velocidades mostraron efectos significativos únicamente para el factor semana, sin diferencias asociadas al producto. Estos cambios parecen responder a la variabilidad propia del juego y a las demandas específicas de cada encuentro.

Completado el análisis de la carga externa durante la pretemporada, se evaluó el posible efecto de la suplementación sobre la recuperación del rendimiento en condiciones de fatiga pospartido. Para ello, se compararon los resultados de las pruebas de CMJ y WAnT realizadas el primer día de pretemporada con los

obtenidos al día siguiente de un partido, tras un periodo de suplementación de cuatro semanas cuya última dosis se administró el mismo día del encuentro. El objetivo fue determinar qué grupo lograba mantener mejor el rendimiento o, en su caso, experimentar un menor descenso bajo condiciones de fatiga acumulada.

El CMJ pre- y post-WAnT se utilizaron para evaluar, respectivamente, la potencia anaeróbica y la fuerza explosiva, así como la fatiga neuromuscular y la capacidad de recuperación bajo condiciones de recuperación pospartido. Ninguna de las variables analizadas mostró diferencias estadísticamente significativas entre grupos. Sin embargo, el grupo NIT + CM presentó valores ligeramente más estables en la altura del salto y una evolución algo más favorable en potencia media y pico, tanto antes como después del WAnT. En ambas pruebas, el grupo placebo mostró un pequeño descenso o una mejora menos marcada en las métricas de potencia. Aunque las diferencias fueron pequeñas y no significativas, este patrón podría reflejar una mejor recuperación del rendimiento anaeróbico y neuromuscular en el grupo que recibió el suplemento. Estos resultados no confirman un efecto claro del producto, pero sugieren una tendencia consistente que justifica una mayor exploración en condiciones similares pospartido.

El WAnT se utilizó para evaluar la potencia y la capacidad anaeróbica en condiciones de recuperación pospartido. Varias variables mostraron diferencias estadísticamente significativas entre grupos, observándose una mejor preservación del rendimiento en el grupo NIT + CM. En concreto, la potencia media y mínima disminuyeron en ambos grupos, pero el descenso fue significativamente menor en las jugadoras que tomaron el suplemento. Asimismo, la pérdida de potencia a lo largo de los 30 segundos de esfuerzo también fue consistentemente menor en el grupo NIT + CM, con diferencias significativas tanto en la primera como en la segunda mitad de la prueba.

Aunque la potencia pico y el índice de fatiga no mostraron diferencias estadísticamente significativas, ambas variables siguieron el mismo patrón observado en el resto de métricas, con una evolución más favorable en el grupo NIT + CM. Por su parte, el tiempo hasta alcanzar la potencia pico no mostró diferencias relevantes entre grupos. En conjunto, estos hallazgos sugieren que la suplementación con NIT + CM podría haber contribuido a preservar la capacidad anaeróbica bajo condiciones de fatiga acumulada. En el contexto del fútbol femenino, este resultado adquiere especial relevancia, ya que una revisión

sistemática con metaanálisis (209) ha demostrado que el rendimiento anaeróbico de alta intensidad, incluida la capacidad de esprintar, puede verse comprometido hasta 48 horas después de un partido. Por tanto, favorecer la capacidad de mantener la potencia anaeróbica al día siguiente de la competición podría tener implicaciones prácticas en la planificación del rendimiento durante calendarios congestionados o periodos de elevada carga de entrenamiento.

Para complementar los datos de rendimiento, se analizaron las concentraciones plasmáticas de NO_3^- y NO_2^- en dos momentos: al inicio del estudio y al día siguiente del partido. El grupo NIT + CM mostró un aumento estadísticamente significativo respecto al valor basal tanto en NO_3^- ($p<0.001$) como en NO_2^- ($p=0.008$). En la medición final, los niveles de NO_3^- fueron significativamente superiores en el grupo NIT + CM en comparación con el grupo placebo ($p<0.001$), mientras que no se observaron diferencias significativas entre grupos en los niveles de NO_2^- .

Es importante destacar que las concentraciones plasmáticas de NO_3^- en el grupo NIT + CM se mantuvieron elevadas al día siguiente de la dosis final. Esto concuerda con investigaciones anteriores que muestran que la suplementación crónica produce aumentos progresivos del NO_3^- plasmático y que las concentraciones se mantienen por encima del nivel basal incluso 24 horas después de la última ingesta. Por ejemplo, en un estudio con una dosis de 19.5 mmol/día durante ocho días, los niveles aumentaron de $37\pm15\ \mu\text{M}$ a $270\pm182\ \mu\text{M}$ un día después de la última administración, lo que refleja una exposición sistémica prolongada (210). Aunque los valores absolutos no son directamente comparables debido a las diferencias de población y dosis, este hallazgo es coherente con las observaciones actuales utilizando 8 mmol/día durante cuatro semanas.

Además, estudios previos han demostrado que la ingesta de NO_3^- durante varios días genera concentraciones plasmáticas más elevadas en comparación con la ingesta de una sola dosis (135) y que los niveles de NO_2^- guardan una relación más estrecha con la cantidad total de NO_3^- consumida a lo largo del protocolo que con la dosis diaria (91). En la presente Tesis, el NO_2^- fue el único biomarcador que no mostró una diferencia significativa entre los grupos en la medición final, a pesar de un aumento significativo con respecto al valor inicial en el grupo NIT + CM. Esta ausencia de diferencias podría deberse a la vida media plasmática mucho más corta del NO_2^- en comparación con el NO_3^- (211), lo que limita su persistencia en la

circulación durante la fase de recuperación. También se ha sugerido que el NO_2^- podría ser tamponado o almacenado en el tejido muscular esquelético (135), lo que reduciría su concentración plasmática sin impedir su participación en la disponibilidad local de NO. Estos mecanismos podrían explicar por qué no se observaron diferencias significativas entre grupos en este biomarcador, incluso en un contexto de elevada disponibilidad sistémica de NO_3^- .

5.4. ARTÍCULO 4

Por otra parte, el cuarto artículo analizó de forma observacional la influencia de las fases del ciclo menstrual sobre la carga externa durante los entrenamientos, monitorizada mediante GPS. Gracias al uso continuado de una aplicación móvil específica (FitrWoman), fue posible estimar la fase de cada jugadora y obtener una muestra contextualizada que refleja la variabilidad individual en un entorno real.

La $V_{\text{máx}}$ mostró diferencias estadísticamente significativas entre fases del ciclo menstrual ($p < 0.001$; $\eta^2 p = 0.058$), con valores más altos durante la fase folicular hasta la ovulación en comparación con las fases lútea y premenstrual (ambas $p < 0.001$). Aunque no se encontraron diferencias significativas respecto a la fase menstrual, los datos descriptivos mostraron que la $V_{\text{máx}}$ fue también inferior a la fase folicular hasta ovulación, con valores similares a los observados en la fase lútea. Se ha observado una tendencia similar en jugadoras profesionales de baloncesto (212), donde el seguimiento con GPS mostró que la fase folicular tardía y la ovulación podrían ofrecer condiciones más favorables para la velocidad y las acciones explosivas. A pesar de las diferencias entre disciplinas, la tendencia observada parece ser coherente con los resultados del presente estudio.

Una posible explicación para este patrón de rendimiento se relaciona con la influencia de las hormonas del ciclo menstrual sobre la función neuromuscular. Durante la fase folicular hasta la ovulación, estimada en este estudio mediante la aplicación utilizada, predomina un perfil hormonal caracterizado por concentraciones elevadas de estrógenos y niveles bajos de progesterona. Esta combinación hormonal podría favorecer la función neuromuscular, ya que el estrógeno ha sido asociado a un efecto neuroexcitador, mientras que la progesterona se relaciona con una reducción de la excitabilidad cortical (213). Estas acciones opuestas podrían influir de manera diferente sobre la función

neuromuscular, favoreciendo la generación de fuerza en presencia de estrógeno y viéndose potencialmente limitada por la progesterona (214, 215). Por ello, se ha propuesto que las capacidades de fuerza y potencia son más propensas a optimizarse bajo perfiles hormonales caracterizados por bajos niveles de progesterona y elevados de estrógeno, como los que se producen durante la fase folicular tardía (216). Por el contrario, el aumento de progesterona propio de la fase lútea podría contribuir a una menor eficiencia neuromuscular.

La capacidad para generar fuerza de forma rápida depende, en parte, de la velocidad inicial de reclutamiento de unidades motoras, un factor clave en el rendimiento explosivo (217). En el contexto específico del fútbol, esta activación neuromuscular cobra especial importancia en los miembros inferiores, ya que sustenta la capacidad de acelerar progresivamente y alcanzar la $V_{\text{máx}}$. Por tanto, es fisiológicamente plausible que las fases del ciclo con este tipo de perfil hormonal favorezcan un mayor rendimiento en acciones explosivas.

Cabe destacar que los valores más bajos de $V_{\text{máx}}$ observados en la fase premenstrual pueden estar relacionados con el aumento de la fatiga y los cambios en el estado de ánimo y la motivación, patrones que se observan con frecuencia durante esta etapa (80). Aunque no se encontraron diferencias significativas para la fase menstrual, los valores descriptivos también se mantuvieron por debajo de los registrados durante la fase folicular hasta la ovulación.

Por otro lado, tanto la TD y distancia caminando mostraron diferencias estadísticamente significativas entre fases del ciclo menstrual. En el caso de la TD ($p < 0.001$; $\eta^2 p = 0.058$), los valores fueron significativamente más altos durante la fase folicular hasta la ovulación en comparación con todas las demás fases, alcanzando sus mínimos en las fases menstrual y premenstrual, y situándose en un punto intermedio durante la fase lútea. La distancia caminando siguió un patrón similar ($p = 0.002$; $\eta^2 p = 0.038$), con el valor más elevado también en la fase folicular hasta la ovulación. En este caso, se registraron diferencias significativas respecto a las fases menstrual y premenstrual, aunque no frente a la fase lútea.

Estos hallazgos podrían reflejar diferencias fisiológicas en la capacidad para sostener una actividad prolongada a lo largo de las distintas fases del ciclo menstrual. El estrógeno se ha asociado con una mayor utilización de lípidos como fuente de energía y una menor dependencia del glucógeno durante el ejercicio submáximo, especialmente a intensidades moderadas (218). También se ha

relacionado con una mejor regulación cardiovascular durante el ejercicio (219). La suma de estos efectos podría traducirse en una mayor eficiencia locomotora sostenida en la fase folicular hasta la ovulación.

Por el contrario, los valores más bajos registrados durante las fases menstrual y premenstrual podrían estar relacionados con los síntomas físicos que se reportan habitualmente en estas etapas. Estas fases se han asociado con mayor frecuencia a dolor menstrual, hinchazón y malestar físico general, factores que podrían influir en la gestión del esfuerzo durante la sesión o en el comportamiento locomotor general de las jugadoras durante los entrenamientos (220). Si bien la fase lútea mostró resultados intermedios, la ausencia de diferencias significativas en la distancia caminando con respecto a la fase folicular hasta la ovulación podría reflejar la variabilidad interindividual en la manifestación de síntomas, la sensibilidad hormonal o las estrategias de adaptación empleadas por cada futbolista a lo largo del ciclo.

Hasta donde alcanza nuestro conocimiento, solo un estudio previo ha analizado las variaciones asociadas al ciclo menstrual en la carga externa de entrenamiento en futbolistas de élite. En dicho estudio, la TD fue significativamente menor durante la semana de la menstruación en comparación con la semana posterior a la ovulación, sin observarse otras diferencias significativas entre fases (221). Aunque estos hallazgos coinciden parcialmente con los del presente estudio, que también mostró valores más bajos en las fases menstrual y premenstrual, ambos trabajos presentan enfoques metodológicos distintos. Una tendencia similar se observó en un estudio controlado con futbolistas de alto nivel, donde el rendimiento en el *Yo-Yo Intermittent Endurance Test* tendió a disminuir durante la fase lútea media en comparación con la fase folicular temprana (77). Pese a que esta diferencia no fue estadísticamente significativa, los autores la interpretaron como un posible indicio de reducción en el rendimiento aeróbico. Si bien las diferencias en el diseño, los protocolos de prueba y la clasificación de las fases limitan la comparabilidad directa entre estudios, ambos trabajos apuntan en la misma dirección: una posible disminución del rendimiento relacionado con la resistencia durante la fase lútea.

Una revisión sistemática reciente sobre los efectos del ciclo menstrual en el rendimiento de futbolistas (222) indicó que varios estudios que evaluaron resultados relacionados con la resistencia aeróbica e intermitente no encontraron

diferencias significativas entre fases. Aunque los resultados en la literatura siguen siendo contradictorios, los hallazgos del presente estudio aportan datos observacionales obtenidos en condiciones de campo que sugieren un posible aumento en la TD y en la distancia caminando durante la fase folicular hasta la ovulación. Estos patrones deben interpretarse con cautela, y se requieren estudios adicionales con metodologías comparables para confirmar su consistencia.

Por último, no se observaron diferencias estadísticamente significativas entre las fases del ciclo en cuanto a la distancia por minuto, trotando, HIR o MIR. Sin embargo, la mayoría de estas variables mostraron una tendencia similar, con valores medios más altos durante la fase folicular hasta la ovulación y más bajos en la fase premenstrual, salvo en el caso de la distancia por minuto, que presentó un patrón inverso. Estas tendencias reflejan una respuesta relacionada con la fase del ciclo, similar a la observada en la TD y la $V_{m\acute{a}x}$. Si bien no todas las variables parecen mostrar la misma sensibilidad, la consistencia de los patrones observados refuerza la utilidad del seguimiento del ciclo menstrual como herramienta para identificar respuestas individuales en la carga externa, aportando un contexto fisiológico valioso para la evaluación del rendimiento en atletas de élite (223).

VI – CONCLUSIONES

VI -CONCLUSIONES

6.1. CONCLUSIÓN GENERAL

La optimización del rendimiento y la recuperación en jugadoras de fútbol profesional durante la pretemporada requiere considerar distintos factores de manera conjunta. La composición corporal se confirma como un elemento clave al condicionar diferentes manifestaciones del rendimiento físico. La posición de juego influye en cómo evoluciona la composición corporal a lo largo del periodo preparatorio. La suplementación con precursores de NO se asocia con un mejor rendimiento y una recuperación más favorable. El ciclo menstrual, por su parte, se relaciona con variaciones en la carga externa entre fases. En conjunto, estos resultados ponen de relieve la necesidad de planificar la pretemporada de manera individualizada, integrando estos factores para afrontar con eficacia las exigencias del fútbol profesional femenino.

6.2. CONCLUSIONES ESPECÍFICAS

Las diferencias entre posiciones al inicio y final de la pretemporada fueron limitadas, observándose solo en variables concretas como la envergadura y el diámetro del tobillo, con las porteras presentando mayor envergadura y menor diámetro en comparación con el resto. En lo referente a la composición corporal, no se hallaron diferencias significativas entre posiciones, sugiriendo que, dentro de este contexto profesional, los perfiles antropométricos parecen ser similares salvo en características muy específicas que podrían estar relacionadas con las demandas propias de la posición.

La evolución de la composición corporal durante la pretemporada varió según la posición de juego. Las centrocampistas fueron las jugadoras que experimentaron una mayor mejora en su composición corporal, mientras que en las defensas los cambios fueron menos evidentes y en las porteras se observaron adaptaciones más limitadas. Parece evidente que, incluso compartiendo un mismo entorno competitivo y de entrenamiento, las jugadoras no responden de forma uniforme, lo que pone de relieve la importancia de contemplar en la planificación

de la pretemporada ajustes específicos en entrenamiento y nutrición acordes a las particularidades de cada rol.

La composición corporal mostró una clara relación con el rendimiento físico al inicio de la pretemporada. Se observaron correlaciones muy altas entre la masa corporal y muscular con la potencia anaeróbica, mientras que la masa grasa presentó asociaciones negativas con la fuerza explosiva y la capacidad aeróbica. Por tanto, un perfil caracterizado por mayor masa muscular y un adecuado control de la grasa puede favorecer un mejor desempeño en acciones clave del fútbol profesional, lo que resalta la importancia de integrar entrenamiento de fuerza y estrategias nutricionales orientadas a optimizar la composición corporal desde el inicio del periodo preparatorio.

El rendimiento físico presentó variaciones entre posiciones en el inicio de la pretemporada. Las porteras alcanzaron los valores más elevados de potencia anaeróbica, mientras que las centrocampistas destacaron en capacidad aeróbica, en contraste con las delanteras y defensas que mostraron valores intermedios, lo que evidencia las demandas específicas de cada rol en el fútbol profesional femenino.

La suplementación crónica con NO_3^- y CM durante la pretemporada pareció asociarse con un mejor desempeño en la carga externa y una recuperación más favorable tras el partido. El grupo que recibió el suplemento alcanzó valores más altos de $V_{\text{máx}}$ tanto en entrenamientos como en partidos, y registró mayores TD y distancias por minuto en entrenamientos. Además, mostró una mejor preservación de la capacidad anaeróbica el día siguiente del partido. Estas respuestas se acompañaron de concentraciones plasmáticas de NO_3^- elevadas 24 h después de la última dosis, lo que indica una disponibilidad sostenida durante la fase de recuperación. Por tanto, la combinación de NO_3^- y CM podría favorecer la tolerancia a las altas cargas de la pretemporada y contribuir a preservar el rendimiento bajo condiciones de fatiga, destacando el potencial de integrar precursores de NO como estrategia nutricional específica en este periodo.

La carga externa de entrenamiento pareció mostrar variaciones en función de la fase del ciclo menstrual estimada con la aplicación FitrWoman. La fase folicular hasta ovulación presentó los valores más altos de $V_{\text{máx}}$, TD y distancia caminando, mientras que las fases menstrual y premenstrual tendieron a reflejar valores más bajos y la fase lútea se situó en un punto intermedio. Aunque el resto de las variables no alcanzaron significación estadística, la mayoría mostraron un patrón

en la misma dirección, lo que sugiere que el ciclo menstrual podría influir en ciertos componentes de la carga externa. Estos resultados apuntan a la utilidad de herramientas de monitorización no invasivas para contextualizar la respuesta de las jugadoras en entornos profesionales.

VII - LIMITACIONES Y FUTURAS LÍNEAS DE INVESTIGACIÓN

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Los artículos que conforman esta tesis presentan limitaciones que deben interpretarse en el marco de la investigación aplicada con futbolistas profesionales. El número de jugadoras fue relativamente reducido, lo que limita la generalización de los resultados. Esta circunstancia, sin embargo, refleja la dificultad de acceso a este tipo de población y pone en valor haber podido trabajar con muestras profesionales reales. La representación por posiciones también fue desigual, especialmente en el caso de las porteras, lo que redujo la potencia estadística en algunos análisis, aunque responde a la propia estructura de las plantillas. Asimismo, todos los estudios se desarrollaron durante la pretemporada, lo que aconseja cautela al extrapolar los hallazgos a otras fases del calendario competitivo.

La participación de dos equipos distintos pudo introducir cierta variabilidad derivada de diferencias en métodos de entrenamiento, decisiones técnicas y protocolos de recuperación. Al tratarse de un entorno real, no fue posible intervenir en la planificación ni estandarizar las cargas, de modo que el estímulo recibido por las jugadoras dependió de las dinámicas propias de cada club. Este hecho, que posiblemente influyó en los resultados, constituye una limitación inherente a la investigación en deportes colectivos, donde el control absoluto de todas las variables externas no es viable. Al mismo tiempo, aporta valor añadido al situar los hallazgos en un contexto auténtico de alto rendimiento.

El uso de pliegues cutáneos como herramienta de valoración de la composición corporal constituye otra limitación. Aunque se trata de un método validado y estandarizado por ISAK, y muy extendido en entornos deportivos, no alcanza la precisión de técnicas de referencia como la absorciometría dual de rayos X (DXA). La elección de este procedimiento respondió a la necesidad de aplicar técnicas prácticas y compatibles con la dinámica de los equipos profesionales, lo que refuerza la aplicabilidad de los hallazgos, pese a la menor exactitud técnica.

En el ámbito de la suplementación, si bien se controlaron ciertos factores externos, como el empleo de ayudas ergogénicas o enjuagues bucales, no se realizó un seguimiento detallado de la ingesta dietética, lo que pudo influir en la disponibilidad de NO_3^- y en la respuesta individual al suplemento.

Respecto al ciclo menstrual, la clasificación de fases se realizó mediante una aplicación móvil sin verificación hormonal, de modo que los periodos considerados fueron siempre una estimación. Esta limitación no responde a un descuido metodológico, sino a la dificultad logística de implementar un control hormonal sistemático en el entorno de equipos profesionales, donde el acceso y la coordinación con las jugadoras hacen muy complejo este tipo de procedimientos. A ello se añade la imposibilidad de estandarizar el contenido de los entrenamientos y la duración relativamente corta del seguimiento, limitada a cinco semanas de pretemporada e inicio competitivo, lo que restringe la extrapolación de los resultados a otros momentos de la temporada.

Para futuras líneas de investigación se proponen los siguientes puntos

- Ampliar el tamaño muestral mediante estudios multicéntricos y en diferentes fases de la temporada.
- Incorporar técnicas de referencia (DXA, ecografía, RM) para la evaluación de la composición corporal.
- Incluir un control nutricional más detallado y biomarcadores musculares y cardiovasculares en los estudios de suplementación.
- Validar aplicaciones de seguimiento del ciclo menstrual con análisis hormonales y extender los periodos de observación.
- Analizar la interacción entre los precursores de NO y el ciclo menstrual.

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