# Muscle Architecture & Sprint Performance in Humans



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# Arquitectura Muscular y Rendimiento en Sprint en Seres Humanos

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Esta tesis es un pequeño tributo a la memoria de mi madre. Gracias por tanto mamá, si hoy estoy aquí es por ti.

Te llevaré siempre en mi corazón y en mis pensamientos. Espero que estés orgullosa de mí.

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## **DEDICATION / DEDICATORIA**

A mis padres, porque si hoy estoy aquí es por vuestro amor incondicional y esfuerzo a lo largo de toda mi vida.

#### A mamá,

Llegó el momento, sin duda la parte más dura de este camino: tener que escribir estas palabras. Esta dedicatoria es para ti, mamá. No hay un solo día en el que no piense en ti, en todo lo que fuiste y en lo que sigues siendo para mí. Estos últimos años han sido los más difíciles de mi vida, y tu ausencia ha dejado un vacío imposible de llenar.

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## LIST OF SCIENTIFIC CONTRIBUTIONS

The present thesis is based on the publications listed below.

#### **Publications**

#### 1. Article 1 published in the Journal of Anatomy:

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4. Article 4 under review in the Scandinavian Journal of Medicine & Science in Sports:

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#### Conferences

**Conference 1.** Oral format. Martin-Rodriguez, S. Seminar of the University Institute of Biomedical and Health Research (Las Palmas de Gran Canaria, 2024). The use of ultrasound in the study of muscle adaptations to exercise.

**Conference 2.** Oral format. Martin-Rodriguez, S. Seminar titled "Exercise Physiology and Sports Training" of the University Institute of Biomedical and Health Research (Las Palmas de Gran Canaria, 2023). Morphological asymmetries of the tibialis anterior in humans: implications of mediation statistical models in the relationships of muscle architecture variables.

**Conference 3.** Poster format. Martin-Rodriguez, S., Galvan-Alvarez, V., Arteaga-Ortiz, R., Calbet JAL., Sanchiz-Moysi, J. Muscle architecture and sprint performance: role of muscle size. Annual Congress of the European College of Sport Science (Online, 2022).

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# LIST OF SYMBOLS AND ABBREVIATIONS

ACSA. Anatomical cross-sectional area

AT, Achilles tendon

BFlh, Biceps femoris long head

CMA, Causal mediation analysis

FL, Fascicle length

GL, Gastrocnemius lateralis

GM, Gastrocnemius medialis

LLM, Lean mass of the lower extremities

LM, Lean mass

MINORS, Methodological Index for Non-Randomised Studies

MT, Muscle thickness

MRI, Magnetic resonance imaging

PA, Pennation angle

PCSA, Physiological cross-sectional area

PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses

PT, Patellar tendon

**RF**, *Rectus femoris* 

**ST**, Semitendinosus

TA, Tibialis Anterior

VL, Vastus lateralis

VM, Vastus medialis

VO2max, Maximal oxygen uptake

 $W_{mean}$ , Mean power output

 $W_{peak}$ , Instantaneous peak power output

W<sub>peak1s</sub>, 1-second peak power output

# CHAPTER I:

# SUMMARY

#### 1. SUMMARY

#### 1.1. Introduction

This thesis explores the basic science of muscle architecture and its applied relevance to sprint cycling performance. The research journey begins with a cross-sectional analysis (Study I), which revisits a fundamental question concerning the morphological symmetry of the tibialis anterior (TA) muscle. This study also explores the relationships between muscle thickness (MT), pennation angle (PA), and fascicle length (FL) using causal mediation analysis (CMA). Study II employs an innovative cross-sectional modelling framework, utilising CMA to investigate how variability in the relationships among MT, PA, and FL manifests across multiple lower limb muscles. This study also examines whether these relationships are influenced by sex and exhibit regional specificity. The latter is followed by a systematic review (Study III), which synthesises and evaluates the intricate interplay between muscle morphology, architecture, and muscle echo intensity, analysing how these variables collectively influence sprint cycling performance across various existing studies. Finally, Study IV examines the relative contributions of lower limb muscle architecture, tendon morphology, and segmental leg variables, establishing how these factors are associated with sprint cycling performance.

Fundamental questions in muscle architecture and anatomy drive this research, as understanding the structural features that underlie function and performance is essential. Basic scientific studies inform clinical and sports applications and challenge anatomical assumptions, thus shaping our knowledge of musculoskeletal function. For over 20 years, the TA muscle has been described as a bipennate structure, with its unipennate regions functioning symmetrically, i.e. displaying similar architectural characteristics on either side of the central aponeurosis. This perspective originates from a study published in 1999 that assessed the predictability of ultrasound-based changes in TA PA from rest to maximal isometric dorsiflexion in a small group of men. Study I sought to re-evaluate the architectural characteristics of the TA muscle by investigating whether its superficial and deep unipennate regions exhibit symmetry in their architectural features at rest. In doing so, Study I partially replicates the 1999 study, expanding on its scope to explore the relationships among key muscle architecture variables using CMA. This approach allowed us to model how these variables interact and vary across individuals, offering a broader perspective based on a large sample including both sexes. Inspired by Study I's findings on the intricate relationships among muscle architecture variables, we questioned whether the results observed in the TA could be extrapolated to a broader range of pennate and nonpennate muscles. This line of inquiry led to the conception of Study II. In this context, skeletal muscles must lengthen throughout human development to accommodate longitudinal bone growth. Consequently, skeletal muscle exhibits a remarkable capacity for adaptation, shaped not only by growth but also by diverse training stimuli. The relationship among MT, FL, and PA is highly dynamic, shaped by both developmental processes and muscle-specific characteristics. However, no study to date has thoroughly examined these interactions in a large sample of males and females. Gaining insight into these complex relationships and the variability observed among individuals could offer a more precise understanding of typical human variability and inform the interplay of muscle architecture variables across both sexes.

Building on the fundamental understanding of muscle architecture addressed in Studies I and II, Study III shifts focus to the applied implications of muscle morphology and architecture in sprint cycling performance. While muscle mass and volume have long been recognised as key determinants of power output, the geometric arrangement of muscle fibres has been also associated with sprint performance. Muscles with longer fascicles can facilitate faster contraction speeds, contributing to power generation. Additionally, greater PAs are associated with increased PCSA, which enhances force-generating capacity, although this effect is partially offset by a reduction in the alignment of force vectors with the line of muscle action. higher PAs enhance force generation capacity. Despite numerous studies investigating how muscle characteristics affect sprint cycling performance, a focused synthesis of the relationships between muscle morphology, architecture, and performance in this context was lacking. Thus, Study III presents a systematic review designed to analyse the existing evidence and clarify these associations. Expanding upon the insights from Study III, Study IV presents a cross-sectional analysis that delves into how lower extremity muscle architecture, tendon morphology, and leg segment variables contribute to sprint cycling performance. Specifically, this study investigates the relative importance of factors such as lower limb lean mass, muscle architecture (including MT, PA, and FL), tendon morphology (with a focus on the Achilles and patellar tendons), and leg segment characteristics, employing advanced statistical methods like elastic net regression and general dominance analysis. By examining these variables in a sex-diverse cohort, Study IV aims to refine our understanding of the key anatomical predictors of sprint cycling performance, challenging the conventional view that places muscle mass at the forefront.

#### **1.2.** Materials and methods

Within the scope of this thesis, a systematic review (**Study III**) and three crosssectional studies were conducted (**Studies I, II, IV**). Real-time ultrasound imaging was employed as the primary technique to assess muscle architecture in a large human sample, focusing on various aspects such as MT, PA, and FL across different muscle groups and conditions. The studies were conducted on large samples of volunteers of both sexes, who participated in ongoing research projects. In all instances, body composition was assessed using dual-energy X-ray absorptiometry, cardiorespiratory fitness was evaluated with a metabolic cart during incremental exercise to exhaustion, and sprint cycling performance was assessed using the Wingate test. Advanced statistical analyses, including CMA, elastic net regression, and general dominance analysis, were utilised to explore the relationships between muscle characteristics and performance outcomes.

#### Study I

This study investigated the architectural anatomy of the human TA, focusing on the morphological asymmetries between the superficial and deep unipennate regions and how these may differ between males and females. A total of 109 physically active individuals, comprising both sexes, were recruited. Measurements of MT, PA, and FL of the TA were obtained at rest in the unipennate regions of both legs using real-time ultrasound imaging. A linear mixed model was utilised, treating MT, PA, and FL as dependent variables, with analyses conducted both with and without covariates for total leg lean mass and shank length. Additionally, CMA analysis was performed to explore the effect of MT on the relationship between FL and PA. This statistical

technique is used to investigate and understand the underlying mechanisms behind the relationship between an independent variable (or predictor) and a dependent variable (or outcome) through one or more mediating variables.

#### Study II

This study serves as an extension of the CMA analysis results from **Study I**, focusing on the intricate relationships among MT, PA, and FL in the muscles of the lower extremities. MT, PA, and FL of the knee flexor and extensor muscles, as well as the plantar flexors, using real-time ultrasound imaging at rest were assessed in the same subjects included in Study I. A mixed-effects model was employed to explore differences based on sex, leg dominance (dominant vs. non-dominant), and muscle region. The analysis aimed to elucidate the interactions between these variables and to apply robust mathematical models, specifically CMA, to assess how MT influences PA, mediated by FL, across a large population sample.

#### Study III

This study presents a systematic review investigating the intricate relationships between muscle morphology, architecture, and echo intensity and their influence on sprint cycling performance across various studies. Following the PRISMA guidelines, extensive four search was conducted across major databases: an MEDLINE/PubMed, Web of Science, CINAHL Complete, and SPORTDiscus. The review was accordingly registered in PROSPERO (CRD42023432824). Additionally, the methodological quality of the included studies was evaluated using the Methodological Index for Non-Randomised Studies (MINORS).

#### Study IV

This study was designed to model how lower extremities' muscle architecture, tendon morphology, and leg segment variables influence sprint cycling performance, establishing their relative relevance. The muscular architecture of several lower limb muscles, along with the morphology of the Achilles and patellar tendons and leg segment variables (i.e., shank length, forefoot length, and the moment arm of the AT), was assessed at rest by real-time ultrasound imaging in a cohort of 101 physically active males and females, which were part of the subjects included in Study I and II. Sprint performance was evaluated through the isokinetic Wingate test. To determine the predictors of sprint cycling performance, a two-stage approach was employed: (I) elastic net regression and the best subset selection algorithm were utilised to mitigate optimisation bias and ensure an unbiased evaluation of predictive performance on unseen data, and (II) to elucidate the contribution of each predictor to the final regression model, a general dominance analysis was conducted.

#### 1.3. Results

#### Study I

This study revealed that the TA muscle exhibits morphological asymmetries between its superficial and deep unipennate regions in both males and females. Specifically, the deep region demonstrates greater MT by 2–4 mm and a higher PA by 1–2° compared to the superficial region. These results contradict the findings of a wellcited publication from over 20 years ago (Maganaris and Baltzopoulos, 1999), which reported no architectural differences between these regions in six males measured at rest and during maximal voluntary contraction. Furthermore, **Study I** found no notable differences in muscle architecture between the dominant and nondominant legs. MT and PA were greater in the deep unipennate region than in the superficial region, with significant values for both sexes (p < 0.001). FL remained similar across both regions. After adjusting for leg lean mass and shank length, sex differences persisted in MT (1.6 mm, p < 0.05) and PA (3.4°, p < 0.001) only in the superficial region. Additionally, the CMA indicated that the coefficient of FL in the mediator model was positive, suggesting that a 10% increase in MT would augment the FL, allowing a 0.38° PA decrease. Overall, **Study I** suggests that the architectural anatomy of the TA displays sexual dimorphism and morphological asymmetries. Lastly, this study also revealed that increments in MT are not always aligned with increments in FL or PA.

#### Study II

This investigation was an extension of the CMA results from **Study I**, focusing on the intricate relationships among MT, PA, and FL in seven muscles of the lower extremities, most of which were evaluated across various regions, in a long cohort of humans of both sexes. As indicated by the CMA analysis in all muscles, a 1 mm increase in MT across subjects was associated with a significant increase (all p < 0.001) in PA (direct effect), ranging from 0.12° in the ST to 1.32° in the GM. Furthermore, both the GM and GL revealed relationships between FL, MT, and PA. Among the muscles measured across multiple regions, the conditional indirect effects (the indirect effects of each muscle region) differ significantly only in the BFlh, as shown by the non-overlapping confidence intervals. This indicates that only in the BFlh was the indirect effect moderated by the muscle region (mediated moderation). Additionally, secondary results revealed that males exhibited increased MT across all examined muscles, ranging from 0.1 to 2.1 cm (p < 0.01), with no significant differences in FL between sexes. The dominant legs displayed greater MT (0.1 cm, p = 0.01) and PA (1.5°, p = 0.01) in the RF, whereas VL exhibited greater FL (1.2 cm, p < 0.001) and PA (0.6°, p = 0.02). Noteworthy regional variations were identified in the VL, RF, and BFlh. Overall, this research elucidates the complex interactions among MT, FL, and PA.

#### Study III

This study comprised a systematic review offering an in-depth overview of the complex relationships among muscle morphology, architecture, echo intensity, and their impact on sprint cycling performance across various human studies. The literature search identified 3971 records across four electronic databases, including ten studies examining the relationship between muscle morphology, architecture, or quality and sprint cycling performance. The total sample comprised 254 participants, predominantly male (85.4%), with ages ranging from 9 to 33 years. Most studies employed ultrasound imaging, with various devices used for evaluating sprint performance, primarily the Wingate test. Heterogeneous results were reported regarding the PA and FL, with some studies showing weak correlations to performance metrics. Regression analyses underscored the robust relationship of quadriceps muscle volume with peak power output (R<sup>2</sup> ranging between 0.65 and 0.82), suggesting its pivotal role in force production. The methodological quality (i.e., MINORS) of the included studies varied, with a mean score of 16/24 for comparative

studies and 11.3/16 for non-comparative studies, highlighting areas for improvement in reporting and design. There were constraints related to the prospective estimation of sample size and the impartial assessment of study endpoints.

#### Study IV

This study demonstrated that lower extremity lean mass is the primary determinant of sprint cycling performance on the cycle ergometer. Additionally, it revealed the significant role of muscle architecture, which accounts for 23-34% of the explained variance in sprint cycling performance, with the BFlh acting as a suppressor of peak power generation. In a cohort of 101 participants aged 20 to 26 years, notable sex differences emerged in physical characteristics and performance metrics. Females exhibited a higher body fat percentage and lower lean mass compared to males. However, when normalising VO<sub>2</sub> max for LM, no significant differences were noted between the sexes. Males outperformed females in absolute values of peak and mean power but not when normalised for whole body and lower extremities lean mass (LLM). Muscle architecture analysis revealed that out of 39 variables assessed, only 14 showed no sex disparities. Males consistently had greater MT and PA across various muscles, while females had more subcutaneous fat in the TA. Differences in tendon morphology showed that males had thicker Achilles and patellar tendons. Further analysis indicated that muscle architecture contributed 24-34% of the explained variance in cycling performance metrics (W<sub>mean</sub>, W<sub>peak1s</sub>, W<sub>peak2</sub>). The most significant predictors for the models included LLM and muscle architecture variables, with LLM consistently demonstrating the highest importance across all models. BFlh FL and PA acted as suppressor variables in the models for peak power. Lastly, the correlation between power variables (W<sub>mean</sub>, W<sub>peak1s</sub>, W<sub>peak</sub>) was similar in both sexes, although mean differences in performance metrics varied between males and females, with men showing greater mean differences across all power comparisons.

#### 1.4. Conclusions

The conclusions drawn from both basic and applied studies provide a multifaceted understanding of muscle architecture and its implications for sprint cycling performance.

#### Study I

In this first study, real-time ultrasonography reveals that the TA presents as a nonsymmetrical bipennate muscle at rest. Small sex differences in TA architecture persist even after adjusting for LLM and shank length. Notably, a suppressive effect of FL on PA is identified, suggesting that increments in MT are not always aligned with increments in FL or PA.

#### Study II

This investigation highlights considerable variations in human muscle architecture within and between muscles, emphasising the complex relationships between MT, PA, and FL. Pronounced sex-related differences are observed, with males exhibiting greater MT and wider PA in specific muscles, while FL did not differ significantly between sexes. Regional architectural differences are noted in several muscles, and the study reveals a suppressive effect of FL on PA in lower limb pennate muscles, with varying effects regionally in the BFlh and concurrent increases in MT and FL in the RF without affecting PA.

#### Study III

The systematic review highlights that muscle morphology, architecture, and echo intensity likely play a role in sprint cycling performance. However, the relationships between these factors are complex and vary across different studies. Muscle volume emerges as a critical determinant of peak power output, with muscle architecture components such as MT, PA, and FL showing varied associations with performance outcomes. Preliminary evidence suggests a link between echo intensity and sprint performance, though this area remains underexplored and requires further investigation. The variability in findings underscores the need for standardisation in measurement methods and performance assessments to enhance comparability.

#### Study IV

Finally, this fourth study provides compelling evidence regarding the pivotal role of muscle mass and architecture in influencing sprint cycling outcomes, with muscle architecture accounting for a significant portion of the variance in performance. Additionally, sex differences in sprint performance were primarily linked to leg lean mass. We also find sex differences in resting muscle architecture, tendon morphology, and leg segments. The study identifies the FL and PA of the BFlh as suppressors, negatively impacting maximal power generation.

Overall, this thesis offers new findings in both basic and applied muscle architecture research, providing significant insights into its role in sprint cycling performance. The research demonstrates that architectural features are nearly as crucial as muscle mass in power generation. By combining studies across foundational and applied contexts, this work elucidates the relationships among key architectural parameters and their complex interdependencies across various muscles and populations. Notably, this thesis also replicates findings from over 20 years ago on the TA muscle, challenging earlier conclusions by uncovering substantial asymmetries between its superficial and deep unipennate regions. These results suggest that prior research may have been affected by a Type I error due to a limited sample size. Furthermore, this thesis introduces the FL and PA of the BFlh as suppressors of maximal power generation, underscoring its relevance in predictive models. The use of advanced statistical techniques enhances previous findings and highlights the multifaceted nature of muscle architecture, bridging foundational anatomical understanding with practical implications. Together, these insights advance our comprehension of muscle architecture's impact on human sprint cycling performance, laying a more precise groundwork for future research and potential applications in sports performance enhancement.
# CHAPTER II:

# RESUMEN

(SUMMARY IN SPANISH)

# 2. RESUMEN

# 2.1. Introducción

Esta tesis explora la ciencia básica de la arquitectura muscular y su relevancia aplicada al rendimiento en el ciclismo de esprint. La tesis comienza con un análisis transversal (Estudio I) que revisita una cuestión fundamental sobre la simetría morfológica del músculo tibial anterior (TA). Este estudio también examina las relaciones entre el grosor muscular (MT), el ángulo de penación (PA) y la longitud de los fascículos (FL) mediante análisis de mediación causal (CMA). El Estudio II emplea un marco de modelización transversal innovador, utilizando CMA para investigar cómo se manifiesta la variabilidad en las relaciones entre MT, PA y FL en múltiples músculos de los miembros inferiores. Este estudio también analiza si dichas relaciones están influenciadas por el sexo y si presentan especificidad regional. Tras este estudio, se desarrolla una revisión sistemática (Estudio III) que sintetiza y evalúa la compleja interacción entre la morfología muscular, la arquitectura y la eco-intensidad del músculo, analizando cómo estas variables influyen colectivamente en el rendimiento en el ciclismo de esprint según los estudios existentes. Por último, el Estudio IV examina las contribuciones relativas de la arquitectura muscular de los miembros inferiores, la morfología de los tendones y las variables segmentarias de las piernas, estableciendo cómo estos factores se asocian con el rendimiento en el ciclismo de esprint.

Esta investigación se centra en preguntas clave sobre la arquitectura y la anatomía muscular, ya que es fundamental entender las características estructurales que determinan la función y el rendimiento. Los estudios de ciencia básica sirven de base para las aplicaciones clínicas y deportivas, además de cuestionar las ideas preconcebidas sobre la anatomía, lo que contribuye a ampliar nuestro conocimiento de cómo funciona el sistema musculoesquelético. Durante más de 20 años, el músculo tibial anterior (TA) ha sido descrito como una estructura bipennada, con regiones unipennadas que funcionan de manera simétrica, es decir, mostrando características arquitectónicas similares a ambos lados de la aponeurosis central. Esta perspectiva se origina en un estudio publicado en 1999 que evaluó la predictibilidad de los cambios en el ángulo de penación del TA, medidos mediante ecografía, desde reposo hasta una dorsiflexión isométrica máxima en un pequeño grupo de hombres. El Estudio I buscó reevaluar las características arquitectónicas del músculo TA investigando si sus regiones unipennadas superficial y profunda exhibían simetría en sus características arquitectónicas en reposo. Al hacerlo, el Estudio I replica parcialmente el estudio de 1999, ampliando su alcance para explorar las relaciones entre las principales variables de arquitectura muscular mediante análisis de mediación causal (CMA). Este enfoque permitió modelar cómo interactúan estas variables y cómo varían entre individuos, ofreciendo una perspectiva más amplia basada en una muestra grande de sujetos que incluye ambos sexos. Inspirados por los hallazgos del Estudio I sobre las complejas relaciones entre las variables de arquitectura muscular, nos preguntamos si los resultados observados en el TA podrían extrapolarse a una gama más amplia de músculos pennados y no pennados. Esta línea de investigación llevó a la concepción del Estudio II. En este contexto, el músculo esquelético debe alargarse durante el desarrollo humano para adaptarse al crecimiento longitudinal de los huesos. Por ende, el músculo esquelético exhibe una notable capacidad de adaptación, influida no solo por el crecimiento, sino también por diversos estímulos de entrenamiento. La relación entre el grosor muscular (MT), la longitud de los fascículos (FL) y el ángulo de penación (PA) es altamente dinámica, mediada tanto por procesos de desarrollo como por características específicas de cada músculo. Sin embargo, hasta la fecha, ningún estudio ha examinado exhaustivamente estas interacciones en una muestra amplia de hombres y mujeres. Comprender estas relaciones complejas y las diferencias entre individuos podría proporcionar una visión más clara de la variabilidad humana y ayudar a explicar cómo interactúan las variables de la arquitectura muscular en hombres y mujeres.

A partir del conocimiento fundamental sobre la arquitectura muscular desarrollado en los Estudios I y II, el Estudio III centra su atención en las implicaciones aplicadas de la morfología y arquitectura muscular en el rendimiento del ciclismo de esprint. Aunque la masa y el volumen muscular han sido reconocidos durante mucho tiempo como factores clave para la generación de potencia, la disposición geométrica de las fibras musculares también se ha asociado con el rendimiento en esprint. Los músculos con fascículos más largos pueden favorecer velocidades de contracción más rápidas, lo que contribuye a la producción de potencia. Además, mayores ángulos de penación están relacionados con un incremento en la sección transversal fisiológica (PCSA), lo que mejora la capacidad de generar fuerza, aunque este efecto se ve parcialmente reducido debido a la menor alineación de los vectores de fuerza con la línea de acción del músculo. A pesar de la abundancia de estudios que han explorado cómo las características musculares afectan el rendimiento en el ciclismo de esprint, era necesario una síntesis que abordara de manera específica las relaciones entre la morfología, la arquitectura muscular y el rendimiento en este contexto. Por ello, el Estudio III presenta una revisión sistemática diseñada para analizar las evidencias existentes y esclarecer estas asociaciones. Basándose en los hallazgos del **Estudio III**, el **Estudio IV** presenta un análisis transversal que examina cómo la arquitectura muscular de las extremidades inferiores, la morfología de los tendones y las características segmentarias de las piernas contribuyen al rendimiento en el ciclismo de esprint. Específicamente, este estudio analiza la importancia relativa de factores como la masa magra de las extremidades inferiores, la arquitectura muscular (incluyendo MT, PA y FL), la morfología del tendón (con énfasis en los tendones de Aquiles y rotuliano) y las características segmentarias de las piernas, empleando métodos estadísticos avanzados como la regresión de red elástica y el análisis de dominancia general. Al investigar estas variables en una cohorte diversa en términos de sexo, el **Estudio IV** busca refinar nuestra comprensión de los principales predictores anatómicos del rendimiento de esprint cíclico, cuestionando la visión convencional que sitúa a la masa muscular como el factor predominante.

# 2.2. Material y métodos

En el marco de esta tesis, se realizaron una revisión sistemática (**Estudio III**) y tres estudios transversales (**Estudios I, II y IV**). La técnica principal utilizada para evaluar la arquitectura muscular en una muestra amplia de individuos fue la ecografía en tiempo real, con un enfoque en diversos aspectos como el MT, PA y FL en diferentes grupos musculares y condiciones. Los estudios se llevaron a cabo en grandes muestras de voluntarios de ambos sexos, quienes participaron en proyectos de investigación en curso. En todos los casos, se evaluó la composición corporal mediante absorciometría de rayos X de energía dual, la condición cardiorrespiratoria se valoró con un carro

metabólico durante un ejercicio incremental hasta el agotamiento, y el rendimiento en ciclismo de esprint se midió utilizando la prueba de Wingate. Se emplearon análisis estadísticos avanzados, como el CMA, la regresión de red elástica y el análisis de dominancia general, para explorar las relaciones entre las características musculares y los resultados del rendimiento.

# Estudio I

Este estudio investigó la anatomía arquitectónica del músculo TA, centrándose en las asimetrías morfológicas entre sus regiones unipennadas superficial y profunda, y cómo estas pueden diferir entre hombres y mujeres. Se reclutaron un total de 109 individuos físicamente activos, de ambos sexos. Se obtuvieron mediciones del MT, PA, y FL del TA en reposo, en las regiones unipennadas de ambas piernas, utilizando ecografía en tiempo real. Para el análisis de los datos, se empleó un modelo lineal mixto, considerando MT, PA y FL como variables dependientes. Los análisis se realizaron tanto con y sin covariables, ajustando por la masa magra total de las piernas y la longitud de la tibia. Además, se llevó a cabo un análisis de mediación causal (CMA) para explorar el efecto del MT sobre la relación entre FL y PA. Esta técnica estadística permite investigar y comprender los mecanismos subyacentes en la relación entre una variable independiente (predictora) y una variable dependiente (resultado), a través de una o más variables mediadoras.

## Estudio II

Este estudio amplía los resultados del análisis de mediación causal realizado en el **Estudio I**, centrándose en las complejas relaciones entre MT, PA y FL en los

músculos de las extremidades inferiores. Se evaluaron estas variables en los músculos flexores y extensores de la rodilla, así como en los flexores plantares, utilizando ecografía en tiempo real en reposo, en los mismos participantes incluidos en el Estudio I. Se utilizó un modelo de efectos mixtos para explorar las diferencias basadas en el sexo, la dominancia de la pierna (dominante frente a no dominante) y la región muscular. El análisis tuvo como objetivo esclarecer las interacciones entre estas variables y aplicar modelos matemáticos robustos, específicamente el análisis de CMA, para evaluar cómo MT influye en PA, mediado por FL, en una muestra poblacional amplia.

## Estudio III

Este estudio presenta una revisión sistemática que investiga las complejas relaciones entre la morfología muscular, la arquitectura y la eco-intensidad, y cómo estas variables influyen en el rendimiento en ciclismo de esprint según diversos estudios. Siguiendo las directrices PRISMA, se llevó a cabo una búsqueda exhaustiva en cuatro bases de datos principales: MEDLINE/PubMed, Web of Science, CINAHL Complete y SPORTDiscus. La revisión fue registrada en PROSPERO (CRD42023432824). Además, se evaluó la calidad metodológica de los estudios incluidos utilizando el Índice Metodológico para Estudios No Aleatorizados (MINORS).

#### Estudio IV

Este estudio fue diseñado para modelar cómo la arquitectura muscular de las extremidades inferiores, la morfología del tendón y las variables segmentarias de la pierna influyen en el rendimiento en ciclismo de esprint, estableciendo su relevancia

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relativa. Se evaluaron la arquitectura muscular de varios músculos de las extremidades inferiores, la morfología de los tendones de Aquiles y rotuliano, así como las variables segmentarias de la pierna (longitud de la tibia, longitud del antepié y el brazo de momento del tendón de Aquiles), utilizando ecografía en tiempo real en reposo. La muestra incluyó a 101 hombres y mujeres físicamente activos, los cuales fueron parte de los participantes de los Estudios I y II. El rendimiento en ciclismo de esprint se evaluó mediante la prueba isocinética de Wingate. Para determinar los predictores del rendimiento, se empleó un enfoque en dos etapas: (I) se utilizaron la regresión de red elástica y el algoritmo de selección del mejor subconjunto, para mitigar el sesgo de optimización y garantizar una evaluación imparcial del rendimiento predictivo en datos no vistos, y (II) se realizó un análisis de dominancia general para esclarecer la contribución de cada predictor al modelo de regresión final.

# 2.3. Resultados

# Estudio I

Este estudio reveló que el músculo TA presenta asimetrías morfológicas entre sus regiones unipennadas superficial y profunda en ambos sexos. Específicamente, la región profunda muestra un MT mayor por 2–4 mm y un PA más alto por 1–2°, en comparación con la región superficial. Estos resultados contradicen los hallazgos de una publicación muy citada de hace más de 20 años (Maganaris and Baltzopoulos, 1999), que no reportó diferencias arquitectónicas entre estas regiones en seis hombres evaluados en reposo y durante una contracción voluntaria máxima. Además, el **Estudio I** no encontró diferencias notables en la arquitectura muscular entre las

piernas dominante y no dominante. MT y PA fueron mayores en la región unipennada profunda que en la superficial, con valores significativos para ambos sexos (p < 0.001). FL permaneció similar en ambas regiones. Tras ajustar por la masa magra de las piernas y la longitud de la tibia, las diferencias entre sexos persistieron solo en la región superficial para MT (1.6 mm, p < 0.05) y PA ( $3.4^\circ$ , p < 0.001). Adicionalmente, el CMA indicó que el coeficiente de FL en el modelo mediador fue positivo, sugiriendo que un incremento del 10% en MT aumentaría el FL, permitiendo una disminución de  $0.38^\circ$  en PA. En conjunto, el **Estudio I** sugiere que la anatomía arquitectónica del TA muestra dimorfismo sexual y asimetrías morfológicas. Por último, este estudio también reveló que los incrementos en MT no siempre están alineados con incrementos en FL o PA.

# Estudio II

Esta investigación fue una extensión de los resultados del CMA del **Estudio I**, centrada en las complejas relaciones entre MT, PA y FL en siete músculos de las extremidades inferiores, la mayoría de los cuales se evaluaron en diferentes regiones, en una amplia cohorte de humanos de ambos sexos. Según el análisis CMA realizado en todos los músculos, un incremento de 1 mm en MT entre los sujetos se asoció con un aumento significativo (p < 0.001 en todos los casos) en PA (efecto directo), que varió desde 0.12° en el ST hasta 1.32° en el GM. Además, tanto el GM como el GL mostraron relaciones entre FL, MT y PA. En los músculos evaluados en múltiples regiones, los efectos indirectos condicionales (efectos indirectos de cada región muscular) solo difirieron significativamente en el BFlh, como lo evidencian los intervalos de confianza no superpuestos. Esto indica que, únicamente en el BFlh, el

efecto indirecto fue moderado por la región muscular (moderación mediada). Adicionalmente, los resultados secundarios revelaron que los hombres presentaron un MT mayor en todos los músculos examinados, con incrementos que oscilaron entre 0.1 y 2.1 cm (p < 0.01), sin diferencias significativas en FL entre sexos. Las piernas dominantes mostraron mayores valores de MT (0.1 cm, p = 0.01) y PA (1.5°, p = 0.01) en el RF, mientras que el VL presentó un mayor FL (1.2 cm, p < 0.001) y PA (0.6°, p = 0.02). Se identificaron variaciones regionales destacables en el VL, RF y BFlh. En conjunto, esta investigación esclarece las complejas interacciones entre MT, FL y PA.

## Estudio III

Este estudio consistió en una revisión sistemática que ofrece una visión detallada de las complejas relaciones entre la morfología muscular, la arquitectura, la intensidad de eco y su impacto en el rendimiento en ciclismo de esprint, según diversos estudios en humanos. La búsqueda bibliográfica identificó 3971 registros en cuatro bases de datos electrónicas, de los cuales se incluyeron diez estudios que analizaron la relación entre la morfología, la arquitectura o la calidad muscular y el rendimiento en ciclismo de esprint. La muestra total abarcó 254 participantes, predominantemente hombres (85.4%), con edades entre 9 y 33 años. La mayoría de los estudios emplearon ecografía como técnica principal, utilizando diversos dispositivos para evaluar el rendimiento en sprint, principalmente mediante la prueba de Wingate. Los resultados fueron heterogéneos en relación con PA y FL, con algunos estudios mostrando correlaciones débiles con las métricas de rendimiento. Los análisis de regresión destacaron la relación sólida entre el volumen del músculo cuádriceps y la potencia pico generada (R<sup>2</sup> entre 0.65 y 0.82), lo que sugiere su papel fundamental en la producción de fuerza. La calidad metodológica de los estudios incluidos (evaluada mediante MINORS) mostró variabilidad, con una puntuación media de 16/24 para estudios comparativos y 11.3/16 para estudios no comparativos, señalando áreas de mejora en el diseño y la calidad del reporte. Además, se identificaron limitaciones relacionadas con la estimación prospectiva del tamaño de muestra y la evaluación imparcial de los resultados de los estudios.

## Estudio IV

Este estudio demostró que la masa magra de las extremidades inferiores es el principal determinante del rendimiento en ciclismo de esprint en el cicloergómetro. Además, destacó el papel significativo de la arquitectura muscular, que explica entre el 23 % y el 34 % de la varianza en el rendimiento en ciclismo de esprint, con el BFlh actuando como un supresor de la generación de potencia pico. En una cohorte de 101 participantes de entre 20 y 26 años, se observaron diferencias significativas entre sexos en las características físicas y métricas de rendimiento. Las mujeres presentaron un mayor porcentaje de grasa corporal y menor masa magra en comparación con los hombres. Sin embargo, al normalizar el VO2 max por LM, no se encontraron diferencias significativas entre sexos. Los hombres superaron a las mujeres en valores absolutos de potencia pico y media, pero no al normalizar por la masa magra corporal total o de las extremidades inferiores (LLM). El análisis de la arquitectura muscular reveló que, de las 39 variables evaluadas, solo 14 no mostraron diferencias entre sexos. Los hombres presentaron consistentemente mayores valores de MT y PA en varios músculos, mientras que las mujeres mostraron más grasa subcutánea en el TA. Las diferencias en la morfología tendinosa indicaron que los hombres tenían tendones de Aquiles y rotulianos más gruesos. Un análisis adicional mostró que la arquitectura muscular contribuyó entre el 24 % y el 34 % de la varianza explicada en las métricas de rendimiento en ciclismo (W<sub>mean</sub>, W<sub>peak1s</sub>, W<sub>peak</sub>). Los predictores más importantes en los modelos incluyeron LLM y variables de arquitectura muscular, con LLM demostrando consistentemente la mayor relevancia en todos los modelos. En particular, FL y PA del BFlh actuaron como variables supresoras en los modelos para potencia pico. Por último, la correlación entre las variables de potencia (W<sub>mean</sub>, W<sub>peak1s</sub>, W<sub>peak</sub>) fue similar en ambos sexos, aunque las diferencias promedio en las métricas de rendimiento variaron, con los hombres mostrando mayores diferencias promedio en todas las comparaciones de potencia.

# 2.4. Conclusiones

Las conclusiones obtenidas tanto de los estudios básicos como aplicados proporcionan una comprensión multifacética de la arquitectura muscular y sus implicaciones en el rendimiento en ciclismo de velocidad.

#### Estudio I

En este primer estudio, la ecografía en tiempo real revela que el TA es un músculo bipennado no simétrico en reposo. Se observan pequeñas diferencias sexuales en la arquitectura del TA, las cuales persisten incluso después de ajustar por LLM y longitud de la tibia. De manera destacada, se identifica un efecto supresor de FL sobre PA, lo que sugiere que los incrementos en MT no siempre están alineados con los incrementos en FL o PA.

# Estudio II

Esta investigación destaca variaciones considerables en la arquitectura muscular humana, tanto dentro de los músculos como entre ellos, subrayando las complejas relaciones entre MT, PA y FL. Se observan marcadas diferencias relacionadas con el sexo, con los hombres mostrando mayor grosor muscular y un PA más amplio en músculos específicos, mientras que FL no presenta diferencias significativas entre sexos. Se aprecian diferencias arquitectónicas regionales en varios músculos, y se identifica un efecto supresor de FL sobre PA en los músculos pennados de las extremidades inferiores, con efectos variables a nivel regional en el BFlh y aumentos concurrentes en el grosor muscular y FL en el RF sin afectar el PA.

# Estudio III

La revisión sistemática destaca que la morfología muscular, la arquitectura y la ecointensidad probablemente jueguen un papel en el rendimiento en ciclismo de esprint. Sin embargo, las relaciones entre estos factores son complejas y varían según los diferentes estudios. El volumen muscular emerge como un determinante crítico de la potencia pico, mientras que los componentes de la arquitectura muscular, como MT, PA y FL, muestran asociaciones variables con los resultados del rendimiento. La evidencia preliminar sugiere una relación entre la eco-intensidad y el rendimiento en esprint, aunque esta área sigue estando poco explorada y requiere más investigación. La variabilidad en los hallazgos subraya la necesidad de estandarizar los métodos de medición y las evaluaciones del rendimiento para mejorar la comparabilidad entre estudios.

## Estudio IV

Finalmente, este cuarto estudio proporciona evidencia convincente sobre el papel fundamental de la masa muscular, y la arquitectura en la influencia de los resultados en ciclismo de esprint, con la arquitectura muscular explicando una parte significativa de la varianza en el rendimiento. Además, las diferencias sexuales en el rendimiento en esprint están principalmente relacionadas con la masa magra de las piernas. También se observan diversas variaciones en la arquitectura muscular en reposo, la morfología de los tendones y los segmentos de la pierna debido al sexo. El estudio identifica la FL y el PA del BFlh como supresores, impactando negativamente en la generación de potencia máxima.

En general, esta tesis aporta nuevos hallazgos tanto en la investigación básica como aplicada de la arquitectura muscular, brindando valiosos conocimientos sobre su papel en el rendimiento en ciclismo de esprint. La investigación demuestra que las características arquitectónicas son casi tan cruciales como la masa muscular en la generación de potencia. Al combinar estudios en contextos de ciencia básica y aplicados, este trabajo aclara las relaciones entre los parámetros arquitectónicos clave y sus complejas interdependencias a través de varios músculos y poblaciones. Es importante destacar que esta tesis también replica hallazgos de hace más de 20 años sobre el músculo TA, desafiando conclusiones anteriores al descubrir asimetrías sustanciales entre sus regiones unipennadas superficial y profunda. Estos resultados sugieren que la investigación previa pudo haber sido afectada por un error de Tipo I debido al tamaño limitado de la muestra. Además, esta tesis introduce la FL y el PA del BFlh como supresores de la generación de potencia máxima, subrayando su relevancia en los modelos predictivos. El uso de técnicas estadísticas avanzadas mejora los hallazgos previos y resalta la naturaleza multifacética de la arquitectura muscular, conectando la comprensión anatómica fundamental con sus implicaciones prácticas. En conjunto, estos conocimientos avanzan nuestra comprensión del impacto de la arquitectura muscular en el rendimiento humano en ciclismo de esprint, estableciendo una base más precisa para futuras investigaciones y posibles aplicaciones en la mejora del rendimiento deportivo.

# CHAPTER III:

# INTRODUCTION

# **3. INTRODUCTION**

# 3.1. Presentation of Thesis Objectives, Published Works, and Thematic Justification

The present thesis investigates both the basic science and applied significance of muscle architecture with human sprint cycling performance. It commences with studies focused on intrinsic characteristics of muscle architecture, followed by research applying these findings to sprint cycling performance.

Firstly, **Study I** examines whether the TA muscle, traditionally classified as bipennate, exhibits symmetry in its superficial and deep unipennate regions at rest. This study revisits a question initially posed over two decades ago in a pioneering study conducted by Professors Constantinos Maganaris, Vasilios Baltzopoulos, and Anthony J. Sargeant, whose work was limited by a small sample size. In the current study, symmetry was analysed in a substantially larger cohort of male and female participants. Employing advanced statistical methodologies, this investigation provides insights into the relationships among MT, PA, and FL in the TA, emphasising implications for muscle growth and adaptation. **Study II** extends this line of inquiry by investigating the relationships between muscle architecture variables across a broader range of muscle groups, assessing their interdependencies within a rigorous analytical framework. This study employs sophisticated statistical analyses to deepen the understanding of how architectural elements interact and influence each other. On the other hand, **Study III** synthesizes current evidence on the complex relationships between muscle morphology, architecture, and echo intensity, and their impact on sprint cycling performance. As a systematic review, it examines the varied findings across existing studies, contextualizing these relationships within the broader field of sprint cycling research. Finally, **Study IV** models the influence of lower limb muscle architecture, tendon morphology, and leg segment variables on sprint cycling performance. Conducted with a large, diverse sample of both male and female participants, this cross-sectional study employs advanced statistical techniques, including elastic net regression, best subset selection, and general dominance analysis, to evaluate the relative significance of each variable. This work challenges conventional perspectives by identifying specific architectural features, such as those of the biceps femoris long head, that substantially contribute to performance outcomes in sprint cycling.

Collectively, the four studies presented in this thesis reveal: (I) the substantial role of muscle architecture in sprint performance; (II) morphological asymmetries within the tibialis anterior muscle between its superficial and deep unipennate regions, as well as evidence of sexual dimorphism; and (III) the intricate interconnections among key architectural variables. These findings offer a valuable basis for advancing research in this field, underscoring the importance of experimental designs that systematically adjust MT, PA, and FL through targeted training interventions. The integration of sophisticated 3D imaging techniques, such as magnetic resonance imaging and ultrasound, could further elucidate these structural influences. However, a full understanding of how these architectural factors interact during sprinting remains limited. As sprint performance in real-world contexts involves continuous

adaptations in muscle length, joint angles, and contraction velocities, future research should prioritize dynamic measurements over static associations, thereby providing a more contextually relevant understanding of muscle architecture with performance.

# 3.2. Background (I): From muscle architecture to function

Muscle architecture refers to the arrangement of muscle fibres relative to the axis of force generation (Lieber and Friden, 2000), encompassing parameters such as FL, PA and PCSA, which collectively influence the functional properties of the muscles. Historically, anatomists from the Renaissance period, such as Andreas Vesalius, laid the groundwork for muscle studies by documenting human anatomy in unprecedented detail, though without the precision needed to capture fibre arrangement (Narici et al., 2016). This period marked a transformative era in medical science, as anatomical dissections unveiled the intricate details of the human body. Before this breakthrough, knowledge of human anatomy was largely derived from Galen's animal dissections, as Roman laws prohibited human dissection since were considered disrespectful and potentially sacrilegious (Ghosh, 2015). The following century saw Giulio Cesare Casseri and Sir Christopher Wren develop more accurate depictions of muscle fibres, but it was through the work of Nicolaus Steno and Giovanni Borelli that the dynamic relationship between muscle architecture and function was better understood (Narici et al., 2016) (Figure 1).



**Figure 1.** A schematic depiction by Nicolaus Steno demonstrates his understanding of how the pennation angle in muscles increases during contraction, described by him as: "*when the muscle contracts, its acute angles become larger*" (translated from Latin). This image is available in the public domain (link).

Since the seminal work of Nobel laureate A.V. Hill in the 1930s, it has been well established that the macroscopic size and arrangement of these fibres, i.e. now collectively termed muscle architecture, play a central role in determining muscle function (Hill, 1938). Muscle architecture can be quantified by calculating the PCSA(Haxton, 1944), which is a major determinant of a muscle's maximum force-generation capacity. However, this relationship is also influenced by factors such as fibre PA and neuromuscular activation. In addition, the relationship between FL, PA, and contraction velocity has proven to be a critical determinant of muscle performance (Aagaard et al., 2001, Charles et al., 2022, Roberts et al., 2019). Muscle fibres can take various structural forms, such as parallel or pennate arrangements, as described by Nicolaus Steno in *Elementorum Mythologiae Specimen* (Narici et al., 2016). Parallel-fibred muscles, often characterised by longer fibre lengths, are thought to

favour high-velocity contractions. In contrast, pennate muscles, with shorter fibres arranged at angles to the line of action, are adapted to generate higher force output due to the greater number of fibres acting in unison (Charles et al., 2022). This variation in structure underscores the importance of accurately measuring FL, PA, and muscle mass when assessing how a muscle functions during dynamic movements (Charles et al., 2022). Regarding PA, Prof. Richard Lieber, a renowned scientist in the field has recently challenged the traditional view that PA plays a critical role in muscle function (Lieber, 2022). He argues that PA is more of a geometrical byproduct, i.e. a structural adaptation that allows muscle fibres to be efficiently packed, rather than a key determinant of force generation. Lieber points to several studies showing that while PA increases during muscle contraction, this does not correspond to a significant decrease in force output, challenging the assumption that PA directly impacts the force transmitted to tendons. He notes that muscle fibres rotate during contraction, which allows the muscle to maintain efficient shortening despite variations in PA. Additionally, the isovolumetric nature of muscle during contraction complicates simple models that attribute excessive importance to PA. Ultrasound studies further reveal that even when PA changes substantially, muscles continue to generate substantial force, indicating that PA has minimal influence on overall force production. Lieber concludes that PA has little functional significance, advocating for a shift in focus towards more relevant architectural parameters such as fibre length and PCSA, which better explain muscle function. Pro. Lieber's assertion to "let the fibres rotate" aligns with the concept of variable gearing introduced by great actual evolutionary biologists Emanuel Azizi, Elizabeth L. Brainerd, and Thomas J. Roberts (Azizi et al., 2008), which demonstrates how pennate muscles adjust their internal structure during contraction. The rotation of muscle fibres allows for a higher gear ratio during low-force contractions, enhancing velocity, while the gear ratio decreases during forceful contractions, favouring strength output. This dynamic adaptation is a critical aspect of muscle function, and it has been hypothesised that gearing in any given contraction is determined by the dynamic interaction of fibre-generated forces, fluid force transmission, and the elastic behaviour of intramuscular connective tissues (Eng et al., 2018). However, this remains underexplored in human studies. Future research should prioritise measuring such variable gearing mechanisms to fully understand their influence on human muscle performance and potential applications in athletic and clinical settings. While Prof. Lieber's arguments are logically sound, robust empirical investigations are still needed to invalidate his proposed hypothesis. Recently, a novel outstanding investigation has been published to solve or partly solve this question (Rockenfeller et al., 2024). The authors develop geometric models to simulate muscle architecture and compare them with experimental data from human lower limb muscles. They find that muscles with a moderate PA and a high lengthto-thickness ratio can significantly enhance force generation. While Prof. Lieber argues that PA is largely a geometrical byproduct with minimal functional significance in terms of force transmission, this paper shows that PA plays a critical role in increasing the effective PCSA and, thus, force production. The results challenge Prof. Lieber's view by demonstrating that PA, though reducing force projection onto the tendon, allows for a higher number of sarcomeres in parallel, which more than compensates for any loss in tendon-directed force.

Adaptation in skeletal muscle architecture is a complex and multifaceted process. Remodelling can occur across the entire motor unit, influencing neuromuscular junctions, motor unit firing patterns, the metabolome, and fibre arrangement (Ferraro et al., 2014, Franchi et al., 2017). These adaptations are not only a response to functional demands but are essential in ensuring the muscle's efficiency in its specific physiological niche, such as in eccentric versus concentric contractions, where different architectural adaptations prevail (Franchi et al., 2017). Such adaptations highlight the dynamic and plastic nature of muscle architecture in response to both chronic loading and unloading (Baar et al., 2006, Franchi et al., 2014, Seynnes et al., 2008).

Muscle architecture is not a static concept but a fundamental determinant of skeletal muscle function. Modern imaging techniques, such as ultrasonography and MRI, have revolutionised our ability to study these structures in vivo, providing crucial insights into how muscles produce force and adapt to different stimuli. Owing to its relatively low cost compared to techniques such as magnetic resonance imaging, 2D ultrasound has been extensively employed in the literature to assess muscle architecture, both at rest (Aagaard et al., 2001, Alegre et al., 2006, Blazevich et al., 2003, Franchi et al., 2018a, Kawakami et al., 1995) and under dynamic conditions (Ryan et al., 2019, Van Hooren et al., 2024, Werkhausen et al., 2022), following or during exercise interventions. However, like any instrument, 2D ultrasound is not without its limitations, chief among them being the estimation of FL (Franchi et al., 2020, Franchi et al., 2018b, Van Hooren et al., 2020). To date, FL measures can be obtained with different ultrasound-based approaches: I) single ultrasound images and linear FL extrapolation, II) single ultrasound images and one of two different trigonometric equations (termed equations A and B), and III) extended field of view (EFOV) ultrasound images. In this regard, a high-quality methodological study on

BFlh aiming to compare these methods to assess FL found that the typical extrapolation methods used for estimating FL from single ultrasound images are reliable within the same session, but not accurate for estimating BFlh FL at rest with a 5-cm field of view, likely because such a narrow field cannot capture the full length of the fascicles, necessitating assumptions that introduce errors. These authors recommend implementing EFOV scans to accurately determine intervention-related FL changes in BFlh. Furthermore, the authors suggested that advancements in techniques, such as freehand three-dimensional ultrasound imaging, should be pursued in the quest for a gold standard to evaluate BFlh FL, allowing the third dimension of muscle architecture to be considered. This is particularly relevant as ultrasound-derived measurements of muscle FL are frequently employed to infer increases (due to chronic stretching or training) or decreases (due to muscle disuse or ageing) in serial sarcomere number. In this regard, *in-vivo* measurements of muscle fibre bundles (fascicles) reflect the organisation of sarcomeres, which ultimately governs the contraction characteristics of the muscle fibres, including their functional range of motion, maximal velocity, and force generation. Consequently, FL is considered indicative of the number of sarcomeres arranged in series, serving as a proxy for the maximal contractile velocity and the muscle's ability to generate force at high contraction speeds (Werkhausen et al., 2023). In this regard, a recent study demonstrated that ultrasound-based FL measurements can overestimate increases in serial sarcomere number by approximately 5% (Hinks et al., 2023).

In conclusion, muscle architecture plays a pivotal role in determining the functional capacity and adaptability of skeletal muscles, influencing not only force generation but also how muscles respond to different mechanical demands. Despite

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significant advancements in imaging techniques, such as 2D ultrasound and MRI, further refinement is necessary to fully capture the complexity of muscle architecture, particularly when considering FL and PA under dynamic conditions. As Professor Lieber and others have highlighted, shifting focus towards parameters such as FL, PCSA, and fibre rotation may provide deeper insights into muscle function. Future research should continue to explore the three-dimensional aspects of muscle structure using emerging technologies like freehand 3D ultrasound. Such developments hold promises for establishing a more comprehensive understanding of muscle behaviour, particularly in the context of training adaptations, injury rehabilitation, and performance enhancement.

# 3.3. Background (II): Sprint cycling performance & muscle architecture

Before the advent of the bicycle in the early 19th century, sprinting was primarily confined to foot-based activities, i.e. sprint running (Mero et al., 1992). The introduction of the bicycle, however, marked a significant shift in training methodologies. The late 19<sup>th</sup> century saw the development of the stationary bicycle, or cycle ergometer, which provided a controlled environment for studying the physiological aspects of both athletic performance and patient rehabilitation (Vandewalle and Driss, 2015). Pioneering research by August Krogh, a Nobel laureate in Medicine, described the bicycle ergometer and respiration apparatus for the experimental study of muscular work (Krogh, 1913). However, it was not until the mid-1970s that the Wingate Anaerobic Test was developed (Ayalon et al., 1974), becoming a standard tool for assessing anaerobic power, drawing inspiration from

the Cumming test (Vandewalle et al., 1987). This 30-second all-out sprint on a cycle ergometer measures power output in short-duration sprints and can be used as a model to provide insights into the physiological and molecular adaptations of anaerobic exercise in humans (Galvan-Alvarez et al., 2024). Despite extensive research on metabolic or biomechanical sprint performance using this test (Beneke et al., 2002, Driss and Vandewalle, 2013, Smith and Hill, 1991), the specific influence of muscle architecture on sprint cycling performance remains a subject of a limited number of investigations (n = 10) (Cesanelli et al., 2023, Coratella et al., 2020, Kordi et al., 2020, Lee et al., 2021a, Lee et al., 2018, Lee et al., 2021b, McCormack et al., 2014, Van Der Zwaard et al., 2018a, van der Zwaard et al., 2018b, Welsman et al., 1997). It is worth noting that most of these studies employed stepwise multiple regression analysis in an attempt to identify which muscle architecture variables most strongly influence sprint cycling performance. However, this method is known to have significant limitations. Stepwise regression is inherently subjective and often produces unstable results, as the final model is highly dependent on the choice of stopping criteria and the sequence in which variables are introduced (Streiner, 1994). This variability can lead to inconsistent outcomes across different analyses. Furthermore, stepwise regression tends to increase the likelihood of false positives (Type I errors), by selecting irrelevant variables, thereby diminishing the overall accuracy of the model (Streiner, 1994). Additionally, it fails to account for interactions between predictor variables, which may be crucial to the model's predictive capability. In light of these shortcomings, dominance weight analysis has emerged as a preferable alternative, offering greater precision and robustness and more effectively identifying the most critical variables even when correlations are present (Mizumoto, 2023).

Meanwhile, other methods as elastic net regression facilitate the identification of the most relevant predictors, significantly enhancing the model's explanatory power (Zou and Hastie, 2005). The integration of these sophisticated statistical methods offers a more robust and precise evaluation of how muscle architecture impacts sprint performance, surpassing previous studies in both methodological rigour and depth of analysis.

It is well-established that muscle mass, cross-sectional area (CSA), and fibre composition are the primary properties influencing maximal muscular power and the power-frequency relationship (Douglas et al., 2021). However, the question remains whether there is something beyond muscle mass itself, such as its geometric structure, that plays a role. As previously discussed, only ten studies have employed correlational or predictive models to explore associations between muscular performance and both muscle architecture (MT, PA, FL) and morphology, such as CSA and muscle volume (Cesanelli et al., 2023, Coratella et al., 2020, Kordi et al., 2020, Lee et al., 2021a, Lee et al., 2018, Lee et al., 2021b, McCormack et al., 2014, Van Der Zwaard et al., 2018a, van der Zwaard et al., 2018b, Welsman et al., 1997). In the absence of a comprehensive review on the topic, an analysis of individual studies suggests that there is no clear consensus regarding the impact of muscle architecture on sprint cycling performance. A systematic review would be necessary to adequately synthesise the findings and draw more definitive conclusions. While the optimal approach to investigate this would involve analysing muscle architecture in vivo during sprinting, such measurements are currently impractical. This would require the simultaneous use of multiple probes, in conjunction with electromyography, across numerous muscles. Future technological advancements may facilitate more comprehensive

dynamic assessments to further elucidate these interactions. To our knowledge, only one study has attempted such an approach (Riveros-Matthey et al., 2023). This study examined the effect of cadence and external power requirements on muscle neuromechanics and joint power during cycling. It found that the VL fascicle shortening velocity increased with cadence and remained consistent across different power outputs. Interestingly, self-selected cadence allowed for optimal fascicle shortening velocity for knee extensor muscle power production. This may suggest that self-selected cadence is associated with minimised muscle activation, aligning with the theory that optimal shortening velocity for maximising power increases with the intensity of exercise and recruitment of fast-twitch fibres.

In summary, while traditional metrics such as muscle mass and fibre composition are well-known determinants of sprint cycling performance, there is growing evidence that the geometric structure of muscles plays a crucial role. However, the complex interplay between muscle architecture and performance remains underexplored due to methodological constraints. As technological advancements emerge, enabling more dynamic and precise assessments of in vivo muscle architecture, future research should aim to delineate these interactions further. Understanding these nuances will be key to refining training protocols and optimizing performance in sprint cycling.

# CHAPTER IV: AIMS & HYPOTHESES

# 4. AIMS AND HYPOTHESES

Within the framework of this thesis, four distinct studies have been conducted, each guided by specific objectives and hypotheses.

The following section outlines the aims and hypotheses of each study in detail.

# Study I: Cross-sectional investigation

# Aims

To determine whether the superficial and deep unipennate regions of the TA muscle exhibit symmetry in their architecture.

To evaluate sex-related differences in the muscle architecture of the TA. To investigate the direct influence of FL on the relationship between MT and

PA in the TA.

# Hypotheses

First, we hypothesized that superficial and deep unipennate regions of the TA would be symmetrical.

Second, we hypothesized that there would be muscle architecture differences in the TA attending to sex variables.

Third, we hypothesized that FL would have a direct effect on the relationship between MT and PA.

# Study II: Cross-sectional investigation

# Aims

To determine whether the relationship between the MT on PA is mediated by FL, in a wide range of pennate and non-pennate muscles.

To assess whether these relationships are influenced by sex and exhibit regional specificity.

# Hypotheses

Due to the exploratory design of this study, formal hypotheses were not predefined.

# Study III: Review article

# Aims

To determine the extent to which muscle morphology, muscle architecture, and muscle echo intensity are associated with sprint cycling performance. To provide a comprehensive overview of the current state of knowledge and identify potential gaps and limitations in the existing research.

# Hypotheses

Systematic reviews, by their very nature, are not designed to test specific hypotheses.

# Study IV: Cross-sectional investigation

# Aims

To determine the relative relevance of lower extremities muscle architecture, Achilles and patellar tendon morphology, and leg segment variables (i.e., shank length, forefoot length, and the moment arm of the AT) for sprint cycling performance.

To find out whether there are sex differences in the predictive relevance of the variables identified.

# Hypotheses

We hypothesized that muscle mass is more determinant than muscle architecture in generating power during sprint cycling performance.

We also hypothesized that predictive models would differ due to expected sex differences.
# CHAPTER V: JUSTIFICATION

### **5. JUSTIFICATION**

The proposed research for this thesis is justified by its potential to elucidate if muscle architecture is a key determinant of sprint cycling performance in humans beyond well-known determinants such as muscle mass, CSA, and fibre type (Douglas et al., 2021). Furthermore, this thesis also pretends to advance our understanding of the intricate relationships between muscle architecture features, which have been shown to contribute to power generation. Moreover, the research addresses gaps in the literature concerning sex differences in muscle architecture, as well as the role of specific muscles, such as the BFlh, which has emerged as a suppressor of power generation. Given the complexity and often contradictory findings in previous studies, this thesis is crucial for establishing more robust, evidence-based models of performance that consider not only morphological traits but also the dynamic interplay of architectural characteristics across various lower extremity muscles. Despite some existing evidence (Cesanelli et al., 2023, Coratella et al., 2020, Kordi et al., 2020, Lee et al., 2021a, Lee et al., 2018, Lee et al., 2021b, McCormack et al., 2014, Van Der Zwaard et al., 2018a, van der Zwaard et al., 2018b, Welsman et al., 1997), well-designed studies employing advanced statistics remain limited.

Additionally, the investigation of TA muscle in this thesis introduces a novel perspective on muscle asymmetry, particularly focusing on the morphological disparities between the superficial and deep unipennate regions. The findings of this study, which challenge a widely cited study from over 20 years ago (Maganaris and Baltzopoulos, 1999), highlight the importance of understanding how such architectural asymmetries could influence muscle function and performance.

Although not directly tied to sprint cycling, these results may have broader implications for understanding muscle mechanics in various physical contexts. Furthermore, this thesis presents an innovative analysis of the relationships between key architectural variables (MT, PA, and FL) across several lower limb muscles. The research offers fresh insights into the interactions between these parameters, showing how they can differ between individuals and specific muscle regions. The application of advanced statistical approaches, such as CMA, underscores the novelty and significance of these findings, particularly in terms of performance prediction. By identifying the suppressive effect of FL on PA and highlighting regional architectural variations in muscles such as the VL, RF, and BFlh, this study lays the groundwork for future explorations into the mechanisms driving performance outcomes.

Collectively, this thesis has the potential to make a significant contribution to the understanding of muscle architecture and its role in sprint cycling performance. Its findings could also inform the development of targeted training interventions and performance optimisation strategies for elite athletes. The integration of these novel insights, especially those related to the tibialis anterior and the broader relationships between MT, PA, and FL, strongly justifies the relevance and impact of this thesis in the field of sports science and performance.

# CHAPTER VI: CONCLUSIONS

### 6. CONCLUSIONS

### Study I: Cross-sectional investigation

- Real-time ultrasonography reveals that the TA, when observed twodimensionally, presents as a non-symmetrical bipennate muscle structure.
- Increases in MT do not consistently correspond with increases in FL or FL, as FL appears to suppress PA increments.
- Small sex differences in TA architecture are observed, with most of these differences persisting even after adjusting for LLM and shank length.

### Study II: Cross-sectional investigation

- This study highlights considerable variation in human muscle architecture within and between muscles, underscoring the complex relationships between MT, PA, and FL.
- Pronounced sex-related differences are identified, independent of height, with males consistently exhibiting greater MT across all muscles and wider PAs in the VL and GMs. FL, however, does not show significant differences between the sexes.
- Regional architectural differences are observed in the VL, rectus femoris, and BFlh.
- A suppressive influence of FL on PA is revealed, particularly in lower limb pennate muscles. Interestingly, this effect varies regionally in the BFlh, while in the RF, both MT and FL increase concurrently without affecting PA.

### Study III: Review article

- Muscle morphology, architecture, and echo intensity likely play a role in sprint cycling performance, though the complexity of these relationships and their variability across studies suggest a more nuanced interplay.
- Muscle volume emerges as a key factor in determining peak power output, underscoring its crucial role in force production and cycling performance.
- Muscle architecture components, such as MT, PA, and FL, demonstrate varied associations with performance outcomes, highlighting the intricate relationship between these parameters in a practical sports context.
- Preliminary evidence indicates a possible link between echo intensity and sprint cycling performance. However, this domain is relatively underexplored (i.e., evidence from only one study) and warrants further investigation.
- The variability across studies highlights an urgent need for standardization in measurement methods and performance tests to improve comparability and facilitate robust meta-analyses.

### Study IV: Cross-sectional investigation

- Muscle mass and muscle architecture play a crucial role in determining sprint cycling performance. Muscle mass accounts for 34-36%, and muscle architecture 23-34% of the relative variance in sprint cycling performance.
- This investigation reveals that sex differences in sprint performance are predominantly attributable to LLM. Although some variations in muscle architecture and morphological characteristics of the AT, PT, and leg segment variables (i.e., shank length, forefoot length, and the moment arm of the AT)

are observed between sexes, these factors contribute minimally to the observed sex dimorphism in sprint performance compared to LLM.

• The BFlh muscle is identified as a negative predictor of maximal power generation, functioning as a suppressor. This effect enhances the predictive accuracy of models by amplifying the significance of other predictive variables.

# CHAPTER VII: REFERENCES

### 7. REFERENCES

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## CHAPTER VIII: APPENDIX

## STUDY I



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ORIGINAL ARTICLE

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### Architectural anatomy of the human tibialis anterior presents morphological asymmetries between superficial and deep unipennate regions

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### Abstract

The tibialis anterior muscle plays a critical role in human ambulation and contributes to maintaining the upright posture. However, little is known about its muscle architecture in males and females. One hundred and nine physically active males and females were recruited. Tibialis anterior muscle thickness, pennation angle, and fascicle length were measured at rest in both unipennate regions of both legs using real-time ultrasound imaging. A linear mixed model was used with muscle thickness, pennation angle, or fascicle length as the dependent variables. All models were carried out with and without total leg lean mass and shank length as covariates. Causal mediation analysis was computed to explore the effect of muscle thickness on the relationship between fascicle length and pennation angle. There were no significant differences between dominant and nondominant legs regarding muscle architecture. Muscle thickness and pennation angle were greater in the deep than the superficial unipennate region in males (1.9 mm and 1.1°, *p* < 0.001) and women (3.4 mm and 2.2°, *p* < 0.001). However, the fascicle length was similar in both regions for both sexes. The differences remained significant after accounting for differences in leg lean mass and shank length. In both regions, muscle thickness was 1-3 mm greater in males and superficial pennation angle 2° smaller in females (both, p < 0.001). After accounting for leg lean mass and shank length, sex differences remained for muscle thickness (1.6 mm, p < 0.05) and pennation angle ( $3.4^\circ$ , p < 0.001) but only in the superficial region. In both regions, leg lean mass and shank-adjusted fascicle length were 1.4 mm longer in females than males (p < 0.05). The causal mediation analysis revealed that the estimation of fascicle length was positive, suggesting that a 10% increase in muscle thickness would augment the fascicle length, allowing a 0.38° pennation angle decrease. Moreover, the pennation angle increases in total by 0.54° due to the suppressive effect of the increase in fascicle length. The estimated mediation, direct, and total effects were all significantly different from zero (p < 0.001). Overall, our results indicate that the

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architectural anatomy of the tibialis anterior shows sexual dimorphism in humans. Tibialis anterior presents morphological asymmetries between superficial and deep unipennate regions in both sexes. Lastly, our causal mediation model identified a suppressive effect of fascicle length on the pennation angle, suggesting that increments in muscle thickness are not always aligned with increments in fascicle length or the pennation angle.

#### KEYWORDS

fascicle length, muscle architecture, muscle thickness, pennation angle, tibialis anterior

#### INTRODUCTION 1

The human tibialis anterior (TA) is the largest muscle in the anterior compartment of the lower leg and accounts for over 60% of the ankle dorsiflexor muscle volume, sharing functions with the extensor hallucis longus, and the extensor digitorum longus (Keith et al., 2006). The main role of the TA is the dorsiflexion and inversion of the foot (Keith et al., 2006). The TA contributes to maintaining the upright posture (Di Giulio et al., 2009) and plays a key role in energy absorption during walking (Maharaj et al., 2019). Consequently, motor disorders affecting TA size and strength, such as cerebral palsy, negatively impact the gait cycle (Bland et al., 2011). Moreover, the age-associated decline in muscle strength of the TA has been found to increase the risk of falls (Perry et al., 2007).

Anatomically, the TA muscle originates on the lateral condyle of the tibia, on the upper two-thirds of the lateral surface of this bone, on the anterior surface of the interosseous membrane, and on the deep surface of the fascia cruris (Keith et al., 2006). On the other end, the distal attachment is typically at the medial cuneiform and first metatarsal bones (Zielinska et al., 2021). From a muscular architectural point of view, the TA is a bipennate muscle (Alexander, 1975) with a superficial and a deep region (Maganaris & Baltzopoulos, 1999), which have been considered symmetrical (Maganaris & Baltzopoulos, 1999) based on the ultrasound analysis of six men. To the best of our knowledge, the study by Maganaris and Baltzopoulos (1999) is unique and has not been replicated. Moreover, it remains unknown whether sex differences exist in the anatomical architecture of the TA in humans.

The architecture of a muscle has important functional consequences. For a given muscle volume, parallel-fibered muscles can generate larger excursions and achieve faster shortening speeds because they have more sarcomeres in series (Eng et al., 2018). In contrast, pennate muscles (e.g., TA) allow for more parallel sarcomeres, leading to large forces for a given muscle length (Powell et al., 1984). The main muscle architecture features are muscle thickness (MT), cross-sectional area, pennation angle (PA), and fascicle length (FL) (Eng et al., 2018). Pennation angles can vary within a muscle, and this influences local fiber strains and hence, gearing, within a muscle (Azizi & Deslauriers, 2014). The simple geometric model used by Azizi and Deslauriers (2014) predicts that fibers with a lower PA undergo greater fiber strains than the more pennate fibers and that this

difference will increase with the magnitude of the muscle strain. This means that under most conditions, fibers with a higher PA work at a higher gear ratio than fibers with a lower PA. However, the relationship between PA with MT, and FL is not always direct or causal. In this regard, discrepancies have been found in the literature when MT and PA are modified after resistance training but not FL (Franchi et al., 2016; Fukutani & Kurihara, 2015). The relationship between MT, PA, and FL could be studied by using causal mediation analysis (Nuzzo et al., 2019), although this technique has not been applied to the TA.

Increased knowledge of the human TA muscle architecture will provide fundamental anatomical information, which may be useful in the clinical setting and to develop specific programs for rehabilitation or to increase sports performance. Based on these previous findings, we hypothesized that superficial and deep unipennate regions of the TA would be symmetrical. Second, we hypothesized that there would be muscle architecture differences in the TA attending to sex variables. Lastly, we hypothesized that FL would have a direct effect on the relationship between MT and PA. This study aimed to test these hypotheses.

#### 2 METHODS

#### Study design and participants 2.1

This is a cross-sectional study comprising two separate measurement sessions. The first measurement session was conducted to perform pretests: anthropometrics and dual-energy X-ray absorptiometry (Lunar iDXA, General Electric) whole-body scans, as previously reported (Calbet et al., 2017). Besides, as a part of the anthropometric measurements, the shank length (i.e., the distance between the proximal head of the fibula to the tip of the lateral malleolus) of both legs was measured as previously reported (Kunimasa et al., 2014). In a second visit, the participant's TA was explored by ultrasound. One hundred nine physically active and healthy males and females volunteered to participate in the study (Table 1). Subjects exercised regularly, performing between 3 and 8h of mostly moderate-intensity physical activity per week. Several subjects had a diverse sports trajectory, with participation in different sports throughout their career, while most of them had practiced soccer during part of their career. The inclusion criteria for participation in this investigation were: age

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TABLE 1 Descriptive characteristics of the study population reported as mean and between bracket the standard deviation.

Variable	Males (n = 64)	Females ( <i>n</i> = 45)	All (n = 109)
Age (years)	23.1 (3.2)	23.0 (2.8)	23.0 (3.0)
Height (cm)	176.7 (6.8)	164.5 (5.9) <sup>†</sup>	171.7 (8.8)
Weight (kg)	74.2 (7.4)	59.4 (8.5) <sup>†</sup>	68.1 (10.7)
BMI (kg/m <sup>2</sup> )	23.8 (2.2)	21.9 (2.7) <sup>†</sup>	23.0 (2.6)
Total BM (g)	3175.3 (332.1)	2444.7 (276.4) <sup>†</sup>	2873.7 (475.3)
Total FM (g)	14346.4 (4486.6)	16481.1 (4527.3) <sup>†</sup>	15227.7 (4614.8)
Total LM (g)	56633.7 (5045.0)	40461.1 (5276.8) <sup>†</sup>	49957.0 (9487.3)
Left leg LM (g)	9977.2 (909.5)	6484.2 (927.3) <sup>†</sup>	9301.2 (1659.9)
Right leg LM (g)	10148.4 (935.5)	6508.5 (946.2) <sup>†</sup>	9443.9 (1744.5)
Both legs LM (g)	20233.4 (1948.3)	14220.7 (2079.6) <sup>†</sup>	17751.1 (3577.6)
Fat (%)	19.1 (4.7)	27.5 (4.7) <sup>†</sup>	22.6 (6.3)
Right dominant leg (%)	0.80 (0.4)	0.96 (0.2) <sup>†</sup>	0.86 (0.4)
Shank length (cm)	37.8 (2.36)	34.5 ( <b>1</b> .80) <sup>†</sup>	36.7 (2.69)
Superficial TA Thickness (cm)	1.23 (0.19)	0.94 (0.17) <sup>†</sup>	1.11 (0.23)
Deep TA thickness (cm)	1.41 (0.22)	1.28 (0.19) <sup>†</sup>	1.36 (0.21)
Total TA thickness (cm)	2.64 (0.30)	2.23 (0.25) <sup>†</sup>	2.47 (0.35)
Superficial TA FL (cm)	6.41 (1.44)	6.46 (1.79)	6.43 (1.59)
Deep TA FL (cm)	6.55 (1.61)	6.52 (1.41)	6.54 (1.53)
Superficial TA PA (°)	11.62 (2.53)	9.52 (2.45) <sup>†</sup>	10.75 (2.70)
Deep TA PA (°)	12.76 (2.65)	11.74 (3.14) <sup>‡</sup>	12.34 (2.90)

*Note*: The shank length and all architectural variables are described as averaged values of both legs. *p*-values presented correspond to comparisons between males and females ( $^{\dagger}p < 0.001$ ;  $^{\ddagger}p < 0.02$ ).

Abbreviations: BM, bone mass; FL, fascicle length; FM, fat mass; LM, lean mass; PA, pennation angle; TA, tibialis anterior.

18–35 years; no chronic diseases or recent surgery; nonsmoking; normal resting electrocardiogram; body mass index above 18 and below 30; no medical contraindications to exercise; and no history of disease requiring medical treatments lasting longer than 15 days during the preceding 6 months. All volunteers signed a written consent after receiving information about the aims and potential risks of the study. The study commenced after approval by the Ethical Committee of the University of Las Palmas de Gran Canaria (CEIH2017/13) and was carried out according to the Declaration of Helsinki. The sex and gender of the participants were defined based on self-reports during participant recruitment, and all participants reported *cis* gender.

### 2.2 | Ultrasound imaging

Real-time two-dimensional B-mode ultrasound (Philips CX50, Philips Medical Systems, Netherlands) with a 38 mm linear-array transducer (12–3 MHz, L12-3 Broadband, Phillips), was used to bilaterally measure the muscle architecture of the TA. An operator with extensive experience in musculoskeletal ultrasonography performed image acquisition. Current guidelines and recommendations for musculoskeletal ultrasound by the European Federation of Societies for Ultrasound in Medicine and Biology were followed (Fodor et al., 2022). The ultrasound depth was adjusted to 4–5 cm depending on the individual participant and the frequency was adjusted



FIGURE 1 Measurement setup. The knee and ankle joint angles were set at 90°, and the angle was verified with a manual goniometer.

between 38–41 Hz. The probe was hand-held, and the measurements were made with the subject in a prone position with the knee flexed at 90° (Maganaris & Baltzopoulos, 1999) while the ankle was kept at 90° (Figure 1). Knee and ankle angles were checked using a manual

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goniometer. The ultrasound probe was placed perpendicular to the skin, and a water-soluble gel was applied to the skin to obtain a highresolution image without losing the detailed anatomical features of the muscles (Ihnatsenka & Boezaart, 2010). Each measurement site was marked on the skin surface with a surgical pen to ensure that the probe was placed in the proper position. The use of the gel meant that the ultrasound probe could be positioned just above the skin surface at each landmark without pressure being applied to the skin. The proximal margin of the TA central aponeurosis was determined from the sagittal images of the TA and was marked on the dermal surface. The measurement site was placed 6 cm below the proximal end of the central TA aponeurosis (Muraoka et al., 2003) (Figure 2). The primary inclusion criterion for ultrasound image analyses was that the aponeuroses were parallel as the angle between the superficial and the intermediate aponeuroses can strongly influence the extrapolation methodologies (Blazevich et al., 2006; Franchi et al., 2018).

### 2.3 | Tibialis anterior architecture assessment

In each unipennate region of the TA, the PA was measured at the fascicle insertions into the superficial (or deep) and central aponeuroses. MT in each unipennate region was measured as the distance between the superficial (or deep) and central aponeuroses in both images' ends. Since TA fascicles were longer than the width of the probe, fascicle length was calculated by linear extrapolation of the visible portion of fascicles to the intersection point with the linearly projected superficial aponeurosis of the muscle (Potier et al., 2009). In total, 6 PAs corresponding to 3 FLs were analyzed in each unipennate region, i.e., 3 PA corresponding to the superficial and 3 PA corresponding to the

central aponeuroses of each unipennate region following previously described procedures to assess TA muscle architecture (Maganaris & Baltzopoulos, 1999). The inclusion criteria for determining appropriate fascicles to analyze were the following: the fascicle insertion point into the central (or deep) aponeurosis must have been visible, and a reasonable portion of the fascicle (~25% or more of the total estimated length) must have been visible within the ultrasound transducer's field of view (Franchi et al., 2020). Muscle architectonic parameters (MT, PA, and FL) were digitized using image-processing software (OsiriX<sup>™</sup> DICOM viewer, Pixmeo). Overall, 218 images and almost ~5000 measures (12 measures per leg) were recorded in all participants. Ultrasound reliability was tested in four males before the start of the study. In brief, the operator acquired one image of the TA of each male at rest in the morning, in a relaxed state and without having exercised or done any vigorous activity in the previous 72h. A person other than the operator segmented the images taken that day, without knowing to whom each image belonged, that is, the images were blinded. This same procedure was performed 3 days later. Thus, the intraclass correlation coefficient (ICC 3.1) was 0.89 for MT, 0.88 for PA, and 0.78 for FL. Our intrarater reliability is in line with the literature (Kwah et al., 2013) and it has been described according to a reference guideline for selecting and reporting for reliability research (Koo & Li, 2016).

### 2.4 | Variables for data analysis

Leg dominance (dominant vs. nondominant), muscle architectural features (MT, PA, and FL), unipennate region (superficial vs. deep), sex, LLM, and shank length were the main variables of data analysis.



**FIGURE 2** Ultrasound imaging protocol used in both unipennate regions of the TA. (a) The proximal margin of the TA central aponeurosis was determined from the sagittal images of the TA and was marked on the dermal surface  $(m_1)$ , then the measurement site was placed 6 cm below the proximal end of the central TA aponeurosis  $(m_2)$  (b) Representative image of the TA and its two unipennate regions (c) Planimetric model of the TA indicating that FL was calculated by linear extrapolation of the visible portion of fascicles to the intersection point with the linearly projected superficial aponeurosis of the muscle. FL, fascicle length; MT, muscle thickness; PA, pennation angle; TA, tibialis anterior.

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### 2.5 | Statistical analysis

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Descriptive data are presented as the mean and standard deviation (SD). Males' and females' general characteristics were compared using an unpaired t-test. A linear mixed model with MT as the dependent variable, fixed effects (sex, leg dominance, both unipennate regions, and the interaction), and random intercept (subjects) was applied to compare both unipennate regions. This linear mixed model introduced lower extremities lean mass (LLM) and shank length as covariates. The same approach was adopted for FL and PA. For all models, marginal means, and standard errors (SEM) were estimated for all factor combinations. Averaged PAs (average of 6 PAs in each unipennate region) and FLs (average of 3 FLs in each unipennate region) values were used for all models to characterize each unipennate region.

A posteriori-planned analysis consisted of exploring the effect of MT on the relationship between FL and PA. Mediation analysis for mixed models was carried out since this study has three variables (i.e., MT, FL, and PA) measured in the same participant in four different conditions, i.e., two factors within participants with two levels: leg dominance (dominant vs. nondominant) and unipennate region (superficial or deep). In the mediator model, FL (i.e., the mediator) was modeled as a linear mixed model, with MT adjusted by the dominant leg, and each unipennate region, sex, and their interaction as predictors. The outcome model, also a linear mixed model with PA as the dependent variable, included MT, the mediator (i.e., FL), and the same set of variables used in the mediator model as explanatory variables. Both the mediator and outcome models have random intercepts (subjects). Since MT is a continuous variable, it is necessary to set two MT values to estimate the effect that this change in MT has on the PA, both directly and indirectly through FL (Imai et al., 2010). The increment in MT was set at 10%. Our mediation analyses are in line with the AGReMA statement (A Guideline for Reporting Mediation Analyses) of randomized controlled trials and observational studies (Lee et al., 2021). The corresponding AGReMA checklist is provided as Data S1.

All statistical analyses were conducted with R 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria). The package "emmeans" for R was used to estimate marginal means (Lenth et al., 2018). The mediation analysis was carried out using the R package "mediation" (Tingley et al., 2014). Given the number of participants included in this study (n=109), we applied the central limit theorem and considered that the data could be analyzed with parametrical statistics. Statistical significance was set at p <0.05.

### 3 | RESULTS

### 3.1 | Study population

This study analyzed one hundred and nine participants from 20 to 26 years old. The descriptive characteristics of the study population are reported in Table 1. Females had a higher body fat percentage,

less total lean mass, and less total leg lean mass than men. Most of the participants were right-legged. Significant differences were found in muscle architecture characteristics (i.e., MT and PA) of the TA between males and females, except for the FL of the superficial and deep unipennate regions. Males had a 3.3 cm longer shank length than females (p < 0.001), while no significant differences were observed between the dominant and nondominant legs in both sexes (Table 1).

### 3.2 | Muscle architecture: Main findings and sex differences

Linear mixed model results are shown in Table 2. There were no significant differences between dominant and nondominant legs regarding muscle architecture. MT and PA were significantly greater in the deep than the superficial unipennate region in men (1.9 mm and 1.1°, p < 0.001) and women (3.4 mm and 2.2°, p < 0.001). However, the FL was similar in both regions for both sexes. The differences remained significant after accounting for differences in LLM and shank length. Compared with females, the superficial and deep regions of the TA were 1.3 and 2.8 mm thicker in men (p < 0.001), and superficial PA was 2.1° greater (p < 0.001). After accounting for LLM and shank length, sex differences remained for MT (1.6 mm, p = 0.02) and PA (3.4°, p < 0.001) in the superficial region. In both regions, LLM and shank-adjusted FL was 1.4 mm longer in females than males (p < 0.05) (Table 2).

### 3.3 | Causal mediation analysis

The estimated mediation, direct, and total effects were all significantly different from zero (p < 0.001) (Table 3). In the mediation model, the estimation of the mediator coefficient (i.e., FL) was positive, suggesting that a 10% increase in MT would increase FL eliciting, through the increase in FL, a 0.38° reduction of the PA. Likewise, this 10% increase in MT has a direct effect of 0.92° on PA, implying that the PA increases by 0.92° per each 10% increase of MT, for a given FL. Since a higher thickness augments the FL, the PA increases in total by 0.54° due to the suppressive effect of the increase of FL.

### 4 | DISCUSSION

The present investigation shows that TA muscle's superficial and deep unipennate regions are morphologically asymmetrical in males and females. In both sexes, the deep region displays higher MT (+2-4 mm) and greater PA (+1-2°) than the superficial region. These findings contrast with the symmetry reported by Maganaris and Baltzopoulos (Maganaris & Baltzopoulos, 1999) who did not find architectural differences between the superficial and deep portions of the TA in six males measured at rest and during a maximal voluntary contraction. We have also modeled how an increase in MT would affect FL and the PA.

Unipennate region	Males			Females			Between sex dif	ferences	
	MT (cm)	FL (cm)	PA (°)	MT (cm)	FL (cm)	PA (°)	MT (cm)	FL (cm)	PA (°)
Non-adjusted model									
Superficial	1.23 (0.02)	6.41 (0.16)	11.62 (0.28)	0.94 (0.02)	6.46 (0.19)	9.52 (0.34)	0.28 (0.03) <sup>†</sup>	-0.05 (0.25)	2.10 (0.44) <sup>†</sup>
Deep	1.41 (0.02)	6.55 (0.16)	12.74 (0.28)	1.28 (0.02)	6.52 (0.20)	11.72 (0.34)	0.13 (0.03) <sup>†</sup>	0.03 (0.25)	1.03 (0.44)
Difference	0.19 (0.02) <sup>†</sup>	0.14 (0.16)	$1.12~(0.26)^{\dagger}$	0.34 (0.02) <sup>†</sup>	0.06 (0.19)	$2.20(0.31)^{\dagger}$	I	I	I
Adjusted model									
Superficial	1.19 (0.03)	5.99 (0.22)	12.03 (0.40)	1.03 (0.04)	7.38 (0.33)	8.65 (0.62)	0.16 (0.05) <sup>‡</sup>	–1.39 (0.47) <sup>‡</sup>	3.39 (0.88) <sup>†</sup>
Deep	1.34 (0.03)	6.04 (0.22)	13.01 (0.40)	1.40 (0.04)	7.46 (0.33)	11.32 (0.62)	-0.06 (0.05)	–1.43 (0.47) <sup>§</sup>	1.69 (0.87)
Difference	0.15 (0.02) <sup>†</sup>	0.05 (0.17)	0.98 (0.29) <sup>†</sup>	0.37 (0.03) <sup>†</sup>	0.08 (0.23)	2.67 (0.39) <sup>†</sup>	I	I	I
Note: Values are presented LLM = 17.751 g (the overall r	as the mean (SEM). mean LLM) and with	. Bonferroni-adjustec h shank length=36.7	d pairwise comparise cm (the overall mea	ons were used ( $^{\dagger}p < i$ an shank length).	0.001; $^{\ddagger}p=0.02$ ; $^{\$}p=$	=0.01). In the adjust	ed model, the marg	inal mean was estim	ated with

Linear mixed model results

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length; MT, muscle thickness; PA, pennation ang

Abbreviations: FL, fascicle

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TABLE 3 Causal mediation analysis on the effect of muscle thickness in the relationship between fascicle length and pennation

		95% interval
Effect	Estimate	confidence
Indirect	-0.38*	(-50, -0.25)
Direct	0.92*	(0.83, 1.01)
Total	0.54*	(0.40, 0.70)

Note: Quasi-Bayesian mediation analyses with one thousand simulations.

\*p<0.001.

#### The superficial and deep regions of the tibialis 4.1 anterior muscle are not symmetrical

For more than 20 years, it has been considered that the TA was a bipennate muscle whose unipennate regions behaved like a mirror, that is, their architectural characteristics were similar at each side of the central aponeurosis. This is based on a pioneer study designed to assess the predictability of in vivo, ultrasound-based changes in human TA pennation angle from rest to maximum isometric dorsiflexion in 6 men (Maganaris & Baltzopoulos, 1999). Using a planimetric model assuming constant thickness between superficial and deep aponeuroses and straight muscle fibers, the authors proposed that if the unipennate regions occupy equal volumes, each region accounts for half the force generated by the whole TA during a dorsiflexor MVC. In agreement with Maganaris and Baltzopoulos (Maganaris & Baltzopoulos, 1999), we observed similar FL in the superficial and deep unipennate regions of the TA. In contrast to Maganaris and Baltzopoulos (1999), we found that MT and PA are greater in the deep than in the superficial region. Our results are robust due to the use of multiple measurements in both legs, in a large sample size, minimizing type II error (Freiman et al., 2019).

Available published data on MT of human TA with similar participant characteristics agree with our findings (McCreesh & Egan, 2011). Compared to Maganaris and Baltzopoulos, our male subjects had ~0.5 cm lower MT than those studied by Maganaris and Baltzopoulos (Maganaris & Baltzopoulos, 1999). This discrepancy can be accounted for by differences in weight and height, which can explain ~50% of the variance in skeletal mass in men and women (Janssen et al., 2000). In this line, it has been shown that body mass index (BMI) is associated with greater MT (Sanz-Paris et al., 2021) (Usgu et al., 2021); so the higher BMI of the subjects analyzed by Maganaris and Baltzopoulos could explain the slightly higher TA MT reported in their study. Another factor that could explain differences in MT between studies relies on the procedure used to measure TA. In the present investigation, we applied the methods described by Muraoka et al. (2003), which seemed the most appropriate given that it allowed for measuring MT in the TA with small variability.

In terms of PA, our results are similar to previously published cadaveric (Sopher et al., 2017) and living human studies (Maganaris & Baltzopoulos, 1999). Although there are published data on FL change of the human TA during walking (Chleboun et al., 2007) and isometric contractions (Raiteri et al., 2016) in healthy subjects, FL data of healthy people at rest is limited (Maganaris & Baltzopoulos, 1999).

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From a functional perspective, the FL should be considered conjointly with the tendon length because biomechanical properties vary depending on the tendon-muscle fiber length ratio  $r_{TEL}$  defined as (tendon + aponeurosis)/(fascicle length) (Morl et al., 2016; Siebert et al., 2017). For example, muscles that act as springs in bouncing gates and contribute to energy conservation have long series elastic components (SEC; aponeurosis and tendons) and short muscle fibers, resulting in high  $r_{TFI}$  (Siebert et al., 2017). This is the case of the plantaris muscle of the wallaby ( $r_{TFL}$ =18.7) (Biewener, 1998). In contrast, muscles with motor function have a comparably short SEC and long muscle fibers like the pigeon pectoralis ( $r_{TFL}$ =0.4) (Biewener, 1998). Since the SEC was not measured in the present investigation, it was not possible to determine precisely the  $r_{\tau \epsilon_1}$ . Nevertheless, we have done some estimations based on anatomical reports (Herbert et al., 2002). For example, the former study reported a TA's distal tendon of  $230 \pm 31$  mm, with FL of  $39 \pm 8$  mm for TA. However, Herbert et al. did not measure the aponeurosis. It should be noted that these FLs are shorter than ours, likely due to differences in the measurement protocol (differences in the leg's position and degree of ankle flexion). Using these data, the TA  $r_{TEL}$ should lie close to 5.9 (or higher after including the aponeurosis in the nominator). This result would correspond to a high  $r_{TEI}$ , meaning that the TA is a muscle with long SEC and short muscle fibers (i.e., spring-like behavior) (Lai et al., 2019; Siebert et al., 2017). Further studies would be needed to compute accurate in vivo  $r_{TEI}$  values for human TA, accounting for human variation and the potential effects of age, height, gender, body composition, and physical activity level.

### 4.2 | Sex differences in tibialis anterior muscle architecture

The present investigation reports for the first time an analysis of the TA architectural sex differences in young, physically active humans. Females have a lower body size than males, which partly explains the smaller muscle mass of females. However, apart from body size, the anabolic effect of testosterone on muscle fibers and the higher proportion of type I fibers in females, which have lower cross-sectional area than type II fibers, account for the sex dimorphism in muscle mass in humans (Simoneau & Bouchard, 1989). As expected, males displayed greater absolute values for MT in both regions of the TA and greater PA in the superficial region, when sex differences in body size are disregarded. However, after accounting for LLM and shank length, it became clear that females had longer fascicles than males in both regions and lower PA and MT in the superficial region.

Sex differences have been reported for other anatomical features of the lower extremity (Shultz et al., 2008). For example, there are sex differences in the Q angle, i.e., the angle between the quadriceps muscle and the patellar tendon, the distal end of the attachment site on the tibia of the TA, and the shape of the tibia. Thus, the variations

in TA's attachment site suggest differences in TA's function and muscle architecture, affecting gait movement and the frequency of lower leg disorders such as chronic exertional compartment syndrome (Kimata et al., 2022). In terms of Q angle, normative Q angle values establish 11° for men and 16° for women (Horton & Hall, 1989). An increment in Q angle over these normative values would cause external rotation of the leg, while the opposite would cause internal rotation. In this regard, a higher Q angle in males has been associated with decreased isokinetic knee strength, power output, and torque (Sac & Tasmektepligil, 2018). Since it is known that joint angular rotation affects muscle architecture (Karamanidis et al., 2005), this could cause different sex-specific muscle adaptations. However, there is controversy about this topic since the accepted, though unproven, explanation until 2005 was that women have greater Q angle than men due to a wider pelvis. However, in 2005 a group of researchers showed that this angle is similar (~2° difference) in men and women (Grelsamer et al., 2005), although contradictory results have also been found showing higher Q angle in women (Mitani, 2017). Nevertheless, caution should be taken since the quadriceps angle is highly sensitive to errors in the definition of the center of the patella and tibial tuberosity. As exposed by some authors, these centers need to be defined with an accuracy of less than 2mm if the error in the quadriceps angle is to remain below 5° (France & Nester, 2001). Our present investigation does not allow us to ascertain whether the Q angle could contribute to explaining the small sex differences in TA FL and PA observed here.

### 4.3 | Fascicle length has a suppressive effect on the pennation angle

Our causal mediation analysis highlighted a suppressive effect of FL on PA, suggesting that if the FL does not increase in length concomitantly with MT, the PA should increase around half a degree. This analysis is a method to dissect the total effect of treatment into direct and indirect effects. The indirect effect is transmitted via a mediator to the outcome. Mediation analyses are inherently causal because the mediation question sets out to explain a mechanism through which the exposure causally operates to affect the outcome. Mediation analysis is, therefore, an important statistical tool for gaining insight into the mechanisms of exposure-outcome effects (MacKinnon, 2012). We have been able to model the relationship between these architectural variables and know how they change concerning each other by analyzing interindividual's differences in a large sample of human beings.

Some authors have found that MT and PA in resistance-trained individuals are larger than in untrained individuals but with no differences regarding FL. These authors indicated that FL was not associated with muscle size, suggesting that FL would not increase with resistance training (Fukutani & Kurihara, 2015). Nevertheless, FL may increase with exercise training depending on the predominant type of muscle contraction (eccentric/concentric) (Franchi et al., 2016). The fact that certain types of exercise training may elicit an increase in MT and FL concurs with our causal mediation model.

The relationship between MT, FL, and PA is not always direct or causal and requires further explanation. As previously explained, an increase in MT with resistance training (Franchi et al., 2018) does not require a direct increase in the other architectural features (Franchi et al., 2016; Fukutani & Kurihara, 2015). An increase in PA allows an increase in the physiological cross-sectional area and, thereby, maximal force-generating capacity (Aagaard et al., 2001; Kawakami et al., 1993). However, with an increase in PA, less force from each fiber is transmitted along the line of action of the muscles (Azizi et al., 2008; Kawakami, 2005). Nevertheless, despite a less efficient transfer of force per muscle fiber, a greater PA allows for more muscle fibers to attach to the tendon as compared to a fusiform muscle (Gans & de Vree, 1987) or an increase of the amount of myofiber inside each fiber (increased fiber cross-sectional area) allowing for the production of more force.

In summary, our causal mediation model identified a suppressive effect of FL on PA, which is in line with some authors suggesting that increments in MT are not always aligned with increments in FL or PA.

#### 4.4 Strengths, limitations, and future directions

The main strengths of this study are the large number of participants analyzed, the inclusion of males and females of similar age and levels of physical activity, the use of modern ultrasound equipment, the assessment of the lean mass of the extremities to account for the effects of body size and the strict identification of anatomical landmarks and standardized procedures for image analysis. This study has also limitations, which relate to its cross-sectional design and the fact that the architectural analysis was limited to specific regions of the muscle. However, our measurement methodology followed the latest guidelines and recommendations for musculoskeletal ultrasound (Fodor et al., 2022), and followed the methodological recommendations made by preceding researchers for TA measurement (Maganaris & Baltzopoulos, 1999; Muraoka et al., 2003).

Fascicle length and PA are major constituents of muscle architecture, and they largely determine the function and shape of the muscle, but current 2D techniques limit their precise measurement. Future studies should identify FL and PA in the TA using state-ofthe-art-3D techniques such as diffusion tensor imaging (Oudeman et al., 2016; Schenk et al., 2013) to better understand the interaction of muscle with surrounding tissue and external forces (Siebert et al., 2014; Yucesoy et al., 2003). Moreover, differences in muscle architecture in the TA's superficial and deep unipennate regions may impact architectural gearing. This concept refers to the relative arrangement of muscle fibers, tendons, and aponeuroses in relation to the joint axis of rotation (Azizi & Brainerd, 2007). For example, differences in FL and PA can affect the torque-generating capability of the muscle and its ability to produce force at different joint angles. Differences in tendon length and stiffness can impact force transmission to the bones and joints, movement efficiency, and performance. The sexual dimorphism in TA architecture could explain a lower torque-generating capacity in females than in males when considering only MT. However, the latter could be compensated by

the lower PA of females. Lastly, architectural gearing is likely to vary between different regions of the TA, contributing to this muscle's anatomical and functional complexity.

#### 4.5 Conclusion

In summary, real-time ultrasonography showed that the tibialis anterior is, two-dimensionally, a nonsymmetrical bipennate muscle at rest in terms of muscle architecture. Moreover, a suppressive effect of fascicle length on pennation angle was identified, suggesting that increments in muscle thickness are not always aligned with increments in fascicle length or pennation angle. Small sex differences exist in tibialis anterior architecture, most of which remain after accounting for the leg lean mass and shank length.

### AUTHOR CONTRIBUTIONS

The contribution of the authors are as follows: SMR, JSM, and JALC contributed to the conception and design of the study and drafted the manuscript; JSM collected the ultrasound data and supervised all analysis; VGA and SCR helped with data collection; JJGH performed the statistical analysis and contributed to the interpretation of the findings; all coauthors critically evaluated and contributed to the manuscript. All authors have approved the final version of the manuscript.

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### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## STUDY II

### scientific reports

### OPEN



### The relationship between muscle thickness and pennation angle is mediated by fascicle length in the muscles of the lower extremities

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Muscle morphological architecture, a crucial determinant of muscle function, has fascinated researchers since the Renaissance. Imaging techniques enable the assessment of parameters such as muscle thickness (MT), pennation angle (PA), and fascicle length (FL), which may vary with growth, sex, and physical activity. Despite known interrelationships, robust mathematical models like causal mediation analysis have not been extensively applied to large population samples. We recruited 109 males and females, measuring knee flexor and extensor, and plantar flexor MT, PA, and FL using real-time ultrasound imaging at rest. A mixed-effects model explored sex, leg (dominant vs. non-dominant), and muscle region differences. Males exhibited greater MT in all muscles (0.1 to 2.1 cm, p < 0.01), with no sex differences in FL. Dominant legs showed greater rectus femoris (RF) MT (0.1 cm, p = 0.01) and PA (1.5°, p = 0.01), while vastus lateralis (VL) had greater FL (1.2 cm, p < 0.001) and PA (0.6°, p = 0.02). Regional differences were observed in VL, RF, and biceps femoris long head (BFIh). Causal mediation analyses highlighted MT's influence on PA, mediated by FL. Moderated mediation occurred in BFIh, with FL differences. Gastrocnemius medialis and lateralis exhibited FL-mediated MT and PA relationships. This study unveils the intricate interplay of MT, FL, and PA in muscle architecture.

Structural remodelling of contractile machinery has been a subject of significant research since the pioneering studies of Giovanni Alfonso Borelli and Niels Stensen during the seventeenth century. Their ground-breaking research on the biomechanics of muscles laid the foundation for understanding the intricate relationship between muscle morphology and function, captivating anatomists, and physiologists since the Renaissance<sup>1</sup>. Using imaging techniques<sup>2</sup> is possible to assess several muscle architecture parameters, including cross-sectional area (CSA), muscle thickness (MT), pennation angle (PA), and fascicle length (FL), which may change with growth, sex, and physical activity. Although it is known that these variables are interrelated, these relationships have yet to be assessed with robust mathematical models and tools like causal mediation analysis (CMA) in ample samples of the population.

During human development, skeletal muscles need to adapt in length as required by the longitudinal bone growth<sup>3</sup>. As a result, skeletal musculature exhibits remarkable adaptability, not only in response to body growth but also to several training stimuli. There is an intricate interplay between MT, FL, and PA which varies not only depending on growth but also on muscle-specific characteristics<sup>4–8</sup>. However, no study has comprehensively explored these relationships in a broad sample of males and females. Understanding the complex interactions among these variables, along with the variability between individuals, could establish a more precise characterization of normal human variability. This would also elucidate the interplay between muscle architecture variables in both sexes.

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To address this question, some authors have advocated the use of CMA<sup>9</sup>, a modern statistical approach for understanding the mechanisms by which an exposure or intervention could explain an outcome. This powerful approach has been used in observational research to gain insights into the underlying processes and pathways that contribute to the observed associations in observational data<sup>10</sup>. Mediation can co-occur with moderation, also called conditional indirect effects. In moderated mediation, the influence of a third variable (moderator) on the mediation effect is explored, adding complexity to the relationship analysis<sup>11</sup>. Our laboratory has recently identified through CMA that the FL of the tibialis anterior muscle seems to have a suppressive effect on the PA, suggesting that increments in MT (i.e., set in 10%) are not always aligned with increments in FL or the PA<sup>12</sup>.

In this study, we employed CMA to model the relationship between MT, PA, and FL and investigate how these parameters influence each other using conventional B-mode ultrasonography, the most used technique to assess muscle architecture<sup>2</sup>. By analysing inter and intra-individual heterogeneity in a large sample of human subjects, our research aimed to address two main objectives. Firstly, we sought to determine whether the relationship between the MT on PA is mediated by FL, in a wide range of pennate and non-pennate muscles. Secondly, we aimed to assess whether these relationships are influenced by sex and exhibit regional specificity.

### Results

### Sex differences in muscle architecture

In all muscles, males had greater MT compared to females (from 0.1 cm in GM to 2.1 cm in GL, p < 0.01) (Table 1). In the VL, sex differences in MT were moderated by the region measured within the muscle (from distal to proximal the differences were 0.3 cm, 0.4 cm, and 0.5 cm, all p < 0.001). For all muscles, there were no sex differences in FL. In terms of PA, males had wider angles than females in the VL (1.3°, p = 0.02), GM (2.1°, p = 0.01), and GL (1.2°, p < 0.001). The dominant leg had higher MT (0.1 cm, p = 0.01) and PA (1.5°, p = 0.01) than the non-dominant one in the RF. In the VL, both FL and PA were higher in the dominant than the non-dominant leg (1.2 cm, p < 0.001; 0.6°, p = 0.02, respectively). The distribution by sex and legs regarding muscle architecture is displayed in Figs. 1, 2, 3.

### **Muscle regional differences**

The FL was longer in the proximal than the distal region (2.6 cm, p < 0.001) of the BFlh. In the RF, the proximal region had greater muscle MT (0.3 cm, p < 0.001), wider PA (6.7°, p < 0.001), and shorter FL (-2.7 cm, p = 0.01) than the distal region. The VL showed regional homogeneity in FL, while in terms of PA, the distal and medial regions were homogeneous but with lower PA (-1.8°, p < 0.001) in the proximal region. In terms of MT, the VL presented regional heterogeneity exhibiting morphological differences between males and females. Muscle thickness in males was 0.28 cm (p = 0.02) greater in the middle region than the most distal region, 0.33 cm (p < 0.001) greater in the most distal region, and 0.03 cm greater in the most proximal than the middle region. In females, the corresponding values were 0.13 cm (p < 0.001), 0.08 cm, and 0.05 cm, respectively. The VL, RF, and BFlh exhibited regional differences regarding MT, FL, and PA while the VM was homogeneous across its regions for all architectural variables (Table 1).

### Causal mediation analyses: mediation, moderated mediation, and mediated moderation

Overall, across subjects, an association between the increase in MT and the increase in PA, which eventually decreased due to an associated increase in FL. All direct and indirect effects were significant (all p < 0.05). Regarding total effects, all were significant (all p < 0.01), except for total effects in the distal and proximal regions of RF. The direct, indirect, and total effects are reported in sexagesimal angle per 1 mm increment in MT across

	Mean ± SD		Sex differences		Regional differences			Leg differences				
Muscle	MT (cm)	FL (cm)	PA (°)	MT (cm)	FL (cm)	PA (°)	MT (cm)	FL (cm)	PA (°)	MT (cm)	FL (cm)	PA (°)
VL	$2.2 \pm 0.4$	$10.9 \pm 3.1$	13.1±3.6	S x R	0.02	1.3 <sup>‡</sup>	S x R	R <sup>1</sup>	R <sup>2</sup>	0.1	$1.2^{\dagger}$	0.6 <sup>‡</sup>
VM	1.9±0.4	9.7±3	$12.9 \pm 3.8$	0.3 <sup>†</sup>	0.6	1.2	0.1	1.1	- 3.2	0.1	-0.3	0.8
RF	$2.1 \pm 0.4$	$10.5 \pm 3.6$	12.5±5	$0.4^{\dagger}$	0.9	0.2	0.3†	-2.7§	6.7†	0.1%	0.5	1.5%
BFlh	$2.1 \pm 0.4$	7.6±2.1	$14.4 \pm 4.1$	0.3†	0.1	0.7	0.1	2.6†	-1.2	-0.1	-0.3	0.6
ST	$2.2 \pm 0.5$	$8.1 \pm 2.4$	12.1±3.8	0.3 <sup>‡</sup>	1.3	1.3	-	-	-	0.02	-0.8	0.6
GM	1.9±0.3	$4.8 \pm 0.8$	$26.7 \pm 4.9$	0.1 <sup>§</sup>	0.1	2.1%	-	-	-	-0.01	0.1	-0.9
GL	$1.5 \pm 0.3$	$8.1 \pm 1.9$	15.7±3.2	2.1 <sup>†</sup>	0.7	$1.2^{\dagger}$	-	-	-	0.02	-0.2	-0.4

**Table 1.** Sex, regional, and leg differences in muscle architecture. *MT* muscle thickness; *FL* fascicle length; *PA* pennation angle; *VL* vastus lateralis; *VM* vastus medialis; *RF* rectus femoris; *BFlh* biceps femoris long head; *ST* semitendinosus; *GM* gastrocnemius medialis; *GL* gastrocnemius lateralis. Sex differences were expressed as men–women. Regional differences were expressed as the proximal–distal regions in muscles with different regions (VM, RF, and BF). Leg differences were expressed as dominant leg–non-dominant leg.  $R^1$  = equal FL across regions;  $R^2$  = PA differences by region: 0.01 for the middle region–most distal region, – 2.3<sup>†</sup> for the most proximal region–most distal region, and – 1.8<sup>†</sup> for the most proximal region–middle region. S x R = interaction between sex and region in MT. A simple effect analysis of this interaction is explained in the results section. († = p < 0.001; ‡ p = 0.02; § p = 0.01).


**Figure 1.** Muscle thickness distribution by sex and leg dominance. The left and right sides of the box correspond to the first (Q1) and third (Q3) quartiles, respectively. The central line indicates the median. The left whisker delimits the smallest data point greater than or equal to Q1 - 1.5 \* (Q3-Q1). The right whisker delimits the largest data point less than or equal to Q3 + 1.5 \* (Q3-Q1). Inside the boxplot, between Q1 and Q3, the mean value is shown with a black dot. See Table 1 for statistical analyses.



**Figure 2.** Pennation angle distribution by sex and leg dominance. The left and right sides of the box correspond to the first (Q1) and third (Q3) quartiles, respectively. The central line indicates the median. The left whisker delimits the smallest data point greater than or equal to Q1 - 1.5 \* (Q3 - Q1). The right whisker delimits the largest data point less than or equal to Q3 + 1.5 \* (Q3 - Q1). Inside the boxplot, between Q1 and Q3, the mean value is shown with a black dot. See Table 1 for statistical analyses.

subjects. In muscles that were measured regionally, the direct, indirect, and total effects were conditioned effects (based on the measured region).

In all muscles, a 1 mm increase in MT across subjects was associated to a significant increase (all p < 0.001) in PA (direct effect), ranging from 0.12° in the ST to 1.32° in the GM (Table 2). Interestingly, the increase in FL was also associated with an increase in MT across subjects. This circumstance caused a significant decrease (indirect effect) in PA (all p < 0.05) in all cases, ranging from 0.02° in the VL(22%) to 0.47° in the distal region (70%) of the BFlh. Therefore, the total effect in all cases was smaller than the direct effect due to the suppressive effect exerted by the increase in FL, ranging from 0.04° in RF(56%) to 0.88° in GM. At the group-level, the percentage of suppressive effect on PA due to the increase in FL when increasing MT by 1 mm. ranges from 5.2% in VL(22%) to 92.3% in RF(56%). Additionally, in the RF, the total effect was not significant, suggesting that changes in MT translated into changes in FL while maintaining PA invariant across subjects. These results remain consistent after adjusting for height in all muscles except for the total effect in the BFlh, which was not significant after accounting for height (Table 3).

Among the muscles measured across multiple regions, the conditional indirect effects (the indirect effects of each muscle region) differ significantly only in the BFlh, as shown by the non-overlapping confidence intervals (Tables 2 and 3). This indicates that only in the BFlh was the indirect effect moderated by the muscle region (mediated moderation). Specifically, for this muscle, the 95% Bootstrap confidence interval for the difference in



**Figure 3.** Fascicle length distribution by sex and leg dominance. The left and right sides of the box correspond to the first (Q1) and third (Q3) quartiles, respectively. The central line indicates the median. The left whisker delimits the smallest data point greater than or equal to Q1 - 1.5 \* (Q3-Q1). The right whisker delimits the largest data point less than or equal to Q3 + 1.5 \* (Q3-Q1). Inside the boxplot, between Q1 and Q3, the mean value is shown with a black dot. See Table 1 for statistical analyses.

Muscle	Region	Indirect effect (95% IC)	Direct effect (95% IC)	Total effect (95% IC)
	22%	$-0.027 \ [-0.059, 0]^{\$}$	$0.514~[0.395, 0.63]^{\dagger}$	$0.487[0.364, 0.61]^{\dagger}$
VL	39%	$-0.058 \ [-0.114, -0.01]^{\circ}$	0.539 [0.424, 0.65]†	0.480 [0.358, 0.60]†
	56%	$-0.112 \ [-0.182, -0.05]^{\dagger}$	0.512 [0.399, 0.62]†	0.401 [0.286, 0.52]†
VM	22%	$-0.127 \ [-0.194, -0.07]^{\dagger}$	0.348 [0.245, 0.45]†	0.221 [0.101, -0.23]†
V IVI	39%	$-0.246 \ [-0.358, -0.15]^{\dagger}$	$0.472~[0.331, 0.62]^{\dagger}$	$0.227~[0.068, 0.38]^{\ddagger}$
DE	39%	$-0.292 \ [-0.530, -0.09]^{\ddagger}$	$0.464~[0.224, 0.71]^{\dagger}$	0.173[-0.117,0.46]
KI <sup>*</sup>	56%	$-0.420 \ [-0.6, -0.24]^{\dagger}$	$0.455~[0.287, 0.62]^{\dagger}$	0.035 [-0.194, 0.26]
PEIL	50%	$-0.466 \ [-0.679, -0.27]^{\dagger}$	$0.714~[0.571, 0.87]^{\dagger}$	0.248 [0.016, 0.49]§
briii	70%	-0.13 [-0.206, -0.06] <sup>†</sup>	0.518 [0.382, 0.65]†	0.389 [0.240, 0.53]†
ST	50%	$-0.04 \ [-0.07, -0.01]^{\ddagger}$	0.12 [0.09, 0.16] <sup>†</sup>	0.08 [0.04, 0.13]‡
GM	-	$-0.44 \ [-0.63, -0.25]^{\dagger}$	1.316 [1.17, 1.45]†	0.88 [0.65, 1.09] <sup>†</sup>
GL	-	$-0.17 \ [-0.27, -0.1]^{\dagger}$	0.43 [0.27, 0.6] <sup>†</sup>	0.27 [0.1, 0.5]‡

**Table 2.** Causal mediation analyses adjusted by leg and sex. In those muscles where the indirect effects are shown by regions, the direct and total effects are conditional effects estimated for the specific region. *VL* vastus lateralis, *VM* vastus medialis, *RF* rectus femoris, *BFlh* biceps femoris long head, *ST* semitendinosus; *GM* gastrocnemius medialis, *GL* gastrocnemius lateralis.  $\dagger = p < 0.001$ ,  $\ddagger p = 0.02$ , \$ p = 0.01.

conditional indirect effects was [-0.54, -0.05], further evidencing the moderation. In all the mediator models tested, the interaction between muscle thickness and muscle region was not significant, indicating the lack of mediated moderation. Concerning the moderation of the direct effect by the muscle region, Tables 2 and 3 show all the confidence intervals for the conditional direct effects within each multi-regionally measured "muscle" almost entirely overlap. The latter indicates that the muscle region does not moderate the direct effects. Comparing GM with GL among subjects, a 1 mm change in MT had a significantly greater impact on the PA, which increased approximately threefold in the GM (1.316°/0.43°) than the GL. It is noteworthy that the GM exhibited the highest PA among the muscles and displayed the largest direct effect (Table 2). The results obtained in the causal mediation analysis were essentially similar when sex was excluded from the models.

#### Discussion

This investigation revealed through modelling that an increase in MT is associated with a widening of the PA, but this effect is influenced by changes in FL, suggesting a suppressive effect of FL on PA in all muscles. Furthermore, this study sheds light on architectural differences across several dimensions: sex, leg dominance, specific muscles, and within individual muscles, specific regions—thereby extending prior knowledge<sup>13–16</sup>. Overall, these findings indicate that the interplay between MT, PA, and FL is specific for different muscles and within a given muscle shows a regional variation (Fig. 4), as discussed elsewhere<sup>5,7,8</sup>.

In all muscles, males had greater MT compared to females (from 0.1 cm in GM to 2.1 cm in GL), as previously reported<sup>17</sup>. The difference in MT between males and females in lower limb muscles could be primarily influenced by biological and physiological factors, including sex hormones, muscle fibre type distribution, and

Muscle	Region	Indirect effect (95% IC)	Direct effect (95% IC)	Total effect (95% IC)
	22%	$-0.030 \ [-0.062, -0.01]^{\ddagger}$	$0.512\;[0.401,0.62]^\dagger$	$0.484~[0.377, 0.60]^\dagger$
VL	39%	$-0.063 \ [-0.117, -0.02]^{\ddagger}$	$0.54~[0.44,0.65]^{\dagger}$	$0.477~[0.364, 0.59]^{\dagger}$
	56%	$-0.115 \ [-0.184, -0.05]^{\dagger}$	$0.513~[0.405, 0.62]^\dagger$	$0.398~[0.281, 0.52]^\dagger$
VM	22%	$-0.131[-0.206, -0.07]^{\dagger}$	$0.345~[0.238, 0.45]^{\dagger}$	$0.214~[0.097, 0.34]^\dagger$
V IVI	39%	$-0.248 \ [-0.358, -0.15]^{\dagger}$	0.471 [0.331, 0.62] <sup>†</sup>	0.223 [0.069, 0.38]‡
DE	39%	$-0.296 \ [-0.539, -0.10]^{\dagger}$	0.468 [0.243, 0.71]†	0.182 [-0.095, 0.45]
Kſ	56%	$-0.427 \ [-0.61, -0.25]^{\dagger}$	$0.464~[0.298, 0.63]^{\dagger}$	0.036 [-0.208, 0.27]
PEIL	50%	$-0.464 \ [-0.666, -0.26]^{\dagger}$	0.693 [0.556, 0.83]†	0.228 [-0.0027, 0.47]
DI III	70%	$-0.133 \ [-0.214, -0.06]^{\dagger}$	$0.498~[0.359, 0.63]^\dagger$	$0.367~[0.223, 0.51]^\dagger$
ST	50%	$-0.042 \ [-0.078, -0.01]^{\ddagger}$	0.123 [0.086, 0.13]†	0.08 [0.034, 0.13]‡
GM	-	$-0.431 \ [-0.62, -0.25]^{\dagger}$	1.328 [1.19, 1.46] <sup>†</sup>	0.90 [0.69, 1.12]†
GL	-	$-0.158 \ [-0.248, -0.08]^{\dagger}$	0.398 [0.232, 0.56] <sup>†</sup>	0.24 [0.066, 0.4]‡

**Table 3.** Causal mediation analyses adjusted for height, leg, and sex. In those muscles where the indirect effects are shown by regions, the direct and total effects are conditional effects estimated for the specific region. *VL* vastus lateralis, *VM* vastus medialis, *RF* rectus femoris, *BFlh* biceps femoris long head, *ST* semitendinosus; *GM* gastrocnemius medialis, *GL* gastrocnemius lateralis.  $\dagger = p < 0.001$ ,  $\ddagger p = 0.02$ , \$ p = 0.01.



**Figure 4.** Different scenarios of the mediation model. 1 = first scenario, muscle initial state; 2 = second scenario, where the pennation angle increases if fascicle length does not change after an increase in muscle thickness (the direct effect accounts for this fact); 3 = third scenario, where the pennation angle decreases due to fascicle length increment (the direct effect partly cancels the direct effect). *MT* muscle thickness; *PA* pennation angle; *FL* fascicle length.

overall body composition. In terms of sex hormones, males typically have significantly higher levels of testosterone compared to females<sup>18</sup>, which can lead to greater muscle hypertrophy and thus increased MT<sup>19</sup>. While MT and FL are related, they are not entirely dependent on each other. In agreement with studies performed using diffusion-tensor magnetic resonance imaging, we found no sex differences regarding FL in all muscles<sup>20</sup>. FL is influenced by factors such as tendon length, joint structure, limb proportions, and age<sup>20–22</sup>. Our study also highlighted sex differences in VL, GM, and GL regarding PA (i.e., males > females), which is also in line with the literature<sup>17,23,24</sup>. Males are taller than females, which has been genetically explained elsewhere<sup>25,26</sup>, so could that alone explain the differences in muscle architecture? While body height could indeed influence certain aspects of muscle architecture, it is not the sole determinant of the observed differences between males and females. Body height could affect absolute muscle size, as larger bodies generally need larger muscles to support and move them. However, our results indicate that when it comes to relative muscle architectural features, body height alone is insufficient to explain the observed differences. This is supported by the fact that after accounting for height in our analyses the results remain essentially unchanged. In agreement, numerous studies have shown that even when adjusted for body size, males typically have greater muscle mass than females, suggesting that factors beyond body size, such as hormonal differences, are contributing to these disparities<sup>27,28</sup>. For example, sex differences in muscle fibre types<sup>29</sup> could also explain sex differences in muscle architecture, although this possibility remains unexplored. Since the observed sex differences remained primarily unchanged after accounting for height, the present findings indicate that height plays a minor role in the observed muscle architectural differences between males and females in the present investigation. Moreover, the results obtained in causal mediation analysis were essentially similar after excluding sex, indicating that the relationships described are robust and similar in males and females.

In terms of leg differences due to dominance, our data show higher MT and PA in the dominant than nondominant leg in the RF. Additionally, the VL showed higher FL and PA in the dominant leg. Such differences could be attributed to the increased mechanical loading<sup>30</sup> and functional demands<sup>31</sup> of the dominant leg vs. the non-dominant leg. In terms of regional differences, the VL, RF, and BFIh exhibited differences regarding MT, FL, and PA while the VM was homogeneous across its regions for all architectural variables. Our VL and RF results concur with Blazevich, et al.<sup>13</sup>. However, in contrast with our findings, these authors found regional differences in MT and PA of the VM. This discrepancy could be attributed to several factors, such as differences in the study population, equipment used, and imaging acquisition techniques.

In the resting BFlh, non-significant differences in FL between the proximal and distal regions have been reported ( $\sim 7.4 \pm 0.5$  cm, and  $\sim 6.4 \pm 1$  cm, respectively)<sup>32</sup> using real-time ultrasound. The latter agrees with data collected from cadavers although limited to elderly males and females (> 80 years)<sup>16</sup>. However, certain cadaveric studies involving individuals aged over 65 years have revealed longer FL proximally than distally ( $\sim 7.1 \pm 0.5$  cm, and  $\sim 6.4 \pm 0.9$  cm, respectively). These regional differences could be attributed to anatomical constraints (e.g., the insertion points of tendons or the shape of the bone attachments)<sup>33</sup>, functional requirements of daily life or during exercise<sup>34</sup>, and mechanical loading<sup>35</sup>. Moreover, it has been suggested that the central nervous system may independently control different regions of the BFlh<sup>36-39</sup>. In agreement, it has been reported that this muscle is innervated by more than one motor nerve branch<sup>40</sup>, allowing a task-specific activation of different regions<sup>41</sup>. The present study is one of the few investigations that has measured the architecture of the BFlh throughout its length in a large sample of volunteers<sup>14,32,42</sup>. Detailed examination of the architectural arrangement of the fibres along muscle length will allow a better understanding of BFlh functional properties.

In our analysis using pooled data, our model predicts that increasing the MT by 1 mm while keeping the FL unchanged should result in a significant widening of the PA in all muscles This increase ranged from 0.12° in the ST to 1.32° in the GM. Nevertheless, the FL increases with muscle thickness. Consequently, our model predicts that a FL increment should be associated with a concurrent decrease in the PA. The term to describe this phenomenon within the field of mediation analyses is defined as "suppression"<sup>43</sup>. Suppression refers to a phenomenon wherein a single causal variable exhibits a relationship with an outcome variable through two distinct mediator variables, with one mediated effect being positive and the other negative. In such instances, each mediator variable suppresses or masks the effect that is transferred through the other mediator variable<sup>43</sup>. These results are in line with a previous investigation of our group which revealed this phenomenon in the tibialis anterior muscle<sup>12</sup>. However, in the RF, our modelling results indicate that concurrent increases in MT and FL should result in no significant alterations in PA. This finding can be attributed to the RF's anatomical arrangement as a fusiform muscle, characterized by its parallel arrangement of muscle fibres.

Some studies have reported that resistance-trained individuals, such as bodybuilders or rugby players, exhibit larger MT and PA compared to untrained individuals, but no significant differences in FL. These findings suggest that FL may not increase with resistance training<sup>8</sup>. The question of whether adaptations to such stimuli manifest in an increase in FL remains a subject of controversy and ongoing debate among scholars. However, it has been observed that the FL may indeed increase with exercise training, depending on the type of muscle contraction (i.e., eccentric, or concentric) involved<sup>4,7</sup>. Additionally, studies have shown that FL is larger in powerlifters<sup>44</sup> and sumo lifters<sup>45</sup>. A study on untrained males<sup>46</sup> showed that, although there was a significant increase in the average cross-sectional area of muscle fibres and PA after resistance training, changes in FL were not significant. These findings again suggest that adaptations in FL may not be a primary contributing factor to training-induced muscle hypertrophy. The observation that specific types of exercise training can lead to increases in both MT and FL aligns with our mediation model. Furthermore, recent insights from a study by Hornberger et al.<sup>47</sup> offer a mechanistic explanation for the observed sex differences in MT and PA. According to their findings, mechanical loading induces changes in fascicle length and diameter, leading to alterations in whole-muscle CSA. In males, resistance training may elicit greater adaptations in fascicle length and diameter compared to females, resulting in larger MT and PA. Specifically, longitudinal growth of fascicles contributes to increased MT, while radial growth leads to a larger PA. Therefore, the observed sex differences in MT and PA could be attributed to the differential response of muscle fascicles to mechanical stimuli between males and females, wherein males may exhibit more pronounced adaptations favouring muscle hypertrophy, which is better captured by MT.

The relationship between MT, FL, and PA is not always direct or causal, and further explanation is required. As previously mentioned, an increase in MT through resistance training does not necessarily entail a direct increase in other architectural features as reviewed by Kruse, et al.<sup>5</sup>. Increasing PA allows for an expansion of the physiological cross-sectional area, and consequently, enhances maximal force-generating capacity<sup>48,49</sup>. However, with an increased PA, the force transmitted along the line of action of the muscle by each fibre decreases<sup>50,51</sup>. Nonetheless, despite the less efficient force transfer per muscle fibre, a greater PA enables more muscle fibres to attach to the tendon compared to a parallel muscle<sup>52</sup> or an increase in the amount of myofiber within each fibre, thereby allowing for the generation of greater force. On the other hand, fibre-type composition could affect muscle architecture features. Slow-twitch (Type I) and fast-twitch (Type II) fibres exhibit distinct contractile properties and metabolic profiles. Muscles with a higher proportion of fast-twitch fibres may exhibit greater MT due to their potential for greater hypertrophy in response to resistance training<sup>53,54</sup>. Additionally, variations in FL and PA may also be influenced by muscle fibre composition. Fast-twitch fibres are typically associated with shorter FL and a greater PA, which can contribute to increased force production<sup>22,55</sup>. However, the mechanisms

underlying the relationship between muscle fibre composition and architectural characteristics warrant further investigation to elucidate their interplay fully.

Lastly, the BFlh exhibited moderated mediation, showing FL differences between its regions. In this regard, a noteworthy 21% increase in BFlh FL after three weeks of eccentric training has been observed in the distal compared to the central region<sup>34</sup>. This finding aligns with emerging evidence suggesting that muscle growth is not uniform throughout the entire muscle, as supported by recent studies<sup>4,56-58</sup>. The mechanism has been attributed to a heterogeneous distribution of fibre strain<sup>59</sup> and muscle activity<sup>60</sup> along the BFlh. The present findings are based on the overall inter-individual heterogeneity and individual departure from the mean is possible<sup>61</sup>. However, our mediation analysis is robust, suggesting that this could be true for pennate muscles but not for parallel muscles such as the RF.

The main strengths of this study are the large number of subjects (n = 109), the inclusion of males and females of similar age, and the employment of robust statistical methods. In addition, we employed 2-D ultrasound (B-mode) to delineate muscle architecture, the most common technique used for this purpose in both crosssectional and longitudinal studies. This study has also limitations, which mainly relate to its cross-sectional design, limiting the extrapolation of our results from the group to the individual. Although we used modern 2D-ultrasound equipment, it is worth mentioning that current state-of-the-art 3D techniques such as diffusion tensor imaging allow an objective measurement of the PA and FL, avoiding some of the limitations associated with current 2D technology<sup>2</sup>. For example, part of the length of the FL had to be estimated, which entails an additional error of measurement for this specific variable. However, the impact of this estimation-associated error on FL assessment should have been similar across subjects, as suggested by the fact that our main conclusion agrees with that reported using diffusion-tensor magnetic resonance imaging<sup>20</sup>. An estimation of the average error using the CMA approach in 2D compared to more direct measurement (e.g., extended field of view or 3D techniques) should be analysed in future studies to consider the curvature of the FL. Future randomized controlled designs should be carried out with designs including concentric and eccentric training groups, as well as controls to verify our results accounting for the complex interplay between MT, PA, and FL. Moreover, studies analysing the effects of muscle atrophy would also add validity to the present findings. Lastly, normalized FL (fascicle length/limb length) should be included in future analyses to control differences in FL produced by differences in limb size.

This study unveils significant intramuscular and intermuscular variations in human muscle architecture, highlighting the intricate dynamics among muscle thickness, pennation angle, and fascicle length. Notably, substantial sex-related differences were observed, which cannot be attributed to sex differences in height. Males consistently exhibited greater muscle thickness across all muscles. Regarding the pennation angle, males revealed wider angles than females in the vastus lateralis, as well as the gastrocnemius medialis and lateralis. However, there were no discernible differences in fascicle length between the sexes. Our study also revealed a suppressive effect of fascicle length on the pennation angle of lower limb pennate muscles. Notably, this suppressive effect was found to be regionally moderated in the biceps femoris long head, wherein distinct differences in fascicle length were observed among its regions. Conversely, in the rectus femoris, concurrent increases in muscle thickness and fascicle length were observed without alterations in pennation angle. This finding can be attributed to the rectus femoris' anatomical arrangement as a fusiform muscle, characterized by its parallel distribution of muscle fibres.

#### Methods

#### Study design and subjects

This cross-sectional study comprises two separate measurement sessions. The first measurement session was conducted to perform pre-tests, as previously reported<sup>12</sup>. In a second visit, the subject's knee extensors, knee flexors, and plantar flexors were explored by ultrasound. A total of 109 physically active and healthy males (n = 64, 59%) and females (n = 45, 41%) volunteered to participate in the study. The descriptive characteristics of the study population, e.g., the body heigh of the subjects, and the inclusion criteria for participation in the study have been reported elsewhere<sup>12</sup>. The volunteers were physically active, engaging in 3 to 8 h of moderate-intensity physical activity weekly. Several participants had a varied athletic background, having participated in various sports throughout their careers. However, the majority had, at some point, played soccer.

Self-selected limb dominance was determined by asking the participants which is their preferred leg to kick a ball as far as possible<sup>62</sup>. Most male (80%) and female (96%) subjects reported right-leg dominance. A written informed consent was obtained and signed by all volunteers after receiving information about the aims and potential risks of the study. The study commenced after approval by the Ethical Committee of the University of Las Palmas de Gran Canaria (CEIH2017/13) and was carried out according to the Declaration of Helsinki. The sex and gender of the subjects were defined based on self-reports during subject recruitment, and all subjects were reported as cisgender.

#### Ultrasound imaging

Real-time two-dimensional B-mode ultrasound (Philips CX50, Philips Medical Systems, Netherlands) with a 38 mm linear-array transducer (12–3 MHz, L12-3 Broadband, Phillips), was used to bilaterally measure the muscle architecture of the knee extensors (rectus femoris, vastus medialis, and lateralis; RF, VM, and VL, respectively), knee flexors (biceps femoris long head and semitendinosus; BFlh and ST, respectively), and plantar flexors (gastrocnemius medialis and lateralis; GM and GL, respectively). All scans started after 15 min of the subject's lying supine on a gurney fully relaxed to allow completion of fluid shifts during changing from the upright position. Image acquisition was performed by an operator with extensive experience in musculoskeletal ultrasonography. Current guidelines and recommendations for musculoskeletal ultrasonography by the European Federation of Societies for Ultrasound in Medicine and Biology were followed<sup>63</sup>. Depending on the subject, the ultrasound depth and frequency were adjusted to 4–5 cm and 38–41 Hz (knee extensors and plantar flexors), while 5–6 cm

and 36–38 Hz (knee flexors). The probe was hand-held, and the measurements were made with the subject in a prone position or supine position, depending on the analysed muscle, checking joint angles with a manual goniometer when was necessary. The ultrasound probe was placed perpendicular to the skin and parallel to the muscle fascicles. A water-soluble gel was applied on the skin to obtain a high-resolution image without losing the detailed anatomical features of the muscles<sup>64</sup>. Each measurement site was marked on the skin surface with a surgical pen to ensure that the probe was placed in the proper position. Using the gel meant that the ultrasound probe could be positioned just above the skin surface at each landmark without pressure being applied to the skin. The primary inclusion criterion for ultrasound image analyses was that the aponeuroses were as parallel as possible since the angle between the superficial and the intermediate aponeuroses can strongly influence the extrapolation methodologies<sup>2,13</sup>. A representative in-house image from the ultrasound data collected is presented in Fig. 5.

#### Muscle architecture assessment

In each muscle, MT was measured as the distance between the superficial and deep aponeuroses at both the beginning and the end of the image, and the average of these distances was taken as the representative value. In addition, the PA and FL were each measured three times at different points along the ultrasound image of the muscle region, and the averages of these measurements were calculated to obtain representative values. Since the muscle's fascicles were longer than the width of the probe, FL was calculated by linear extrapolation of the visible portion of fascicles to the intersection point with the linearly projected superficial aponeurosis of the muscle<sup>65</sup>. The inclusion criteria for determining appropriate fascicles to analyze were the following: the fascicle insertion point into the central aponeurosis must have been visible, and a reasonable portion of the fascicle (~ 25% or more of the total estimated length) must have been visible within the ultrasound transducer's field of view<sup>66</sup>. Muscle architectural parameters (MT, PA, and FL) were digitized using image-processing software (OsiriX<sup>™</sup> DICOM



**Figure 5.** A demonstrative ultrasound image corresponding to the gastrocnemius lateralis of a male participant is shown. The white straight line indicates a fascicle, and  $\theta$  shows the pennation angle. (A) Fascicle length was calculated by linear extrapolating the visible portion of fascicles to the intersection point with the linearly projected superficial muscle aponeurosis. (B) Original ultrasound image.

viewer, Pixmeo, Geneva, Switzerland). Overall, 2616 images and 20.928 measures (96 measures per leg) were recorded in all subjects. Ultrasound reliability was tested in four males before the start of the study. In brief, the operator acquired one image of all the muscles of each male at rest in the morning, in a relaxed state and without having exercised or done any vigorous activity in the previous 72 h. A person other than the operator segmented the images taken that day without knowing to whom each image belonged, that is, the images were blinded. This exact procedure was performed three days later. The intraclass correlation coefficient (ICC 3.1) and the confidence interval of each muscle are shown in Table 4. The latter is in line with the literature<sup>67</sup>, and it has been described according to a reference guideline for selecting and reporting for reliability research<sup>68</sup>.

#### **Knee extensors**

The subjects laid supine, their knees flexed to 45°, legs supported, and muscles relaxed. To standardize the ultrasound probe positions, the thigh length was measured from the superior border of the patella to the anterior superior iliac spine. Distal to proximal anatomical landmarks were marked upon the skin at 22, 39, and 56% of the measured length<sup>13</sup>. Ultrasound images of the RF (39 and 56%), VL (22, 39, and 56%), and VM (22 and 39%) were captured for later analysis (Fig. 6a).

Muscle	Region	Muscle thickness	Pennation angle	Fascicle length
	22%	0.998 [0.995, 0.999]	0.827 [0.573, 0.936]	0.977 [0.935, 0.992]
VL	39%	0.984 [0.954, 0.994]	0.875 [0.679, 0.954]	0.827 [0.574, 0.936]
	56%	0.929 [0.808, 0.974]	0.808 [0.533, 0.928]	0.924 [0.796, 0.973]
VM	22%	0.997 [0.991, 0.999]	0.975 [0.929, 0.991]	0.975 [0.929, 0.991]
V IVI	39%	0.99 [0.971, 0.996]	0.946 [0.853, 0.981]	0.938 [0.833, 0.978]
DE	39%	0.999 [0.998, 1]	0.944 [0.847, 0.98]	0.989 [0.732, 0.963]
Kr	56%	1 [0.999, 1]	0.952 [0.869, 0.983]	0.951 [0.865, 0.983]
PEIL	50%	0.996 [0.998, 0.999]	0.849 [0.62, 0.944]	0.936 [0.828, 0.977]
DFIII	70%	0.996 [0.988, 0.999]	0.939 [0.835, 0.978]	0.836 [0.593, 0.939]
ST	50%	0.999 [0.997, 1]	0.972 [0.922, 0.99]	0.983 [0.952, 0.994]
GM	-	0.999 [0.996, 1]	0.935 [0.826, 977]	0.962 [0.895, 987]
GL	-	0.813 [0.543, 0.93]	0.929 [0.809, 0.975]	0.782 [0.482, 0.918]

**Table 4.** Intraclass correlation coefficients with 95% confidence intervals of ultrasound measurements. *VL* vastus lateralis; *VM* vastus medialis; *RF* rectus femoris; *BFlh* biceps femoris long head; *ST* semitendinosus; *GM* gastrocnemius medialis; *GL* gastrocnemius lateralis.



**Figure 6.** Muscle's scanning sites. Muscle's scanning sites. X1 = measurement zone for the gastrocnemius medialis; X2 = measurement zone for the gastrocnemius medialis. (A) vastus lateralis, medialis and rectus femoris: (B) biceps femoris long head and semitendinosus; (C) gastrocnemius medialis and lateralis.

#### **Knee flexors**

The subjects laid prone with the hip and knee angles at 0° (full extension). To standardize the ultrasound probe positions, the common proximal BFlh and ST tendon at the ischial tuberosity and the distal myotendinous junctions were determined and marked on the skin, as reported<sup>42</sup>. Ultrasound images of BFlh and ST were taken at 50% and 70% along the line from the measured distal to proximal anatomical landmarks (Fig. 6b).

#### **Plantar flexors**

The subjects laid prone with feet overhanging the gurney's edge. To standardize the ultrasound probe position for the GM, the insertion on the medial condyle of the femur and the distal end of the muscle belly was determined and marked on the skin. Ultrasound images were obtained on the mid-longitudinal axis at two-thirds of the measured muscle belly length from the origin<sup>69</sup>. For the GL, images were acquired proximally, at 30% of the distance between the knee joint interline and the centre of the lateral malleolus, as previously reported<sup>70</sup> (Fig. 6c).

#### Statistical analysis

For each muscle, the mean and standard deviation (SD) of the overall sample is presented. A mixed-effects model was used to investigate differences among sexes, legs (dominant vs. non-dominant), and muscle regions (distal and proximal regions in BFlh, RF, VM, and three regions—distal, medial, and proximal—in the VL) in each parameter of muscle architecture. The subjects were considered random factors, while the complete model included fixed factors such as sex, leg, and region. For muscles measured in a single region (GM, GL, and ST), the same procedure was followed, excluding the region variable. In cases where a significant interaction was found, a simple effects analysis was performed using the "emmeans" package for R<sup>71</sup>.

A mediation analysis for mixed models was conducted for each muscle (Fig. 7). For muscles with different regions measured within the same muscle (VL, VM, RF, and BFlh), the fixed part of the mediator model used FL as the dependent variable and was modelled as a linear mixed model. The model included adjustments for leg, region, sex, body heigh, and the interaction between region and MT (for the study of mediated moderation and moderated mediation). The outcome model, which used PA as the dependent variable, was also a linear mixed model and included MT and the mediator (FL), adjusted for leg, region, sex, body heigh, and the interactions: region x MT, as well as region x FL (for the study of mediated moderation and moderated mediation). Both the mediator and outcome models included random intercepts (i.e., subjects). For muscles without more than one region, the same procedure was followed, but the region variable was omitted in all models. In all cases, the estimated direct, indirect, and total effects were calculated for each 1 mm increment in MT. It is worth mentioning that in muscles measured at different regions along their length, the effects (direct, indirect, and total) are conditioned effects. The mediation analysis was performed using the "mediation" package for R<sup>72</sup> (for further details, see Supplementary statistical methods). Due to the limitations of the "mediation" package in studying moderated mediation and mediated moderation, which can be induced by the region variable in muscles with different regions, bootstrap methods for mixed models were employed<sup>73</sup>. To assess whether the mediation analysis was influenced by sex, the model was run again after excluding sex.



**Figure 7.** Mediation model diagram for muscle architecture variables taken from multiple regions in different muscles. For single region measurement, remove the node representing the muscle region variable and all its associated edges. The red edges indicate the pathway through which the indirect effect is exerted, whereas the blue edge indicates the pathway for the direct effect.

Our mediation analyses adhere to the AGReMA statement (A Guideline for Reporting Mediation Analyses) for randomized controlled trials and observational studies<sup>74</sup>. The corresponding AGReMA checklist is provided as Supplementary information.

All statistical analyses were performed using R 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria). When multiple comparisons were necessary, p-values were adjusted using the Bonferroni correction. Considering the sample size of this study (n = 109), we applied the central limit theorem and deemed the data suitable for parametric statistics. Statistical significance was defined as p < 0.05.

#### Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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#### **Author contributions**

The contribution of the authors is as follows: S.M.R., J.J.G.H., J.A.L.C., and J.S.M. contributed to the conception and design of the study and drafted the manuscript; J.S.M. collected the ultrasound data and supervised all analysis; J.C.D.C. helped with data collection; J.J.G.H. performed the statistical analysis and contributed to the interpretation of the findings; all co-authors critically evaluated and contributed to the manuscript. All authors have approved the final version of the manuscript.

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#### **Competing interests**

The authors declare no competing interests.

#### Additional information

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# STUDY III

# SYSTEMATIC REVIEW

**Open Access** 

# Interplay of Muscle Architecture, Morphology, and Quality in Influencing Human Sprint Cycling Performance: A Systematic Review



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## Abstract

**Background** This systematic review aimed to discern the relationships between muscle morphology, architecture, and quality with sprint cycling performance while considering the multifaceted nature of these relationships across diverse studies.

**Methods** Employing the PRISMA guidelines, an exhaustive search was performed across four primary databases: MEDLINE/PubMed, Web of Science, CINAHL Complete, and SPORTDiscus. The Methodological Index For Non-Randomised Studies (MINORS) was used to assess the methodological quality of the included studies. Out of 3971 initially identified records, only 10 studies met the eligibility criteria.

**Results** These investigations underscored the robust relationship of quadriceps muscle volume with peak power output (R<sup>2</sup> from 0.65 to 0.82), suggesting its pivotal role in force production. In muscle architecture, the pennation angle and fascicle length showed varied associations with performance. Furthermore, muscle quality, as denoted by echo intensity, showed preliminary evidence of a potential inverse relationship with performance. The methodological quality assessment revealed varied scores, with the most consistent reporting on the aim, endpoints, and inclusion of consecutive patients. However, limitations were observed in the prospective calculation of study size and unbiased assessment of study endpoints.

**Conclusion** Our findings indicate that muscle volume is a major determinant of sprint cycling performance. Muscle architecture and quality also impact performance, although in a more intricate way. The review calls for standardised methodologies in future research for a more comprehensive understanding and comparability of results.

**PROSPERO registration number** CRD42023432824 (https://www.crd.york.ac.uk/PROSPERO/display\_record. php?RecordID=432824).

### **Key Points**

• Muscle size matters: greater muscle volume and cross-sectional area correlate strongly with peak power output, emphasising the importance of muscle size in sprint cycling.

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• Pennation angle positively correlates with peak power output, but varied associations indicate the existence of other influencing factors, highlighting the complexity of muscle architecture in sprint performance.

• Preliminary evidence suggests lower echo intensity, indicating higher muscle quality, may be linked to better sprint performance, urging further exploration of this relationship in athletes.

Keywords Muscle volume, Cross-sectional area, Pennation angle, Fascicle length, Muscle Thickness

#### Background

Sprint cycling is a high-intensity, explosive discipline, where the ability to generate maximal power is crucial for success [1]. Bicycling requires the coordinated extension and flexion of multiple joints, notably encompassing the hip, knee, and ankle [2]. By combining electromyography and force measurements during pedalling it has been shown that the knee extensors, hip extensors, ankle plantar flexors, knee flexors, and hip flexors muscles contribute 39, 27, 20, 10, and 4%, respectively, to the power generated during cycling [3]. Monoarticular thigh muscles - comprising the gluteus maximus, vastus lateralis, vastus medialis, tibialis anterior, and soleus - are predominantly responsible for generating the forces transmitted to the pedals. Of these, the vastus medialis and vastus lateralis manifest peak activity commencing from the top dead centre (0°) of the pedal cycle and continuing halfway (90°) through the propulsion phase (0-180°) [4-6]. Conversely, biarticular muscles, including the biceps femoris, semitendinosus, semimembranosus, rectus femoris, and the medial and lateral heads of the gastrocnemius, primarily exert control over the direction of the forces applied to the pedals [6-8]. Thus, the muscles of the lower extremities are submitted to specific mechanical demands during cycling and some morphological and architectural features are likely more suitable for maximising sprint performance. However, little is known about the morphological and architectural determinants of peak power output during sprint cycling [9].

Muscle morphology (e.g., muscle volume or mass) and architecture (e.g., the geometric arrangement of muscle fibres) are crucial for force production in cycling [9, 10]. Muscle volume refers to the total amount of muscle tissue within a specific anatomical region, and it is typically calculated by integrating cross-sectional area (CSA) measurements along the length of the muscle. Muscle volume and anatomical CSA are measurements of muscle size often determined by magnetic resonance or ultrasound imaging that present a strong relationship (r>0.73) with muscle strength [11, 12]. Functional magnetic resonance imaging (MRI) indicates that the quadriceps muscle is the main contributor to power generation [13], however, peak power during sprinting cycling can also be predicted by the sum of the lean mass of the lower extremities [14]. Muscle architectural features include muscle thickness (MT), pennation angle (PA), and fascicle length (FL) [15]. Muscles with longer fibres and greater pennation angles can typically produce more power [16]. This is because longer fibres have more sarcomeres in series, allowing faster contraction speeds, while a higher pennation angle reflects a higher number of muscle fibres and/or increased cross-sectional area of the individual fibres (thus more sarcomeres) in parallel with an enhanced capacity for force generation [17]. More recently, indirect indices of muscle quality, like echo intensity, have been proposed to assess the composition of the muscle which may influence its performance potential. Echo intensity is a B-mode ultrasound-derived measure related to the strength of the returned signal after sending an ultrasonic wave through tissue. Several factors may influence echo intensity values and interpretation, such as methodological factors (i.e., subcutaneous fat correction and probe tilt), previous exercise (muscle damage), hydration, water distribution between intra and extracellular compartments, age, sex, ethnicity, adiposity, muscle size, strength, and connective tissue, among others [ [18, 19]. Additionally, muscle temperature and fascicle angle have been shown to be negatively associated with echo intensity [20]. These findings underscore the importance of considering both physiological and methodological factors when interpreting echo intensity measurements. Low echo intensity has been related to superior muscle quality [21], whereas high echo intensity has been associated with muscle impairment and disease [22]. Some authors have observed an inverse relationship between echo intensity and cardiorespiratory performance in aged persons, suggesting that connective and adipose tissue accumulation may be detrimental to cardiorespiratory capacity [23]. Echo intensity is negatively associated with functional capacity in the elderly [24-27] and lower muscle power [26, 28, 29].

While some studies have explored the impact of muscle characteristics on athletic performance [30–34], a comprehensive synthesis of the literature focusing specifically on sprint cycling performance is lacking. This systematic review offers an opportunity to critically analyse the existing body of evidence, identify trends, and assess the overall strength of associations between muscle morphology, muscle architecture, and muscle quality with sprint cycling performance. This systematic review aimed to determine the extent to which muscle morphology, muscle architecture, and muscle quality are associated with sprint cycling performance. By synthesising findings from various studies, we seek to provide a comprehensive overview of the current state of knowledge and identify potential gaps and limitations in the existing research.

#### Methods

#### **Study Design**

The design of this systematic review was developed through the Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement guidelines [35]. The protocol was pre-registered on PROSPERO (CRD42023432824) before searches and data extraction. Before the registration, a detailed search was performed on PROSPERO (https://www.crd.york.ac.uk/PROS-PERO/) to identify similar reviews. The PRISMA checklist is available in Supplementary File 1.

#### Search Strategy

The primary search focused on studies reporting on the associations of muscle architecture features with sprint cycling performance. An electronic database search for the articles published online or in print up to July 2023 was performed in four databases: MEDLINE/PubMed, Web of Science, CINAHL Complete, and SPORTDiscus (via EBSCOhost). The Cochrane database was also searched for a potentially similar review. To reduce publication bias, the search was performed with no restrictions on date or language. Search strings for electronic databases are shown in Supplementary File 2.

The Participants, Interventions, Comparators, Outcomes, and Study Design (PICOS) framework [36] was used to build search criteria for electronic databases. The PICOS consists of terms related to sprint performance, muscle morphology, architecture, and diagnostic imaging techniques. The search strings used for the other databases were adapted using the Polyglot Search Translator Tool (https://sr-accelerator.com/#/polyglot) [37]. These search strings are reported in Supplementary File 1. An automatic online deduplicator tool (https://sraccelerator.com/#/deduplicator) was employed to identify and remove duplicate publications. After removing duplicates, the resulting non-duplicated references were uploaded to a reference management tool (EndNote 20, Clarivate Analytics, PA, USA) to manually search for two reasons: (1) to manually eliminate possible duplicates that the online tool did not identify because they were in another language, and (2) to identify and manage the articles of interest by title and abstract. From the initial search, the titles and abstract were reviewed to exclude any irrelevant study. The full texts of the remaining studies were then retrieved and read independently by two authors (SMR and IJB) to determine whether the studies met the inclusion criteria. Any disagreement was resolved by consensus with a third author (JJGH).

#### **Eligibility Criteria**

The main selection of studies was performed by applying the following inclusion criteria: a) articles must be strictly focused on investigating associations, i.e., using the Pearson product-moment correlation coefficient or Spearman's rank correlation coefficient, between muscle morphology, architecture, or quality and sprint cycling performance, (b) articles must contain at least one measurement of muscle architecture and one measurement of sprint cycling performance, (c) articles must evaluate muscle morphology, architecture, or quality with the following diagnostic imaging devices: MRI or ultrasound imaging. Grey literature was excluded, i.e., studies were required to be published in a peer-reviewed journal indexed in the Journal of Citations Reports or the SCImago Journal & Country Rank. Conference abstracts were also excluded due to the difficulty in obtaining full methods and complete data sets. Finally, studies were excluded if they included individuals with known pathologies and/or injuries. Due to the limited number of studies, we decided not to restrict by sex. In the case of studies measuring muscle architecture in several regions of the muscle of interest, only the assessments obtained in the mid portions, i.e., 50% of the length, were retained for data analysis unless most articles incorporate evaluations in multiple sites of the muscles of interest. A complete list of reports excluded for eligibility is displayed in Supplementary File 3.

#### **Data Extraction**

To identify and extract representative data from all the included articles, publications were analysed by one author (SMR) and cross-checked by a second (IJB). The following data were extracted and coded for authors in an Excel spreadsheet: year of publication, research design (i.e., observational study), sample size, sex, age, body mass, the Pearson Product Moment Correlation (r) or Spearman's rank correlation coefficient (rho) of muscle morphology (i.e., volume), muscle architecture features (i.e., PA, FL, MT, echo intensity, volume, CSA), and the devices used to assess sprint performance and muscle morphology, architecture, and quality. Where data extracted were not available from tables, figures, or the results section, authors were contacted a maximum of three times over 4 weeks to request the information and allow a final decision on inclusion to be made. Data extraction was completed in duplicate independently by two of the co-authors (SMR and IJB). A third co-author checked the similarity between the data extracted by these two co-authors (JJGH). Any discrepancies were reviewed and agreed upon by all assessors after discussion. This method was tested on the first five studies chosen at random before commencing data extraction. The data were extracted in the form of percentages,

ranges, correlations, and regressions, aligning with the outcomes reported by the studies analysed. Pearson's and Spearman's rho correlations were interpreted as reported elsewhere [38, 39].

#### Assessment of Methodological Quality

Two researchers (SMR and IJB) independently assessed the methodological quality of the studies using the Methodological Index For Non-Randomized Studies (MINORS) [40]. This scale consists of 12 items grouped into three sections: study design, analysis, and outcomes. Each item is scored on a scale of 0 to 2, with a higher score indicating better methodological quality. The global ideal score is 16 for non-comparative studies and 24 for comparative studies. A third evaluator (JJGH) made the final decision in cases of disagreement between the scores provided. Finally, the risk of bias was not assessed due to the lack of validated tools to assess the risk of bias in the type of studies included in this review.

#### Results

#### **Characteristics of Included Studies**

The literature search resulted in 3971 records identified through four electronic databases. Before the screening, 193 records were removed from SPORTDiscus under the criterion of non-academic journals. A total of 3778 records were screened, and 689 were excluded due to duplication. From the remaining 3089, just 40 articles accomplished the eligibility criteria. Of these, 30 were excluded for different reasons (see Supplementary File 3). Finally, 10 studies analysing the relationship between muscle morphology, architecture, or quality with sprint cycling performance were included in the qualitative synthesis of this systematic review. All studies included in this systematic review were cross-sectional observational studies published between 1997 and 2022. The flowchart is shown in Fig. 1.

#### Participants

The total sample was composed of 254 participants [222 males (85.4%) and 32 females (15.6%)]. Sample sizes ranged between 10 [41] and 58 [42]. The ages of the subjects ranged between 9 [43] and 33 years [44]. Three articles included elite athletes [45–47], while six included cyclists [44, 45, 47–50]. Only one study evaluated the maximum oxygen consumption [45].

#### **Diagnostic Imaging and Sprint Performance Devices**

All the studies employed ultrasound imaging devices except one [43]. Two studies used 3D ultrasound imaging [45, 46], while the rest employed 2D technology. Two articles included MRI devices of 0.5 and 1.5 T, respectively [43, 47].

Half of the studies (50%) used the Monark 894 E Peak Bike [44–46, 49] or the 814 E [43]. The rest employed the Lode Excalibur [41, 42], the Watttbike [48, 50], or the Schoberer ergometer [47].

# Muscle Morphology, Architecture, and Muscle Quality Variables

All articles reported the evaluation of lower extremities, while only one article also reported muscles from the upper extremities [48]. All the studies analysed the dominant leg, whereas just one study analysed the non-dominant leg [41]. None of the included studies reported the criteria to establish leg dominance. The most reported muscles were the rectus femoris and the vastus lateralis (Table 1). All the included articles analysed muscle architecture at only one site (i.e., 50% of the vastus lateralis), except for two that included several measurement sites [42, 50].

Muscle volume was only assessed in four studies, of which two employed MRI [43, 47] and the other two employed 3D ultrasonography [45, 46]. Three studies determined the CSA using 3D [45, 46] or 2D ultrasonography [47]. Four studies reported MTs [41, 42, 48, 50], six PAs [41, 42, 45–47, 49], and five FLs [45–47, 49, 50]. Only one article reported the echo intensity [44]. Regarding FL calculation, just three of the five studies indicated the procedures applied for its estimation. One study used the linear extrapolation method [47], another the extended field of view procedure [49], and the other a trigonometric equation [50].

#### **Sprint Performance Outcomes**

Sprint performance was evaluated with the Wingate test in all the studies except for one [47] (Table 1). Most studies employed the classical 30-s Wingate test, while two used 15-s [44] and 20-s [50] all-out sprints. All the studies evaluated the peak power output, but only six assessed the mean power output [41, 43, 44, 46, 48]. Peak power output measurement varied among studies, i.e., peak (highest 1-s averaged) power output, maximum anaerobic power over 5-s, or peak power during the test. Only two studies reported the performance outcomes normalised by body weight [42, 49]. No study normalised the power output to the lean mass of the lower extremities.

#### Associations between Muscle Morphology, Architecture, and Quality and Sprint Performance

The following associations shown here are only displayed if there were  $n \ge 2$  studies with common variables. Moderate (r=0.59) to very strong (0.83) significant correlations were found between the muscle volume of the vastus lateralis with peak power output in absolute values [45, 46]. The CSA of the vastus lateralis showed moderate



Fig. 1 PRISMA flow chart. The reasons for excluding reports (bottom right-hand box) are detailed below. Reason 1: Studies not investigating associations, i.e., using the Pearson product-moment correlation coefficient or Spearman's rank correlation coefficient, between muscle architecture, morphology, or quality, and cycling sprint performance; Reason 2: Articles not evaluating muscle morphology, architecture, and quality with the following diagnostic imaging devices: computed tomography, magnetic resonance imaging, or ultrasound; Reason 3: Review articles

Reference	Study design	N (M/F)	Popula- tion (age (years))	Dominant/ Non-Dom- inant leg	Muscle analysed	Muscle mor- phology	Muscle architecture outcomes	Fascicle length US calculation method	Type of sprint	Sprint performance outcomes
				measures		outcomes				
Welsman et al. [43]	CR	32 (16/16)	9-10	Yes/No	Right thigh	Volume	N.A.	N.A.	Wingate 30 s	1-s peak power, 30 s mean power
McCormack et al. [41]	CR	10 (0/10)	19.5±1	Yes/Yes	٨L	N.A.	MT, PA	N.A.	Wingate 30 s	Peak power during the Wing- ate, 30 s mean power
Lee et al. [48]	CR	12 (12/0)	21±0.9	Yes/No	RF, VL, rectus abdomi- nis, erector spinae	N.A.	MT	N.A.	Wingate 30 s	5-s peak power, 30 s mean power
van der Zwaard et al. [46]	CR	18 (12/6)	27±2	Yes/No	٨L	Volume	pCSA, FL, PA	Not reported	Wingate 30 s	1-s peak power, 30 s mean power
van der Zwaard et al. [45]	CR	28 (28/0)	25±7	Yes/No	٨L	Volume	pCSA, FL, PA	Not reported	Wingate 30 s	Peak power during the Wingate at 100 Hz
Kordi et al. [47]	CR	35 (35/0)	22±4	Yes/No	Quadriceps, ham- strings, VL	Volume	FL, PA	Linear extrapolation	Sprint cycling test	Peak power during the sprint cycling test
Coratella et al. [49]	CR	21 (21/0)	24±4	Yes/No	VL, GM	N.A.	ACSA, FL, PA	Extended field of view	Wingate 30 s	Peak power during the Wingate
Lee et al. [50]	CR	24 (24/0)	20.7±1	Yes/No	RF, VL, VI, VM, GM, GL	N.A.	MT, FL, PA	Trigonometric equation	Wingate 20 s	Mean power output
Lee et al. [42]	CR	58 (58/0)	20.1±1.4	Yes/No	RF, VL, VM, TA, GM, GL	N.A.	MT, PA	N.A.	Wingate 30 s	Peak power during the Wingate
Cesanelli et al. [44]	CR	16 (16/0)	32.8±8.2	Yes/No	RF, VL, VM	N.A.	N.A.	N.A.	Wingate 15 s	Peak power during the Wing- ate, 15 s mean power
Abbreviations: CR=cross anterior; N.A. = not appli mean±standard deviatio	s-over trial; M cable; MT=m in, or as age ra	l=male; F=fe uscle thickne: inge, if not otl	emale; VL=va ss; FL=fascicl herwise repo.	istus lateralis; V e length; PA = pe rted in the studi	M = vastus medialis; VI = va ennation angle; pCSA = phy ies	astus intermedi siological cross	ius; RF=rectus f∉ -sectional area; A	:moris; GM=gastrocnemi CSA=anatomical cross-se	us medialis; GL= ctional area. Age	gastrocnemius lateralis; TA=tibialis is presented as overall or groupwise

Table 1 Study characteristics

significant associations (r=0.41 and 0.45) with peak power output in absolute values [45, 46].

Heterogeneous results regarding muscle architecture were found among the analysed studies. In this regard, a weak (r=0.26) to very strong (r=0.81) positive significant correlation was found between PA of the vastus lateralis with absolute peak power output [45, 47]. In contrast, two studies found non-significant associations (r=-0.02 to -0.015) between the PA of the vastus lateralis with absolute peak power output [42, 45]. Very weak negative non-significant (r=-0.15) [47] to a positive weak (r=0.27) [42] and moderate (r=0.60) [45] significant associations were reported between the FL of the vastus lateralis and absolute peak power output.

A weak (r=0.32) to very strong significant correlation (Spearman's rho=0.87) was found between the MT of the rectus femoris and peak power output [42, 48]. Similar results were reported for MT of the vastus lateralis and peak power output (r=0.162 to Spearman's rho=0.90) [42, 48]. Moderate (r=0.58 and 0.59) to very strong (Spearman's rho=0.91) significant associations were observed for MT of the rectus femoris and mean power output [42, 48, 50]. Similarly, moderate (r=0.37and 0.48) to very strong (Spearman's rho=0.90) significant associations were shown for MT of the vastus lateralis and mean power output [42, 48, 50].

Lastly, one study reported associations between echo intensity and sprint performance variables [44]. This article found moderate negative significant associations between echo intensity and peak power output ranging from r = -0.54 to r = -0.62 depending on the evaluated muscle, i.e., vastus lateralis, rectus femoris, and vastus medialis.

Given that a subset of studies included regression analysis investigating the relationship between muscle architecture and cycling performance, a post hoc examination of these findings was conducted despite not being stipulated originally within the eligibility criteria. Only regressions featuring a minimum of two regressors were addressed based on the recognition that previous results have primarily depicted raw associations between two variables, whereas the inclusion of regressions allows for the presentation of adjusted associations.

In terms of the relationship between muscle volume and power output, one of the studies showed a significant association, indicating that vastus lateralis muscle volume largely accounted for peak power output ( $R^2$ =0.82) in 12 males and 6 male and female Olympic rowers analysed conjointly [46]. The same authors reported a regression model to predict lean body mass (LBM)-normalised sprint performance, in which LBM was allometrically corrected by raising its value to the 2/3 exponent, i.e., sprint performance was expressed as W/kg LBM<sup>2/3</sup> [45]. In the latter study, fast fibre-type percentage alongside vastus lateralis muscle volume explained 65% of the variance in normalised sprint performance [45]. Building on this, it has been shown that both quadriceps volume (76%) and vastus lateralis PA (11%) collectively explained 87% of the variance in peak power output in cyclists [47]. In agreement with the previous findings, it has been reported that the anatomical cross-sectional area of the vastus lateralis and gastrocnemius medialis accounted for 85% of peak power (normalised per body mass) variance in 21 amateur cyclists with large differences in muscle cross-sectional areas [49].

Using stepwise multiple regression, a close linear relationship between vastus lateralis, erector spinae, and rectus femoris MT and 5-s peak power output (absolute values) has been reported ( $R^2$ =0.993, adjusted  $R^2$ =0.989) in 12 cyclists with large differences in power output and muscle thickness [48]. Additional research using multiple regression analyses has reported that the MT of vastus lateralis and gastrocnemius medialis emerged as significant predictors of peak power in absolute values [42]. In the same study, rectus femoris MT (50% region) and gastrocnemius medialis MT were significant predictors of mean power in absolute values. Nevertheless, the authors did not provide information regarding the coefficients of determination or the percentage of variance explained by each muscle in their multiple regression analyses.

Furthermore, regarding FL, only one study identified the FL of the rectus femoris as a significant predictor of 20-s mean cycling power (absolute values) in varsity cyclists [50].

#### Methodological Quality

The quality of the studies was scored based on the MINORS scale designed for non-randomised studies (Fig. 2). Only three studies were classified as non-comparative studies, meaning it was not possible in these articles to evaluate the last four items. The mean score of the comparative studies (n=7) was 16/24 (range: 14 to 18). The mean score of the non-comparative studies (n=3) was 11.3/16 (range: 11 to 12). All studies scored 2 (i.e., reported and adequate) on items 1, 2, 4, 6, and 7. In addition, items 3 and 5 relating to the prospective data collection and unbiased assessment of the study endpoint scored 0 (i.e., not reported). Lastly, item 8 and the last four items related to additional criteria in the case of comparative studies were mostly scored with 1 (i.e., reported but inadequate).

#### Discussion

This systematic review revealed that muscle morphology, architecture, and quality probably contribute to sprint cycling performance, but the nature of these relationships appears to be multifaceted and somewhat varied across studies. More specifically, our findings underscored the



Assessment of methodological quality (MINORS)

Fig. 2 Methodological Index For Non-Randomized Studies (MINORS) plot. Each item is scored on a scale of 0 (red = not reported), 1 (yellow = reported but inadequate), and 2 (green = reported and adequate), with a higher score indicating better methodological quality. Black dot = not applicable

importance of muscle volume, as indicated by strong correlations with peak power output, emphasising the role of muscle size in force production and, therefore, cycling performance. The muscle architecture elements, including muscle thickness, pennation angle, and fascicle length, showed mixed associations with performance outcomes, reflecting the complex interplay of muscle architecture parameters in an applied sports setting. Finally, preliminary evidence on muscle quality, as assessed by echo intensity, suggested a potential negative association with performance, meaning that a lower echo intensity is associated with better performance. However, this domain is relatively underexplored (i.e., evidence from only one study) and warrants further investigation. The diversity of methodologies across the included studies, from diagnostic imaging techniques to sprint performance assessment protocols, added complexity to the interpretation of the findings.

#### **Muscle Morphology**

The critical role of muscle morphology, particularly muscle volume and CSA, in physical performance has been underscored in a plethora of studies. The findings from this review are consistent with this established knowledge, showing a substantial correlation between these morphological parameters of the vastus lateralis and peak power output in sprint cycling. However, it should be noted that these associations are limited to a small number of articles that evaluated these associations [43, 45–47].

The correlation between muscle volume and peak power output aligns well with prior research. For instance, Fukunaga et al. [51] have highlighted the relationship between muscle volume and strength, demonstrating that larger muscles housing a higher number of muscle fibres have a greater capacity for generating force. This principle is particularly relevant in sprint cycling, which demands high-intensity, explosive force generation for success [1]. Additionally, previous research has shown that the larger the muscle volume, the higher the proportion of fast-twitch muscle fibres, which are responsible for explosive movements, as required in sprint cycling [52]. In addition, it has been observed that resistance training could lead to hypertrophy of these fast-twitch fibres [53], thereby increasing muscle volume and potentially enhancing peak power output in activities like sprint cycling. However, it is crucial to note that muscle volume and CSA do not exist in isolation and are influenced by numerous factors, such as training status, age, sex, and nutrition [51, 54]. For instance, muscle hypertrophy resulting from resistance training can increase muscle volume and CSA, contributing to enhanced performance in athletes [55]. It is essential to distinguish between physiological (pCSA) and anatomical (ACSA) cross-sectional areas since an increase in PA is associated with a larger increase in pCSA than ACSA [56]. While the pCSA was determined in two studies [45, 46], only one reported the ACSA [49], and most associations reported in the literature between muscle strength and CSA have used ACSA. Finally, some studies included multiple regression analyses showing a predominant role of quadriceps muscle volume, especially the vastus lateralis, as indicated by the high  $R^2$  of the relationship between quadriceps muscle volume (or vastus lateralis muscle volume) and peak power output ( $\mathbb{R}^2$  from 0.65 to (0.82) [45–47]. However, it is essential to interpret these findings cautiously, considering variations in sample characteristics and measurement methods across studies.

Additionally, other authors found that the anatomical cross-sectional areas of the vastus lateralis and gastrocnemius medialis accounted for 85% of peak power (normalised per body mass) variance [49]. However, the low number of regressors included in these articles could lead to a selection bias, as mentioned in the literature [57, 58].

While the findings of the studies included in this review are insightful, it is crucial to consider the methodological differences. For instance, the techniques used to measure muscle volume and CSA, such as 2D or 3D ultrasonography and MRI, can lead to substantial variation in measurement errors [59]. Unfortunately, these variations prevent a meaningful meta-analysis of the published studies. This underscores the urgent need for standardisation of measurement techniques in future research, a crucial step towards enhancing the reliability and comparability of findings in this field.

#### **Muscle Architecture and Quality**

Key architectural features such as the PA and FL directly impact the force and velocity of muscle contractions, respectively, and could, therefore, potentially influence sprint cycling performance [16, 17]. This review found a weak to very strong positive correlation mainly between the PA of vastus lateralis and absolute peak power output. However, some studies reported very weak associations, indicating the presence of other influencing factors or variability in measurement techniques [17].

The association between PA and force production has been substantiated in the literature [56, 60]. Greater PAs are usually associated with a larger pCSA, potentially enhancing force production [16, 56]. A study by Aagaard et al. [56] demonstrated that resistance training can increase PA, potentially leading to greater force production. However, the relationship between PA and cycling performance may be influenced by other factors, such as the cyclist's technique, fibre type distribution, and the type and intensity of training, which should be investigated in future research [61, 62]. The PA changes dramatically during muscle contraction and has been thought to influence muscle force generation [63]. However, Lieber challenges this long-held belief, arguing that PA is more of a packaging strategy, allowing short fibres to be packed into a confined volume. He suggests that, despite measurable changes in PA during muscle contraction, it may not have any significant functional impact. Therefore, the author recommends revising current biomechanical models to cease incorporating PA as a functionally significant factor. This recommendation is based on experimental studies showing considerable muscle fibre rotation during contraction, as well as studies showing a complex muscle-connective tissue composite structure [64]. However, this perspective might not apply to all muscles and simplifies the complex interplay within muscle mechanics.

Additionally, the results from this review indicate a strong relationship between MT of the vastus lateralis and rectus femoris with both peak power output and mean power output in sprint cycling. This is expected since MT is just a unidimensional indirect assessment of the CSA. In agreement, a close linear relationship between ultrasound-assessed MT and MRI-derived ACSA and volume before and after 12 weeks of resistance training (r=0.82, P<0.001 and r=0.73, P<0.001, respectively) has been reported [65]. These data support the use of MT as a surrogate of muscle ACSA. The robust association between MT and power output supports the use of resistance training to elicit muscle hypertrophy and enhance sprint cycling performance, as previously reported [66].

On the other hand, the present review shows varying results concerning the relationship between the FL of the vastus lateralis and absolute peak power output, ranging from very weak negative to moderate positive associations. This discrepancy may stem from the complex relationship between FL and cycling performance. Longer muscle fibres have more sarcomeres in series, which can produce faster contraction and benefit sprint cycling performance [15, 17]. However, the influence of FL on cycling performance may be confounded by several factors, including the cyclist's specific pedalling technique and the distribution of muscle fibre types, which have been shown to influence cycling performance significantly [8].

Lastly, in this review, only one study assessed echo intensity in relation to sprint cycling performance [44]. This study found moderate negative associations between echo intensity and sprint performance variables, indicating that lower echo intensity (suggestive of higher muscle quality) might be associated with increased sprint performance. Previous research in older adults has shown an inverse relationship between echo intensity and physical performance, suggesting that higher levels of intramuscular fat and connective tissue (both of which increase echo intensity) might be detrimental to muscle function [23]. Similarly, in a study of young, healthy individuals, echo intensity was negatively associated with muscle strength and positively correlated with body fat percentage, indicating that lower muscle quality may be associated with reduced strength and higher adiposity [28]. Together, these findings point to a potentially relevant role of echo intensity in sprint cycling performance. However, given the limited research in this area, particularly in athletic populations, more studies are needed to explore this relationship further and determine the utility of echo intensity as a marker of muscle quality in athletes.

On the other hand, some of the included studies conducted multiple regression analyses, indicating that both MT and FL can predict peak and mean power output in sprint cycling, both in absolute and relative values, depending on the study [42, 48, 50]. In general, high regression coefficients were reported in studies with high heterogeneity in body sizes and performance levels in the analysed groups, mainly when power output was included in the regression models in absolute values. However, as with the muscle morphology variables, the low number of regressors used in these articles should be noted, which could lead to selection bias [57, 58]. Therefore, these findings should be interpreted cautiously, considering variations in sample characteristics and measurement methods across studies. Our eligibility criteria aimed to ensure consistency in data analysis methods, particularly regarding muscle architecture assessments.

These divergent findings underscore the need for further investigation using precise and standardised methodologies to assess FL. For instance, the linear extrapolation method, extended field of view, and the trigonometric equation have been used to calculate FL in the included studies, potentially influencing the variability in reported associations [67].

#### **Methodological Quality**

The methodological quality of the included studies, as indicated by the MINORS scale, generally varied. All studies adequately reported the aim, endpoints, and inclusion of consecutive volunteers, indicating the rigorous design of these studies. However, prospective calculation of the study size and an unbiased assessment of the study endpoints were areas of limitation. The variation in imaging and sprint performance testing methods across studies adds complexity to comparing and interpreting the findings. Using standardised, reliable, and valid methods in future research would enhance the comparability of results across studies and improve the overall quality of the evidence [68].

#### **Limitations and Future Research**

The present systematic review has uncovered notable heterogeneity across studies regarding several key methodological aspects. Differences in diagnostic imaging tools (MRI vs. ultrasound) [59], the lack of normalisation of power output to lean mass, muscle mass, or body weight [69, 70], the method employed for the estimation of fascicle length [67], or the utilisation of different performance tests impact the calculation of mean power output substantially (e.g., Wingate test or others), explain significant variation in the field. Given the scarcity of studies employing high-quality fascicle length measurements, their impact on sprint cycling performance remains elusive. Additionally, while it is well-documented that certain ergometers, such as the Lode Excalibur (Groningen, The Netherlands) and SRM (Jülich, Germany), are associated with low measurement errors, the variability in equipment across studies may introduce inconsistency in power and performance measurements. Since other ergometers exhibit higher measurement errors, this variability should be acknowledged as a limitation of interpreting and generalising our findings. This methodological divergence limits the ability to synthesise data effectively and perform meta-analyses. The heterogeneity underscores the necessity for adopting more standardised methodologies in future research to ensure comparability and validity of results. Future studies should consider all these limitations to enhance experimental designs and effectively address research questions.

#### **Practical Applications**

The identified relationships between muscle morphology, architecture, and quality with sprint cycling performance offer valuable insights for athletes, coaches, and sports scientists. Understanding the importance of muscle volume, particularly its robust association with absolute peak power output, highlights the significance of muscle size in force production and, consequently, cycling performance. Coaches and athletes may benefit from this knowledge by incorporating targeted resistance training programs to increase muscle volume, especially in muscles like the vastus lateralis, to enhance sprint cycling performance. Additionally, insights into muscle architecture parameters such as muscle thickness, pennation angle, and fascicle length could inform the development of more specific training interventions tailored to individual athlete characteristics. For instance, athletes with lower muscle thickness or suboptimal pennation angles may benefit from targeted strength training exercises to improve these architectural features, and some studies indicate that eccentric strength training and stretching may increase fascicle length in specific muscles [71, 72]. However, it is essential to note that further research is needed to validate these practical applications and establish specific guidelines for implementation in athletic settings.

#### Conclusions

This systematic review identified associations between muscle morphology, architecture, and sprint cycling performance, as evidenced by studies utilising varying methodologies. Key findings underscored: (1) the significant role of muscle volume and cross-sectional area in peak power output, while the influence of muscle architectural features appeared more complex; (2) while preliminary evidence suggests a potential association between echo intensity and sprint cycling performance, caution is warranted until this finding is replicated in the literature. Further investigation is needed to elucidate the role of echo intensity as a performance determinant in sprint cycling; (3) the heterogeneity among studies underlines a pressing need for standardisation in measurement techniques and performance tests to enhance comparability and enable robust meta-analyses, thereby providing clearer direction for future investigations and practical applications; (4) only 20% of the included studies normalised sprint performance outcomes by body weight, and none by the muscle mass of the lower extremities.

Given the reliance on simple analyses such as Pearson-product and Spearman correlations, the intricate nature of these relationships may not be fully captured. Advanced statistical techniques like mediation analysis may offer a more comprehensive understanding of the complex interplay between muscle characteristics and sprint cycling performance. In the end, intervention studies modifying the muscle mass and the rest of the architectural variables would be required to establish a link between changes in muscle phenotype and sprint performance. However, given the complexity of the variables that may influence sprint performance, including muscle phenotype [73] and neuromuscular factors [74], future research should adopt a more integrative approach.

#### Abbreviations

ACSA	Anatomical cross-sectional area
CSA	Cross-sectional area
FL	Fascicle length
LBM	Lean body mass
MINORS	Methodological Index For Non-Randomized Studies
MRI	Magnetic resonance imaging
MT	Muscle thickness
PA	Pennation angle
pCSA	Physiological cross-sectional area
PRISMA	Reporting Items for Systematic Reviews and Meta-analysis

#### Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40798-024-00752-2.

Supplementary Material 1: PRISMA checklist

Supplementary Material 2: Search strings for electronic databases

Supplementary Material 3: Reports excluded for eligibility

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Not applicable.

#### **Author Contributions**

SMR registered the review protocol, wrote the manuscript, took part in study selection and study quality rating, and created tables and figures; IKB performed literature screening and study selection; IKB and JJGH were involved in conceptualisation, performed, and revised the study quality rating; JALC and JSM were involved in conceptualisation, supervision, and revision of the manuscript. All authors have read and approved the final version of the manuscript and agree with the order of presentation of the authors.

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#### Data Availability

The data that support the findings of this study (data collection forms; data extracted from included studies; data used for all analyses; and any other materials) are available from the corresponding author upon reasonable request.

#### Declarations

**Ethics Approval and Consent to Participate** Not applicable.

#### **Consent for Publication**

Not applicable.

#### **Competing Interests**

The authors declare that they have no competing interests.

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# STUDY IV



## The contribution of muscle mass, muscle architecture, and tendon morphology to sprint cycling performance in males and females

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Keywords:	muscle mass, muscle thickness, pennation angle, fascicle length, Wingate test
Section Specialties:	

# SCHOLARONE<sup>™</sup> Manuscripts

2		
3 4	1	The contribution of muscle mass, muscle architecture, and tendon
5 6	2	morphology to sprint cycling performance in males and females
7 8	3	Martin-Rodriguez, S <sup>1,2</sup> , Gonzalez-Henriquez, JJ <sup>2,3</sup> , Arteaga-Ortiz, R <sup>4</sup> , Galvan-Alvarez, V <sup>1,2,5</sup> ,
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# 29 Abstract

30	Previous research has highlighted the association between muscle architecture (MA)
31	and sprint cycling performance (SP), though most studies were limited by small sample
32	sizes and focused on male participants. This study models the influence of lower limb
33	lean mass, MA, tendon morphology, and leg segment variables (LSG) on SP, examining
34	their relative importance. A cross-sectional study was conducted with 101 physically
35	active participants (60 males and 41 females). Leg lean mass (LLM), MA, Achilles
36	(AT) and patellar tendon morphology, and LSG (i.e., shank and forefoot length and the
37	moment arm length of the AT) were assessed using ultrasound imaging. SP was
38	measured via isokinetic Wingate tests. Predictors were identified using Elastic Net
39	Regression, followed by Best Subset Selection, and the contribution of each predictor
40	was assessed by General Dominance analysis. The LLM emerged as the strongest
41	predictor of mean and peak power output in both sexes. However, MA variables
42	explained 23-34% of the variance in SP. Forefoot length and AT moment arm
43	accounted for 15% of the variance in mean Wingate test power output. The biceps
44	femoris long head (BFlh) fascicle length and pennation angle explained 1-3% of the
45	variance in peak power output, acting as suppressors, i.e., negatively impacting peak
46	power. While LLM is the dominant predictor of SP in both sexes, MA accounts for
47	nearly as much as muscle mass, challenging the traditional view that attributed a minor
48	role to MA. This study, for the first time, identifies a small suppressive influence of the
49	BFlh on SP.

50 Keywords: muscle mass, muscle thickness, pennation angle, fascicle length, Wingate
51 test

# **1. Introduction**

53	The relationship between lower extremities' muscle characteristics and peak power
54	output during maximal sprinting on the cycle ergometer has received considerable
55	attention. It has been reported that thigh muscle volume <sup>1-4</sup> , lower extremities lean mass
56	<sup>5,6</sup> , lower extremities volume <sup>7</sup> , and the percentage of myosin heavy chain type II (MHC
57	II), together with several muscle phenotypic features $^8$ , could explain >60-70% of the
58	variance in sprint performance. However, despite these associations, a significant
59	proportion (~ 20-30%) of the variability in sprint performance remains unexplained $^{9,10}$ .
60	About ten studies have been published addressing the potential predictive value
61	of muscle architectural features for sprint cycling performance, mostly carried out in
62	small samples of males <sup>10</sup> . These studies have concluded that the quadriceps muscle
63	volume explains 65 to 82% of the variance of peak power output, while muscle
64	architecture associates with peak and mean power output more intricately <sup>10</sup> , reflecting
65	the complex interplay of muscle architecture features <sup>11,12</sup> . Moreover, only one study
66	evaluated the main variables of muscle architecture <sup>13</sup> , i.e., muscle thickness (MT),
67	pennation angle (PA), and fascicle length (FL), while the others included only one or
68	two of these variables <sup>10</sup> . Lastly, only three of the five articles that assessed FL
69	mentioned how it was calculated, limiting the interpretation of the associations reported
70	14

In general, the role that tendons may play as determinants of cycling sprint performance has been overlooked <sup>15</sup>. During sprint cycling, the calculated mechanical load on the knee and ankle tendons is substantial <sup>16</sup>, which may explain the incidence of patellar and Achilles tendinitis in cyclists <sup>17,18</sup>. Moreover, *in vivo* assessments have identified sex differences in patellar (PT) and Achilles tendon (AT) mechanical properties in humans <sup>19,20</sup>. However, whether resting morphological characteristics of

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77 the AT and PT are associated with sprint cycling performance in males and females remains unknown<sup>21-23</sup>. Additionally, leg segment variables, including shank and 78 79 forefoot length, and the moment arm length of the AT (MA<sub>AT</sub>), may significantly 80 influence sprint cycling performance. While specific studies on the shank and forefoot 81 length are lacking, limb length has been shown to provide a mechanical advantage in generating power during cycling <sup>24,25</sup>. Theoretically, a longer AT moment arm may 82 allow higher power generation with the same force <sup>26,27</sup>. Nevertheless, the relative 83 84 impact of human variability in AT moment arm length on cycling sprint performance 85 has not been empirically assessed.

Therefore, the main objective of this investigation was to determine the relative relevance of lower extremities muscle architecture, AT and PT morphology, and leg segment variables for sprint cycling performance. A secondary objective was to find out whether there are sex differences in the predictive relevance of the variables identified.

# 90 2. Methods

# 91 2.1. Study design: participants and pre-testing

92 This is a cross-sectional study comprising two separate measurement blocks. The first 93 block was conducted to perform pre-tests and familiarisation, while the second block 94 was dedicated to ultrasonography imaging acquisition and evaluating sprint cycling 95 performance. During pre-testing and familiarisation phases, subjects reported to the 96 laboratory early in the morning, following a 12-h overnight fast for assessment of their 97 body composition using dual-energy X-ray absorptiometry (Lunar iDXA, GE 98 Healthcare, Milwaukee, WI, USA), as previously reported <sup>28</sup>. The anatomical regions of 99 interest were manually delineated from the whole-body DXA scans <sup>29</sup>. On subsequent 100 visits to the laboratory, the volunteers performed an incremental exercise to exhaustion

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2 3 4	101	and two isokinetic Wingate tests at 80 rpm for familiarisation purposes, as well as an
5 6	102	incremental exercise test until exhaustion in normoxia (Vyntus, Jaeger-CareFusion,
7 8 9	103	Höchberg, Germany), as previously reported <sup>8</sup> , including a verification phase <sup>30</sup> . The
10 11	104	seat and handlebar were carefully adjusted to the subject's anthropometric
12 13	105	characteristics during the first visit and were kept consistent throughout the following
14 15	106	visits. Participants were familiarised at least twice with sprinting in isokinetic mode
16 17 18	107	while remaining seated on the saddle. Exercise tests took place in an air-conditioned
19 20	108	laboratory with an ambient temperature of $\sim 21^{\circ}$ C, a relative humidity of 60%–80%,
21 22	109	and $\sim$ 735 mmHg atmospheric pressure. Participants were instructed to avoid caffeine-
23 24 25	110	containing drinks and strenuous exercise in the 24 hours preceding the tests.
25 26 27	111	The second block was conducted to bilaterally measure the resting muscle
28 29	112	architecture of the knee extensors, knee flexors, plantar flexors, tibialis anterior muscle
30 31 32	113	(TA), and the AT and the PT. In addition, leg segment parameters, described in detail
33 34	114	further below, were also bilaterally measured. Limb dominance was determined by
35 36 27	115	asking the participants which leg they preferred to kick a ball as hard as possible <sup>31</sup> . In
37 38 39	116	this block, an isokinetic Wingate test was performed to assess sprint performance.
40 41	117	Given that power measurements were not obtained from each leg individually but from
42 43	118	both conjointly, all the measurements were averaged across both legs.
44 45 46	119	Although 109 physically active and healthy males $(n = 64)$ and females $(n = 45)$
47 48	120	volunteered to participate in the study, complete data were obtained for 101
49 50 51	121	participants. The inclusion criteria for participation in the study have been previously
52 53	122	reported <sup>32</sup> . All volunteers signed a written consent after receiving information about the
54 55	123	aims and potential risks of the study, which commenced after approval by the Ethical
56 57	124	Committee of the University of Las Palmas de Gran Canaria (CEIH2017/13) and was
59 60	125	carried out according to the Declaration of Helsinki. The sex and gender of the
participants were defined based on self-reports during participant recruitment, and allparticipants reported cisgender.

128 2.2. Wingate test

The Wingate test consisted of a 30-s all-out sprint performed on a separate testing day, at least 1 h after a light meal (Lode Excalibur Sport 925 900, Groningen, The Netherlands). The Wingate test was preceded by a standardised warm-up consisting of 1 min of unloaded pedalling, 2 min at 40 W or 60 W, 3 min at 60 or 80 W, 1 min at 80 or 100 W, 1 min at 100 or 120 W, and 1 min at 120 or 140 W for females and males, respectively. At the end of the warm-up, the participants recovered by pedalling at a low cadence (20-40 rpm) for 5 min with the ergometer unloaded. The Wingate test was performed in isokinetic mode at a fixed cadence of 80 rpm. During the Wingate test, the subjects received strong verbal encouragement to ensure maximum effort was provided from the start to the end, while the subjects were requested to remain seated on the saddle for the whole Wingate test. About 30 s before the beginning of the sprint, participants accelerated the flywheel with the ergometer unloaded near the prescribed cadence and waited, ready to sprint as hard and fast as possible after a 5-s countdown. On isokinetic mode, this cycle ergometer exclusively permits a consistent pedalling cadence of 80  $(\pm 1)$  rpm, ensuring that any diminution in the force applied to the pedals leads to a corresponding reduction in the ergometer's resistance and, conversely, an augmentation in force is counteracted by an increase in resistance. Regardless of the sport cycling footwear chosen, all participants were tightly attached to the pedals using additional fastening straps. Instantaneous peak power values (W<sub>peak</sub>) and 1-second peak power averages (W<sub>peak1s</sub>) were obtained using Lode Ergometry Manager Software (LEM; Lode BV, Groningen, The Netherlands). The mean power output (W<sub>mean</sub>) was calculated as the average power developed during the 30-s sprint.

#### 2.3. Imaging acquisition

6 7	152	Real-time two-dimensional B-mode ultrasound (Philips CX50, Philips Medical
8 9	153	Systems, Netherlands) with a 38 mm linear-array transducer (12-3 MHz, L12-3
10 11	154	Broadband, Phillips), was used to bilaterally measure the muscle architecture of the
12 13	155	knee extensors (rectus femoris, vastus medialis, and lateralis; RF, VM, and VL,
14 15 16	156	respectively), knee flexors (biceps femoris long head and semitendinosus; BFlh and ST,
17 18	157	respectively), plantar flexors (gastrocnemius medialis and lateralis; GM and GL,
19 20	158	respectively), and <i>tibialis anterior</i> (TA) (Figure 1). In addition, the AT thickness and
21 22	159	cross-sectional area, as well as the PT thickness, were also assessed. Lastly, the
23 24 25	160	subcutaneous fat thickness over the tibialis anterior (TA FAT) measuring point was
26 27	161	determined as the distance from the skin surface to the superficial boundary of the
28 29	162	tibialis anterior fascia <sup>33</sup> . All scans started after a 15-minute resting period in the supine
30 31	163	position to standardise the potential impact of fluid shifts due to changes in body
32 33 34	164	posture. Image acquisition was performed by the same operator, who has extensive
35 36	165	experience in musculoskeletal ultrasonography. Current guidelines and
37 38	166	recommendations for musculoskeletal ultrasonography by the European Federation of
39 40	167	Societies for Ultrasound in Medicine and Biology were followed <sup>34</sup> . Depending on the
41 42 43	168	subject, the ultrasound depth and frequency were adjusted to 4-5 cm and 38-41 Hz
44 45	160	(knee extensors and plantar flexors) 5-6 cm and 36-38 Hz (knee flexors) 4-5 cm and
46	109	(knee extensors and plantal flexors), 5-0 cm and 50-58 Hz (knee flexors), 4-5 cm and
47	170	38-41 Hz (TA), and 2-3 cm and 43-45 Hz for the AT and the PT. The probe was hand-
49 50	171	held, and the measurements were made with the subjects in a prone or supine position,
51 52 53	172	depending on the muscle or tendon explored, checking joint angles with a manual
55 55	173	goniometer when necessary. Muscle images were acquired using water-soluble gel <sup>35</sup>
56 57	174	without applying pressure, with the ultrasound probe oriented perpendicular to the skin
58 59 60	175	and parallel to the muscle fascicles. Each measurement site was marked on the skin

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surface with a surgical pen before performing the measurements. The primary inclusion
criterion for muscle image analysis was that the aponeuroses were parallel since the
angle between the superficial and the intermediate aponeuroses can strongly influence
the extrapolation methodologies <sup>36,37</sup>.
\*\*\*INSERT FIGURE 1 HERE\*\*\*

181 *2.4. Leg segment parameters* 

182 The shank length was defined as the distance between the proximal head of the fibula to 183 the tip of the lateral malleolus, as previously reported <sup>38</sup>. Gastrocnemius and soleus AT 184 lengths were measured bilaterally from the ultrasound images using the analysis 185 software (OsiriX<sup>™</sup> DICOM viewer, Pixmeo, Geneva, Switzerland). In brief, soleus and 186 gastrocnemius AT lengths were measured from the AT insertion point on the calcaneus 187 bone to the distal end of the soleus muscle and the AT junction between the medial and 188 lateral gastrocnemius muscles <sup>38</sup>, respectively. The distal end of the soleus muscle was 189 identified as the point where the *soleus* muscle disappeared while scanning the 190 transverse ultrasound images along the AT, from the proximal to distal.

191 Lastly, the forefoot and the  $MA_{AT}$  lengths were measured. The forefoot length 192 was defined as the distance between the vertical projection from the lateral malleolus tip 193 to the distal head of the first metatarsal bone <sup>38</sup>. The  $MA_{AT}$  was measured in a sitting 194 position as described in the literature <sup>39</sup>.  $MA_{AT}$  was considered as the mean value of the 195 horizontal distances measured on the left and right feet, from the posterior aspect of AT 196 to the most prominent aspect of the medial and lateral malleoli.

197 2.5. Achilles and patellar tendon morphology

198 To assess the AT, the subjects laid prone with their heels overhanging the examination199 stretcher to enable free movement of the feet. Ultrasound scans were performed on both

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200	legs. The examined ankle was passively flexed at 90° to avoid waving of the tendon <sup>40</sup> .
201	A longitudinal scan was obtained at the deep insertion of AT with the calcaneus to
202	determine the AT thickness. Using image-processing software (OsiriX <sup>TM</sup> DICOM
203	viewer), three perpendicular lines from the deep towards the superficial peritenon were
204	drawn at 0.5 (AT <sub>proximal</sub> ), 1 (AT <sub>central</sub> ), and 1.5 cm (AT <sub>distal</sub> ) from the calcaneus
205	attachment, enabling the determination of the AT thickness at these three subregions.
206	To determine the AT cross-sectional area (CSA), a transverse scan was obtained just
207	distal to the <i>soleus</i> muscle belly insertion end <sup>41</sup> . The CSA was measured by tracing a
208	line surrounding the entire geometry of the AT using the software's closed polygon tool.
209	The peritenon was not included in any analyses.
210	To assess the PT thickness, the subjects were positioned supine on an
211	examination table, with their knees flexed at 30° by placing a pillow beneath the
212	popliteal space. This position stretches slightly the extensor elements, preventing
213	potential anisotropy that could result from a concave quadriceps-patellar profile which
214	may occur if the tendons are not fully extended. For each subject, six ultrasound images
215	were analysed (three per leg). These images captured the PT proximal insertion on the
216	patella (at 0.5 cm and 1 cm from the insertion, referred to as proximal $PT_{0.5cm}$ and

217 proximal  $PT_{1cm}$ , respectively), the distal insertion on the tibial tuberosity (distal  $PT_{0.5cm}$ 

218 and distal  $PT_{1cm}$ ), and three central regions, located half the length of PT (medial

219  $PT_{central}$ ), 0.5 cm towards the proximal insertion (medial  $PT_{proximal}$ ) and 0.5 cm towards

the distal insertion (medial  $PT_{distal}$ ) (**Supplementary Figure 1**). To ensure accuracy, the

ultrasound probe was repositioned before each measurement. This allowed for a

222 comprehensive analysis of the medial portion of the tendon thickness across multiple

- regions. The specific procedures for obtaining these measurements have been
- 224 previously detailed in the literature 42.

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## 225 2.6. Muscle architecture

 In each muscle, thickness (MT) was measured as the distance between the superficial and deep aponeuroses at the beginning and the end of the captured image. The average of these distances was taken as the representative value of MT. Additionally, the pennation angle (PA) and fascicle length (FL) were each measured three times at different points along the ultrasound images, and the averages of these measurements were calculated to obtain representative values for each muscle. When the muscle fascicles were longer than the width of the probe, the FL was calculated by linear extrapolation of the visible portion of fascicles to the intersection point with the linearly projected superficial aponeurosis of the muscle <sup>43</sup>. The inclusion criteria for determining appropriate fascicles for analysis were as follows: the fascicle insertion point into the central aponeurosis had to be visible, and a reasonable portion of the fascicle ( $\sim 25\%$  or more of the total estimated length) needed to be discernible within the ultrasound transducer's field of view <sup>44</sup>. Muscle architectural parameters (MT, PA, and FL) were digitised using image-processing software (OsiriX<sup>TM</sup> DICOM viewer). Ultrasound reliability was tested before the start of the study, as reported previously 11,32. The specific details for knee extensors, knee flexors, plantar flexors, and TA measurements have been published elsewhere <sup>11,32</sup>. A representative image of the muscle architecture assessment is shown in Figure 2. \*\*\*INSERT FIGURE 2 HERE\*\*\* 

245 2.7. Statistical methods

A total of 101 volunteers (60 males and 41 females) had a complete set of
measurements, to which the central limit theorem was applied and deemed the data
suitable for parametric statistics. For all muscle architecture, tendon morphology, and

sprint performance variables (W<sub>peak</sub>, W<sub>peak1s</sub>, and W<sub>mean</sub>), the mean and standard
deviation (SD) are presented by sex. Sex differences were analysed using a two-tailed
unpaired t-test, with appropriate correction in case of inequality of variances as assessed
by Levene's test.

We employed a two-stage procedure to identify the predictors (67 possible variables) of cycling sprint performance for each dependent variable (W<sub>peak</sub>, W<sub>peakls</sub>, and W<sub>mean</sub>). First, the Elastic Net Regression (EN) method, a regularised (penalised) regression, was used to select the first set of predictors. The EN analysis was carried out using the R package "glmnet"<sup>45</sup>. A nested cross-validation procedure was carried out, splitting the dataset into a 10-fold outer loop to evaluate model performance and a 10-fold inner loop to tune hyperparameters using a grid search within each inner fold. This approach mitigates the optimisation bias and ensures an unbiased assessment of predictive performance on unseen data. From the first selected set of predictors, we carried out a second selection of predictors using the best subset (BS) selection algorithm <sup>46</sup>, with the Bayesian information criterion as an estimate of test error. The package "leaps" for R was used to apply the BS selection algorithm <sup>47</sup>. To clarify the contribution of each predictor to the final regression model, we carried out a general dominance (GD) analysis using the 'yhat' <sup>48</sup> package in R. All assumptions of the linear regression model were met.

All statistical analyses were performed using R 4.3.2 (R Foundation for

Statistical Computing, Vienna, Austria). Statistical significance was defined as p < 0.05.

#### **3. Results**

# *3.1. Physical characteristics and performance*

The descriptive characteristics of the study population are reported in **Table 1**. Females exhibited a higher body fat percentage and lower whole-body lean mass (LM) and leg lean mass than males. When the VO<sub>2</sub>max was expressed normalised to the LM and the lean mass of the lower extremities (LLM), no significant sex differences in VO<sub>2</sub>max values were observed (p = 0.350 and p = 0.970, respectively). Males showed higher W<sub>peak</sub> and W<sub>mean</sub> than females in absolute values. However, no statistically significant sex differences were observed after normalisation of  $W_{peak}$  and  $W_{mean}$  for LM and LLM (Supplementary Table 1).

### \*\*\*INSERT TABLE 1 HERE\*\*\*

3.2. Sex differences in muscle leg segments, muscle architecture, and tendon
morphology.

Muscle architecture results are shown in Supplementary Table 2. Merely fourteen out of thirty-nine muscular architecture variables (i.e., PAs and FLs of diverse muscles) exhibited no sex differences. Males had greater MT in all muscles than females (with differences ranging from 5.3% in GM to 25.0% in superficial TA, respectively, all p<0.05). Regarding PA, males had greater angles than females (ranging from 7.0% in BFlh to 14.8% in the superficial TA, respectively, all p < 0.05). Sex differences were observed in only two out of eight measured muscles regarding FL, wherein men exhibited greater VL (9.5%, p = 0.011) and VM FL (11.5%, p < 0.001) than females, both measured at 39% of the distal insertions to the proximal origins. Lastly, females displayed a wider subcutaneous fat depot than males in the TA (50%, p < 0.001). In the

1		
2 3 4	296	whole group, the thickness of the TA subcutaneous depot was correlated with the
5 6 7	297	percentage of whole-body fat (r = 0.67, $p < 0.001$ , n = 101).
8 9	298	Tendon's morphology and leg segment results are shown in Supplementary
10 11	299	<b>Table 3</b> . Sex differences were observed across all morphological variables of the AT,
12 13 14	300	PT, and leg segments (i.e., shank length, forefoot length, and $MA_{AT}$ ). Males exhibited
15 16	301	greater Achilles thickness (11.9-18.0%, $p < 0.001$ , depending on the region) and PT
17 18	302	thickness (9.5-12.2%, $p < 0.001$ , depending on the region) than females. While the
19 20 21	303	differences in MA <sub>AT</sub> were significant ( $p = 0.018$ ), the magnitude of the difference was
21 22 23 24	304	only 5.7%. The AT CSA was 16.7% greater in males than females ( $p < 0.001$ ),
25 26	305	3.3. Muscle architecture accounts for 24-34% of the explained variance in sprint
27 28 29	306	cycling performance
30 31 32	307	3.3.1. Description of the variables selected by each method, along with the correlations
33 34	308	between the predictors and the dependent variable
35 36 37	309	To identify the optimal set of predictors for three distinct power variables ( $W_{peak}$ ,
38 39	310	$W_{peak_{1s}}$ and $W_{mean}$ ), the EN method was first applied by introducing the 67 available
40 41 42	311	variables, followed by the BS method. For $W_{mean}$ , the EN method selected a set of 19
42 43 44	312	variables, subsequently refined to 7 predictors by the BS method. For $W_{peak1s}$ , the EN
45 46	313	method singled out a set of 25 variables, refined to 8 predictors by the BS method. For
47 48 40	314	$W_{peak}$ , the EN method selected a set of 24 variables, refined to 8 predictors by the BS
49 50 51	315	method. The models with outcome variables $W_{mean}$ , $W_{peak1s}$ , and $W_{peak}$ will be referred
52 53	316	to as the $W_{mean}$ model, $W_{peak1s}$ model, and $W_{peak}$ model, respectively.
54 55 56	317	For the $W_{mean}$ model, the only predictor inversely correlated with mean power
57 58	318	output was the thickness of subcutaneous fat above the TA (TA FAT, $r = -0.49$ , $p <$
59 60	319	0.001); the rest of the predictors displayed a direct correlation (from $MA_{AT}$ with $r =$

320	0.32, $p < 0.01$ to LLM with $r = 0.84$ , $p < 0.001$ ). For the W <sub>peak1s</sub> model, the regional PA
321	and FL of the BFlh were not significantly correlated with $W_{peak1s}$ ; the remaining
322	predictors exhibited a direct correlation with $W_{\text{peakls}}$ (ranging from VM PA, $r = 0.29$ , $p$
323	< .01 to LLM, $r = 0.8$ , $p < 0.001$ ). For the W <sub>peak</sub> model, the sole predictor with an
324	inverse correlation was the thickness of subcutaneous fat above the TA ( $r$ = -0.44, $p$ <
325	0.001). The remaining predictors directly correlate with the outcome variable, ranging
326	from $r = 0.24$ , $p < 0.05$ for VM PA to $r = 0.76$ , $p < 0.001$ for LLM.
327	***INSERT TABLE 2 HERE***
328	3.3.2. Variance explained by the models and relative importance of predictors
329	According to the general dominance analysis $^{49}$ , the $R^2$ value is decomposed into the
330	contributions of each predictor (GD weight) to explain the variability in the outcome
331	variable in a regression model. The variance explained by the $W_{mean}$ model ( <b>Table 2</b> )
332	was 82% ( $R^2 = 0.82$ , 95% CI [0.77, 0.89] decomposed in 34% for LLM, 11% for VL
333	MT, 10% for forefoot length, 9% for TA FAT, 7% for GM PA, 6% for GL PA, and 5%
334	for MA <sub>AT</sub> . Bootstrap confidence intervals for the differences in general dominance
335	between any pair of variables were calculated to assess the relative importance among
336	the variables. The pairwise comparison of predictor importance showed that the general
337	dominance of LLM was significantly higher than that of all other variables. Its
338	confidence interval did not overlap with any other. For all the other combinations of
339	variables, such as VL MT and forefoot length, the GDs were not statistically different
340	among them since their confidence intervals overlapped. This indicates that, except for
341	LLM, the remaining variables do not show substantial differences in their general
342	dominance statistics, suggesting they had comparable levels of importance.

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2 3 4	343	The W <sub>peak1s</sub> model ( <b>Table 3</b> ) accounted for 77% of the variance ( $R^2 = 0.77, 95\%$ )
5 6	344	CI [0.73, 0.86]) decomposed in 34% attributed to LLM, 17% to ST MT, 13% to VL
7 8 9	345	MT, 10% to GL PA, 7% to soleus AT length, 5% to VM PA, 3% to BFlh FL, and 1% to
9 10 11	346	BFlh PA. In this model, LLM is significantly more important than all other variables;
12 13	347	ST MT is more important than BFlh FL, BFlh PA, soleus AT length, and VM PA; GL
14 15	348	PA and VL MT played a more relevant role than BFlh PA. The remaining pairs of
16 17 18	349	variables showed similar relative importance. The variance explained by the model for
19 20	350	the W <sub>peak</sub> model ( <b>Table 4</b> ) was 73% ( $R^2 = 0.73$ , 95% CI [0.66, 0.81]), decomposed in
21 22	351	36% attributed to LLM, 11% to VL MT, 8% to TA FAT, 6% to GL PA, 5% to proximal
23 24 25	352	$PT_{0.5cm}$ , 3% to VM PA, 3% to BFlh PA, and 1% to BFlh FL. In this model, LLM is
26 27	353	significantly more important than all other variables; VL MT is more important than
28 29	354	BFlh PA and BFlh FL; GL PA, TA FAT, and proximal PT <sub>0.5cm</sub> are more important than
30 31 22	355	BFlh FL.
33 34 35	356	***INSERT TABLE 3 HERE***
36 37 38	357	***INSERT TABLE 4 HERE***
39 40 41	358	3.3.3. Variance explained by the architecture block relative to leg lean mass in each
42 43	359	model
44 45	360	In the $W_{mean}$ model, the LLM explained 34% of the general dominance (GD = 0.34,
46 47 48	361	95% CI [0.28, 0.39]). The architectural variables considered as a block (VL MT, GM
49 50	362	PA, and GL PA) accounted for 24% of the general dominance (GD = $0.24$ , 95% CI
51 52	363	[0.16, 0.27]). In comparison, the set of variables comprising forefoot length and MA <sub>AT</sub>
53 54 55	364	explained a 15% of the general dominance (GD = 0.15, 95% CI [0.12, 0.21]). Both
55 56 57	365	intervals presented a moderate overlap, suggesting that these two blocks of variables
58 59 60	366	hold a similar relative importance within the model. Nevertheless, both sets of

367	architectural variables exhibited lower relative importance than the LLM. These
368	findings suggest that, given two groups with an equivalent amount of LLM, the
369	configuration of muscle architecture determines the $W_{mean}$ of each group.
370	In the $W_{\text{peak}_{1s}}$ model, the LLM accounted for 38% (GD = 0.38, 95% CI [0.28,
371	(0.41]), and the architectural variables as a block accounted for approximately $34%$ (GD
372	= $0.34$ , 95% CI [ $0.28$ , $0.41$ ]) of the variability. The remaining variability in the model
373	was attributed to the <i>soleus</i> AT length, contributing 5% (GD = $0.05$ , 95% CI [ $0.02$ ,
374	0.1]). The considerable overlap in the confidence intervals for GD between architecture
375	and LLM suggests that architectural characteristics and LLM hold comparable relative
376	importance in predicting W <sub>peak1s</sub> .
377	In the $W_{peak}$ model, the LLM explained 36% (GD = 0.36, 95% CI [0.26, 0.44])
378	of the variability, while the architectural variables as a block explained 23% (GD =
379	0.23, 95% CI [0.17, 0.32]). Again, given the moderate overlap of the confidence
380	intervals, it can be inferred that architecture is as relevant as LLM in predicting
381	instantaneous peak power.
382	In summary, across all three power variables ( $W_{mean}$ , $W_{peak1s}$ , and $W_{peak}$ ), LLM
383	consistently shows higher relative importance, while muscle architecture also plays a
384	crucial role, particularly evident in its comparable dominance to LLM in some models.
385	3.3.4. BFlh FL and BFlh PA as suppressor variables in the models of the two peak
386	power output measures. Identification of the predictors affected by suppression in each
387	model.
388	A suppressor variable influence occurs when the square of the standardised regression
389	coefficient of a predictor variable is greater than the square of its correlation with the
390	dependent variable <sup>50,51</sup> . BFlh FL and BFlh PA act as a suppressor variable in the

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391	$W_{peak1s}$ and $W_{peak}$ models (classical suppression). A suppressor variable enhances the
392	predictive power of a model by increasing the importance of other predictors, even
393	though it may not be directly correlated with the outcome itself. In both models,
394	although the two predictors (BFlh FL and BFlh PA) were not correlated with the
395	dependent variable, the prediction of the outcome variable was significantly enhanced
396	when each variable was incorporated into the model (2% for BFlh FL and 1% for BFlh
397	PA for $W_{peak1s}$ , and 3% for BFlh PA and 1% for BFlh FL for $W_{peak}$ ). The suppressor
398	variables eliminate irrelevant predictive variance from the other predictors, thereby
399	enhancing the standardised regression coefficient of the predictors and consequently
400	improving the overall predictability of the model.
401	To better understand how the suppression of BFlh FL and PA affects sprint
402	cycling performance, we calculated 95% bootstrap confidence intervals to determine the

cycling performance, we calculated 95% bootstrap confidence intervals to determine the 402 403 differences in the importance of other predictors with and without BFlh FL and PA in 404 the model. For W<sub>neak1s</sub>, the variables that enhanced the standardised regression 405 coefficient were LLM (0.028, 95% CI [0.012, 0.045]); VL MT (0.027, 95% CI [0.005, 406 0.049]); and VM PA (0.032, 95% CI [0.008, 0.056]). For W<sub>peak</sub>, the variables that 407 enhance the standardised regression coefficient were LLM (0.08, 95% CI [0.04, 0.11]); 408 GL PA (0.04, 95% CI [0.02, 0.06]); and VM PA (0.06, 95% CI [0.03, 0.09]). The 409 predictors affected by the suppression were those that had a significant relationship with 410 BFlh FL and PA, indicating a functional interaction between the BFlh and other 411 muscles during sprint cycling. 412

### **4. Discussion**

#### *4.1. Overview*

The present investigation revealed that the primary determinant of sprint cycling performance on the cycle ergometer is the lean mass of the lower extremities, which is in line with previous research<sup>8</sup>. As a novelty, we have also shown that several architectural variables contribute to explaining 23-34% of the variability in sprint performance by applying a novel modelling technique that allows for specifically comparing the contributions made by each variable or combinations of variables to predicting sprint performance. Moreover, sex differences could not properly be addressed in previous studies due to the small sample sizes or the inclusion of only males. The present investigation demonstrates that sex differences in sprint performance are explained mainly by LLM; despite some sex differences in muscle architecture and morphological variables of the AT, PT, and leg segment lengths, these do not contribute to the sex dimorphism in sprint performance more than the lean mass of the lower extremities. Last, we have shown that the BFlh fascicle length and pennation angle negatively influence maximal power generation, acting as suppressor variables and enhancing the predictive power of the models tested by tuning the importance of other predictors.

# 433 4.2. Sex differences related to performance, muscle architecture, morphological 434 variables of tendons, and leg segments

This study revealed several sex differences regarding performance, muscle architecture,
and morphological variables of the AT, PT, and leg segments. First, males developed
more W<sub>peak</sub>, W<sub>peak1s</sub>, and W<sub>mean</sub> than females in absolute values, although these sex
differences disappeared after normalisation to LM and LLM, which aligns with the

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43	9 literature <sup>5,8</sup> . Second, as previously reported, males had greater MT and PA than females
44	<sup>52</sup> . The observed sex disparity in lower limb MT likely stems from a confluence of
44	biological and physiological factors. In this regard, sex hormones play a significant role,
442	2 with males typically exhibiting higher testosterone levels compared to females <sup>53</sup> . This
44	hormonal advantage translates into a more significant potential for muscle hypertrophy,
444	4 ultimately influencing MT <sup>54</sup> . It should be highlighted that while MT is related to
44	overall FL and PA, they are not directly proportional due to their complex interaction,
44	as recently revealed by mathematical modelling <sup>11</sup> . In terms of PA, males had greater
44	angles than females, which agrees with the literature <sup>52,55,56</sup> . Although height differences
44	8 naturally exist between males and females <sup>57,58</sup> and could be considered as an
44	explanation for the observed sex differences in muscle architecture, the reality is more
45	complex. Analyses controlling for height have revealed a minimal impact <sup>59,60</sup> . While
45	1 muscle fiber types could explain sex differences in muscle architecture, the only
45	2 available investigation indicates that, at least in young females, the associations between
45	3 muscle fiber types (based on histochemical myosin ATPase fiber typing) and muscle
454	4 architecture are weak <sup>61</sup> .

455 In line with the literature, sex differences were observed across all 456 morphological variables of the AT <sup>62</sup>, PT <sup>63</sup>, and leg segments (i.e., shank length, forefoot length, and MA<sub>AT</sub>)<sup>64-66</sup>. These differences could be attributed to greater muscle 457 mass in males <sup>60</sup> and differences in skeletal growth development <sup>67</sup>, partly explained by 458 459 hormonal factors <sup>68</sup>. Males had longer  $MA_{AT}$  (5.7%) than females. A longer  $MA_{AT}$ endows a mechanical advantage to generate a higher plantar flexion torque for a given 460 muscle mass <sup>26,27</sup>. Interestingly, we have found the forefoot and MA<sub>AT</sub> lengths are novel 461 462 performance predictors during prolonged cycling sprints. Although the mechanism that

463 links forefoot and  $MA_{AT}$  lengths to  $W_{mean}$  remains to be elucidated, it may be related to 464 their potential effects on cycling efficiency.

Because muscle moment arms are intrinsic to musculoskeletal system build-up
and can only be altered by surgery, understanding how they may influence human
performance can be challenging <sup>69</sup>.

Lastly, the thicker subcutaneous adipose tissue in the TA observed in females corresponds with the higher percentage of body fat and the female-specific regional distribution of subcutaneous fat in the lower extremities <sup>70</sup>. Since the body fat percentage is generally lower at the highest fitness level, its negative association with sprint power is likely indirect.

473 4.3. The way muscles are geometrically structured is just as crucial as their size for
474 power generation

A recent systematic review underscored the robust relationship between quadriceps muscle volume and peak power output ( $R^2$  from 0.65 to 0.82)<sup>10</sup>, suggesting the pivotal role of this set of pennate muscles in force production, reinforcing the notion that the active muscle mass is the primary determinant of sprint cycling performance <sup>5,8</sup>. Some associations between PA and FL with performance have been reported <sup>10</sup>. Our models have demonstrated that muscle architecture as a whole is a much more relevant predictor of sprint performance than previously thought, accounting for 23-34% of the explained relative variance in sprint cycling performance. The included muscle architecture predicting variables were essentially similar for Wpeak, Wpeakls, and Wmean, with slight nuances for peak and mean power. Specifically, for the W<sub>peak</sub> model, the predictive value was enhanced by including the proximal PT<sub>0.5cm</sub> thickness. In the case of W<sub>mean</sub>, MA<sub>AT</sub>, forefoot length, and TA FAT added predictive value to the model. 

487 Lastly, for W<sub>peak1s</sub>, the *soleus* AT and ST MT improved the predicting capacity of the
488 model.

The difference mentioned above between the predictors of W<sub>peak</sub> and W<sub>mean</sub> highlights the distinct physiological demands of achieving peak power versus maintaining a high average power output during a prolonged all-out sprint. This could be explained by considering that during pedalling, a longer MA<sub>AT</sub> may increase the mechanical advantage of the calf muscles on the ankle joint, requiring less force to generate the same amount of ankle torque during push-off and potentially reducing the energy cost, as shown in running studies <sup>71</sup>. In addition, a longer forefoot is advantageous for sprint running performance <sup>72</sup>. Although sprint running and cycling differ, a longer forefoot might provide a larger surface area for power transfer from the leg muscles to the pedals. This could allow for a more forceful push-off during each pedal stroke, contributing to maintaining a higher average power output throughout the sprint. Although the TA muscle is an antagonist to the plantar flexors during the push phase of each pedal stroke, it may generate upward forces in the rest of the pedal cycle, together with the hip flexor muscles <sup>73</sup>. Lastly, excess TA FAT could add unnecessary weight, increasing the inertia moment of the lower leg and, hence, the energy required to maintain a given power output <sup>74,75</sup>.

It should be highlighted that the models for  $W_{peak}$  and  $W_{peak1}$  were practically similar. They shared the LLM and a block of muscle architectural variables explaining power generation (i.e., VL MT, GL PA, BFlh PA, and BFlh FL). The ST MT and *soleus* AT length seem important for  $W_{peak1s}$ , while the proximal  $PT_{0.5cm}$  influences (negatively) power generation in  $W_{peak}$ . The ST MT and AT length may contribute significantly to  $W_{peak1s}$  through their elastic properties. Tendons, including the AT, function as biological springs, storing elastic energy during muscle lengthening

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(eccentric phase) and releasing it during shortening (concentric phase) <sup>76</sup>. A longer AT, coupled with the ST MT, can potentially store more elastic energy, augmenting muscle force production and, thereby, enhancing power output during a little more than a complete revolution in the case of  $W_{peakls}$ . Conversely, the proximal  $PT_{0.5cm}$  primarily influences  $W_{peak}$  by impacting the lever arm length <sup>77</sup>. A thinner proximal  $PT_{0.5cm}$  could be a proxy for a shorter PT and lever arm, leading to greater muscle force requirements to generate a given torque. Research suggests that greater tendon thickness is often associated with increased tendon length. For instance, studies using cadaveric and imaging models have shown a positive correlation between PT thickness and length, with Spearman's coefficients ranging from 0.33 to 0.36<sup>78</sup>. This relationship indicates that a thicker PT is also likely to be longer. Longer tendons can affect the lever arm by extending the distance over which force is applied. This mechanical advantage allows for greater efficiency in force transmission during rapid movements, which could enhance performance in high-speed tasks such as sprint cycling. In contrast, shorter tendons may reduce the lever arm length, necessitating more muscle force to achieve the same torque level. While this could benefit slower, high-force movements, it may hinder tasks requiring rapid force generation and explosive power, such as those involved in sprint cycling.

530 Unsurprisingly, the main architecture variables with a positive predictive value 531 for sprint cycling performance were the VL MT, VM PA, GM, and GL PAs since their 532 activation patterns, function, muscle recruitment, and estimated contribution to torque 533 generation during cycling are well-established <sup>79-84</sup>. The latter has been corroborated by 534 a recent review <sup>10</sup>. Some anatomical regions have higher predictive value for cycling 535 sprint performance, reflecting their functional specialisation, with areas closer to the 536 tendons, such as VL 22% and VM 39%, having a stronger influence on power

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537	generation. These tendon-proximal regions contribute more efficiently to force
538	transmission and elastic energy storage, enhancing explosive movements like sprint
539	cycling. This is further supported by findings showing that both mid- and tendon-
540	proximal regions, particularly in muscles like the VL, demonstrate higher contributions
541	to power generation due to their structural advantage in transmitting force efficiently <sup>85</sup> .
542	In contrast, regions farther from tendons are less involved in immediate force
543	production, explaining their weaker predictive value. Lastly, the GM and GL are
544	responsible for plantar flexion of the ankle and play a crucial role in power generation
545	during the push-off phase of the pedal stroke <sup>84</sup> .
546	Strikingly, the BFlh FL and PA were found to negatively contribute to
547	performance in our $W_{peak}$ and $W_{peak1s}$ models, which is addressed in the next section.
548	Unlike other muscles, the BFlh shows predictive power in the 70% region, closer to its
549	origin at the ischial tuberosity rather than the knee insertion. This may be due to its role
550	in hip extension and pelvic stabilisation, critical for power transfer and force control
551	during sprint cycling <sup>86,87</sup> , rather than direct force transmission to the knee.
552	4.4. The biceps femoris long head acts as a suppressor in the peak power output models
553	Despite a small contribution of BFlh FL and PA to predicting $W_{peak}$ and $W_{peak1s}$ , their
554	inclusion in the model clarifies the relationship between other predictors and peak
555	power output by eliminating irrelevant predictive variance, thus enhancing the model's
556	overall predicting ability. The BFlh is a biarticular and pennate muscle that flexes the
557	knee and extends the hip while exerting control over the direction of the forces applied
558	to the pedals <sup>73,86,87</sup> . During the push-off phase of the pedal stroke, the BFlh acts
559	synergistically with the hip extensor muscles (such as the gluteus maximus) while
560	opposing the knee extensor muscles (such as the VL, VM, and RF) <sup>73,86,87</sup> . A large BFlh
561	PA and long BFlh FL may negatively impact sprint performance by several

mechanisms. A large PA can result in less efficient force transmission in the direction of
movement because a portion of the force generated by the muscle fibers is not aligned
with the mechanical line of action <sup>88</sup>. Longer fascicles can shorten more quickly than
shorter ones <sup>88</sup>, which may entail a higher energy expenditure and more fatigue <sup>89</sup>.
During prolonged sprinting, the hamstring muscle group fatigues quickly <sup>90</sup> and may be
unable to effectively assist in hip extension, leading to a less forceful push-off phase.

#### 568 4.5. Strengths and limitations

 This study benefits from several methodological strengths. First, we have included a large and diverse study population (n=101) encompassing males and females with similar age and activity levels. This enhanced the generalizability of the findings, at least to physically active participants, since differences with elite or trained cyclists are expected to exist <sup>91</sup>. Second, the study employed modern ultrasound equipment for muscle architecture assessment, ensuring accurate and reliable data collection with strict adherence to standardised image acquisition and analysis procedures, minimising measurement errors, and ensuring consistency across participants. Finally, the combination of a comprehensive evaluation of muscle architecture, tendons, and segment lengths on a large population with the use of advanced statistical methods provided the most robust and accurate assessment of the influence of muscle architecture on cycling sprint performance so far undertaken <sup>10</sup>.

Some limitations of this study should be acknowledged. The current study is limited because each leg's power output during the Wingate test was not determined individually. While averaging bilateral measurements provides a valuable performance assessment, it overlooks potential asymmetries in leg power output between the left and right sides <sup>92</sup>, which could influence sprint cycling performance <sup>93</sup>. Additionally, measurements of muscle architecture were obtained at rest rather than during actual

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587 sprinting. Thus, it remains to be determined how these architectural variables interact 588 under sprint conditions, which are dynamic regarding muscle length, joint angles, and 589 shortening velocities <sup>94</sup>. However, measuring muscle architecture dynamically would 590 require using multiple probes simultaneously across many muscles, which seems hard 591 to implement.

#### 592 **5.** Conclusion

593 We have confirmed that muscle mass is a crucial determinant of sprint cycling 594 performance in males and females. As a novelty, we have shown that muscle 595 architecture and leg and foot morphology contribute almost as much as muscle mass to 596 explain the human variability in cycling sprint performance. Including a large sample of 597 subjects of both sexes has allowed the identification of forefoot length and Achilles 598 tendon moment arm as new anatomical predictors of mean power output during 599 prolonged cycling sprints. Finally, we have shown that the BFlh fascicle length and 600 pennation angle negatively influence maximal power generation. While future research 601 is warranted to further elucidate the complex relationship between muscle architecture 602 and sprint cycling performance dynamically, our findings provide valuable insights for 603 athletes, coaches, and sports scientists seeking to optimise training and performance 604 strategies.

#### 605 **6. Perspective**

Our study offers a robust methodological approach that advances the understanding of
muscle architecture's role in sprint cycling performance. Previous studies have
highlighted the critical role of muscle mass and volume as primary determinants of
power output in cycling sprints <sup>10</sup>. However, our research uniquely demonstrates that
muscle architecture variables explain a substantial proportion (23-34%) of the variance
in performance, approaching the predictive capacity of lean mass itself. These results

> challenge the traditional view of muscle mass as the dominant factor and underline the importance of considering muscle architectural configurations when assessing sprint potential. Furthermore, our comprehensive analysis of muscle architecture, tendon morphology, and leg segment lengths, combined with advanced statistical methods, represents the most thorough examination of these variables' collective impact on sprint performance to date. The assessments of muscle architecture were conducted at rest, not during the dynamic conditions of a sprint, leaving open questions about how these variables behave under high-speed contractions and changing joint angles. Addressing these challenges would require novel imaging solutions, which are currently difficult to implement <sup>94</sup>. Future research should strive to capture these dynamics and explore the implications for training and performance.

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628 Authors' contributions

The contribution of the authors is as follows: SMR, JJGH, JALC, and JSM contributed to the conception and design of the study and drafted the manuscript; JSM collected the ultrasound data; RAO, VGA, AGS, AAA, and MMR helped with data collection of sprint performance and leg segment variables; JJGH performed the statistical analysis and mathematical modelling; all co-authors critically evaluated and contributed to the manuscript. All authors have approved the final version of the manuscript.

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3	636	Competing interests
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2 3 4	919	Tables
5 6	920	<b>Table 1.</b> Physical characteristics and ergospirometric variables (mean $\pm$ SD).
7 8	921	Table 2. Mean power best predictors and the general dominance of sprint cycling
9 10 11	922	performance.
12 13	923	Table 3. Instantaneous peak power best predictors and the general dominance of sprint
14 15	924	cycling performance.
16 17	925	Table 4. One-second peak power best predictors and the general dominance of sprint
18 19 20	926	cycling performance.
20 21 22	927	
23 24	928	Figures
25 26 27	929	<b>Figure 1.</b> Muscle scanning locations: $X_1$ indicates the measurement site for the
28 29	930	gastrocnemius lateralis, with X <sub>2</sub> corresponding to the gastrocnemius medialis. This
30 31	931	figure represents all measured regions, including the vastus lateralis, vastus medialis,
32 33 24	932	rectus femoris, biceps femoris long head, semitendinosus, tibialis anterior $(Z_1)$ , and
35 36	933	both the medial and lateral gastrocnemius. Specific details of all the measurements can
37 38	934	be found in the Materials and Methods section.
39 40	935	Figure 2. A demonstrative ultrasound image corresponding to the vastus lateralis (VL)
41 42 43	936	22% region of a male participant. Above the ultrasound image, a schematic
44 45	937	representation of the vastus lateralis illustrates the proximal (56%), medial (39%), and
46 47	938	distal (22%) regions, with the 22% region located near the knee.
48 49	939	
50 51	940	Supplementary material
52 53	941	Supplementary Table 1 Wingste test performance (mean + SD)
54 55	0.42	Supplementary Table 1. Wingate test performance (mean ± 5D).
50 57 58 59	942	Supplementary 1 able 2. Muscle architecture characteristics by sex (mean $\pm$ SD).

943 Supplementary Table 3. Tendon's morphology and leg segment characteristics (mean
944 ± SD).

Supplementary Figure 1. Patellar tendon architecture: The figure highlights three distinct regions of the patellar tendon (PT). Region A1 encompasses the proximal insertion on the patella, including measurements taken at 0.5 cm and 1 cm from the insertion. Region A2 represents the medial portion of the tendon, where thickness was assessed at three points: the medial PT central, medial PT proximal (0.5 cm towards the proximal insertion), and medial PT distal (0.5 cm towards the distal insertion). Region A3 marks the distal insertion on the tibial tuberosity, with measurements taken at 0.5 cm and 1 cm from the insertion.





129x129mm (300 x 300 DPI)

	Males (n = 60)	Females (n = 41)	р
Age (years)	$23.0 \pm 3.2$	$23.1 \pm 2.9$	0.866
Height (cm)	$176.2 \pm 6.3$	$164.4 \pm 6.0$	0.000
Body weight (kg)	$74.1\pm7.6$	$59.0\pm8.4$	0.000
Total BM (kg)	$3.16 \pm 0.33$	$2.44\pm0.29$	0.000
Total FM (kg)	$14.59\pm4.51$	$16.36 \pm 4.56$	0.057
Total LM (kg)	$56.43 \pm 5.11$	$40.23\pm5.39$	0.000
LLM (kg)	$20.20\pm2.01$	$14.11 \pm 2.09$	0.000
Body fat (%)	$19.4 \pm 4.6$	$27.4\pm4.8$	0.000
VO <sub>2</sub> max (mL·min <sup>-1</sup> )	$3294\pm446$	$2307\pm450$	0.000
VO <sub>2</sub> max (mL·kg <sup>-1</sup> ·min <sup>-1</sup> )	$44.6 \pm 5.9$	$39.1 \pm 5.7$	0.000
VO <sub>2</sub> max (mL·kg LM <sup>-1</sup> ·min <sup>-1</sup> )	58.3 ± 5.9	$57.1 \pm 6.8$	0.350
VO <sub>2</sub> max (mL·kg LLM <sup>-1</sup> ·min <sup>-1</sup> )	$163.3 \pm 17.7$	$163.5 \pm 21.3$	0.970

**TABLE 1.** Physical characteristics and ergoespirometric variables (mean  $\pm$  SD).

Abbreviations: BM, bone mass; FM, fat mass; LM, lean mass; LLM, lean mass of the lower extremities; VO<sub>2</sub>max, maximal oxygen consumption.

2															
4		<b>TABLE 2.</b> Mean power best predictors and general dominance analysis.													
5- 6		1	2	3	4	5	6	7							
7 8	Variables				r				b	Beta	GD (%)	GD 95% CI			
9- 10	1. W <sub>mean</sub>														
11 12	2. LLM (gr)	0.84†	_						$0.02^{\dagger}$	0.49†	0.34 (41.5%)	(0.28,0.39)			
13 14	3. VL MT (22%) (cm)	0.51†	$0.47^{\dagger}$						68.22†	0.18 <sup>†</sup>	0.11 (13.4%)	(0.06,0.19)			
15 16	4. Forefoot length (cm	) 0.46†	0.43 <sup>†</sup>	0.05	_				29.36 <sup>†</sup>	0.18 <sup>†</sup>	0.10 (12.2%)	(0.04,0.15)			
17 18	5. TA FAT (mm)	-0.49†	-0.46†	-0.20§	-0.20	_			-136.65‡	-0.15‡	0.09 (11%)	(0.05,0.15)			
19 20	6. GM PA (°)	0.42†	0.25 <sup>§</sup>	0.21§	0.11	-0.21§	_		4.3‡	0.15‡	0.07 (8.5%)	(0.04,0.13)			
21	7. GL PA (°)	$0.42^{\dagger}$	0.27‡	0.12	0.25 <sup>§</sup>	-0.14	$0.47^{\dagger}$	_	5.76 <sup>§</sup>	0.12§	0.06 (7.3%)	(0.02,0.11)			
23	8. MA <sub>AT</sub> (cm)	0.32‡	0.32‡	0.12	-0.09	-0.01	0.07	0.03	44.44‡	0.14‡	0.05 (6.1%)	(0.01,0.10)			
2 <del>4</del> 25	Intercept = $-595.11^{\dagger}$ ; $R^2 = 0.82$ , 95% CI [0.77,0.89] $R^2 = 0.82(100\%)$														

<sup>26</sup>  $W_{mean}$ , mean power output; LLM, lean mass of the lower extremities; VL MT (22%), regional muscle thickness of vastus lateralis; GM PA, pennation angle of 27 gastrocnemius medialis; GL PA, pennation angle of gastrocnemius lateralis; TA FAT, thickness of subcutaneous fat above tibialis anterior; MA<sub>AT</sub>, Achilles 28 endon moment arm; Beta, standardized regression coefficients; r, zero-order correlation; GD, general dominance statistic. <sup>†</sup>p < .001; <sup>§</sup>p < .05. 29

	1	2	3	4	5	6	7	8				
Variables				r					b	Beta	GD (%)	GD 95% C
1. W <sub>peak</sub> (w)	_											
2. LLM (gr)	0.76†	_							0.05†	$0.7^{+}$	0.36 (49.3%)	(0.26,0.44)
3. VL MT (22%) (cm)	$0.5^{+}$	0.47 <sup>†</sup>							151.03†	0.22 <sup>†</sup>	0.11 (15.1%)	(0.04,0.22)
4. TA FAT (mm)	-0.44†	-0.46†	-0.2§	—					-285.22§	-0.17§	0.08 (11%)	(0.03,0.14)
5. GL PA (°)	0.35†	0.27‡	0.12	-0.14	_				16.97†	0.20†	0.06 (8.2%)	(0.02,0.14)
6. Proximal PT <sub>0.5cm</sub>	$0.4^{\dagger}$	0.58†	0.38†	-0.49†	0.06	—			-908.61‡	-0.20‡	0.05 (6.8%)	(0.03,0.1)
7. VM PA (39%) (°)	0.24§	0.24§	0.07	-0.10	0.05	0.13	_		12.26 <sup>§</sup>	0.13 <sup>§</sup>	0.03 (4.1%)	(0.01,0.08)
8. BFlh PA (70%) (°)	0.04	0.25‡	0.03	-0.14	0.23§	0.02	0.28‡	—	-29.73†	-0.29†	0.03 (4.1%)	(0.01,0.06)
9. BFlh FL (70%) (cm)	0.01	0.09	0.24§	0.04	-0.05	0.19	-0.03	-0.33†	-27.27§	-0.15 <sup>§</sup>	0.01 (1.4%)	(0.004,0.04)
	170.088	(n=0.01)	3) $\cdot R^{2}$	=0.73	25% CI	[0.66.0	).81]				$R^2 = 0.73(100\%)$	

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3												
4	TABL	E <b>4</b> . One-s	second r	eak no	wer be	est pre	dictors	and gener	al domina	nce analv	sis.	
5				,eun po				unu gener			0101	
6 7	1	2	3	4	5	6	7	8				
8 Variables 9				r					b	Beta	GD (%)	GD 95% CI
10 <b>1. W</b> <sub>1s</sub>	—											
12 <b>2. LLM (gr)</b> 13	$0.8^{\dagger}$	_							0.03†	0.56†	0.34 (44.2%)	(0.27,0.42)
1 <b>3. ST MT (70%) (cm)</b> 15	0.59†	$0.6^{\dagger}$							62.7§	0.15§	0.13 (16.9%)	(0.08,0.20)
1 <b>6.</b> VL MT (22%) (cm)	0.49†	$0.47^{\dagger}$	0.37†	—					87.8‡	0.19‡	0.10 (13%)	(0.03,0.21)
18 <b>5.</b> GL PA (°)	$0.42^{\dagger}$	0.27‡	0.27‡	0.12	—				12.21†	$0.20^{\dagger}$	0.08 (10.4%)	(0.03,0.14)
<b>6</b> 0Soleus AT length (cm)	0.36†	0.29‡	0.22§	0.02	0.16	—			15.47 <sup>§</sup>	0.12§	0.05 (6.5%)	(0.02,0.1)
<sup>21</sup> 22 <sup>7</sup> . VM PA (39%) (°)	0.29‡	0.24§	0.1	0.07	0.05	0.15	—		9.0‡	0.14‡	0.04 (5.2%)	(0.01,0.1)
23 24 BFlh FL (70%) (cm)	-0.06	0.09	0.1	0.24§	-0.05	-0.08	-0.03		-26.35†	-0.2†	0.02 (2.6%)	(0.01,0.06)
<sup>25</sup> <sub>26</sub> . BFlh PA (70%) (°)	0.17	0.25 <sup>§</sup>	0.2 <sup>§</sup>	0.03	0.23 <sup>§</sup>	0.17	0.28‡	-0.33†	-12.59‡	-0.18‡	0.01 (1.2%)	(0.01,0.03)
27 Inte	ercept=18	8.59 (p=0.	$(27); R^2$	= 0.77,	95% (	CI [0.73	3,0.86]				$R^2 = 0.77(100\%)$	)

y<sub>9</sub>, one-second peak power output; LLM, lean mass of the lower extremities; ST MT (70%), regional muscle thickness of semitendinosus; VL MT (22%), regional muscle thickness of yastus lateralis; GL PA, pennation angle of gastrocnemius lateralis; Soleus AT length, aquilles tendon length from the AT insertion point on the calcaneus to the distal end of the soleus muscle; VM PA (39%), regional pennation angle of vastus medialis; BFlh FL (70%) (cm), regional fascicle length of biceps femoris long head; BFlh PA (70%), regional pehnation angle of biceps femoris long head; Beta, standardized regression coefficients; r, zero-order correlation; GD, general dominance statistic.  $^{\dagger}p < .001$ ;  $^{\ddagger}p < .01$ ;  $^{\$}p < .05$ .


162x121mm (300 x 300 DPI)

	Males (n = 60)	Females (n = 41)	р
W <sub>mean</sub> (W)	$629.7 \pm 100.0$	$440.9\pm78.4$	0.000
W <sub>peak</sub> (W)	$1085.5 \pm 196.0$	$764.2 \pm 143.3$	0.000
W <sub>peak 1-s</sub> (W)	$858.7 \pm 124.9$	$619.5\pm103.2$	0.000
W <sub>mean</sub> (W·kg BW-1)	$8.5 \pm 1.0$	$7.5 \pm 1.1$	0.000
W <sub>mean</sub> (W·kg LM <sup>-1</sup> )	$11.1 \pm 1.3$	$10.9 \pm 1.3$	0.318
W <sub>mean</sub> (W·kg LLM <sup>-1</sup> )	$31.2 \pm 3.9$	31.1 ± 3.9	0.857
W <sub>peak</sub> (W·kg BW <sup>-1</sup> )	$14.6 \pm 2.4$	$12.9 \pm 1.8$	0.000
W <sub>peak</sub> (W·kg LM <sup>-1</sup> )	$19.3 \pm 3.2$	$18.8 \pm 2.4$	0.438
W <sub>peak</sub> (W·kg LLM <sup>-1</sup> )	$54.0 \pm 9.3$	$53.8 \pm 6.5$	0.878
W <sub>peak 1-s</sub> (W·kg BW <sup>-1</sup> )	$11.6 \pm 1.6$	$10.5 \pm 1.4$	0.000
W <sub>peak 1-s</sub> (W·kg LM <sup>-1</sup> )	$15.2 \pm 2.0$	$15.3 \pm 1.7$	0.913
W <sub>peak 1-s</sub> (W·kg LLM <sup>-1</sup> )	$42.8 \pm 6.0$	$43.7 \pm 5.0$	0.421

<b>SUPPLEMENTARY TABLE 1</b>	. Wingate test	performance	$(\text{mean} \pm \text{SD})$	).
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Abbreviations: BW, body weight; LM, whole-body lean mass; LLM, lean mass of the lower extremities;  $W_{mean}$ , mean power output;  $W_{peak}$ , instantaneous peak power output;  $W_{peak}$  l-s, 1-s peak power output.

## **SUPPLEMENTARY TABLE 2.** Muscle architecture characteristics (mean ± SD).

	Males (n = 61)	Females (n = 40)	р
GM MT (cm)	$1.9 \pm 0.2$	$1.8 \pm 0.2$	0.026
GM PA (°)	$27.5 \pm 4.6$	$25.7 \pm 4.0$	0.052
GM FL (cm)	$4.8 \pm 0.6$	$4.7 \pm 0.6$	0.698
GL MT (cm)	$1.5 \pm 0.2$	$1.3 \pm 0.1$	0.000
GL PA (°)	$16.3 \pm 2.6$	$14.7 \pm 2.6$	0.003
GL FL (cm)	$9.7 \pm 5.3$	$8.7 \pm 3.1$	0.257
Superficial TA MT (cm)	$1.2 \pm 0.1$	$0.9 \pm 0.1$	0.000
Superficial TA PA (°)	$11.5 \pm 2.0$	$9.8 \pm 2.0$	0.000
Superficial TA FL (cm)	$6.4 \pm 1.2$	$6.2 \pm 1.3$	0.611
Deep TA MT (cm)	$1.4 \pm 0.2$	$1.2 \pm 0.1$	0.000
Deep TA PA (°)	$12.6 \pm 1.7$	$12.0 \pm 2.2$	0.150
Deep TA FL (cm)	$6.4 \pm 0.8$	$6.5 \pm 0.9$	0.611
TA FAT (mm)	$0.2 \pm 0.1$	$0.3 \pm 0.1$	0.000
BFlh MT (50%) (cm)	$2.1 \pm 0.3$	$1.8 \pm 0.2$	0.000
BFlh PA (50%) (°)	$15.0 \pm 2.9$	$14.7 \pm 2.9$	0.559
BFlh FL (50%) (cm)	$6.3 \pm 0.9$	$6.3 \pm 1$	0.835
BFlh MT (70%) (cm)	$2.1 \pm 0.3$	$1.9 \pm 0.2$	0.000
BFlh PA (70%) (°)	$14.2 \pm 2.1$	$13.2 \pm 2.4$	0.035
BFlh FL (70%) (cm)	$8.8 \pm 1.0$	$8.8 \pm 1.5$	0.868
ST MT (70) (cm)	$2.6 \pm 0.3$	$2.2 \pm 0.3$	0.000
ST MT (50%) (cm)	$2.3 \pm 0.5$	$2.1 \pm 0.4$	0.029
RF MT (39%) (cm)	$2.0 \pm 0.2$	$1.7 \pm 0.2$	0.000
RF PA (39%) (°)	$9.6 \pm 2.2$	$8.9 \pm 2.7$	0.195
RF MT (56%) (cm)	$2.4 \pm 0.2$	$2.0 \pm 0.2$	0.000
RF PA (56%) (cm)	$16.0 \pm 3.4$	$14.8 \pm 3.1$	0.065
RF FL (56%) (cm)	$10.8 \pm 2.7$	$9.7 \pm 2.7$	0.053
VL MT (22%) (cm)	$2.1 \pm 0.3$	$1.8 \pm 0.2$	0.000
VL PA (22%) (°)	$14.4 \pm 3.0$	$13.1 \pm 2.4$	0.023
VL MT (39%) (cm)	$2.4 \pm 0.3$	$2.0 \pm 0.2$	0.000
VL PA (39%) (°)	$14.1 \pm 2.6$	$12.6 \pm 2.3$	0.003
VL FL (39%) (cm)	$10.5 \pm 2.1$	$9.5 \pm 1.5$	0.011
VL MT (56%) (cm)	$2.4 \pm 0.3$	$1.9 \pm 0.3$	0.000
VL PA (56) (°)	$12.0 \pm 3.0$	$11.7 \pm 3.0$	0.628
VM MT (22%) (cm)	$2.0 \pm 0.3$	$1.7 \pm 0.2$	0.000
VM PA (22%) (°)	$14.9 \pm 2.5$	$13.0 \pm 2.9$	0.000
VM FL (22%) (cm)	$9.7 \pm 2.1$	$9.6 \pm 2.1$	0.826
VM MT (39%) (cm)	$1.9 \pm 0.2$	$1.5 \pm 0.2$	0.000
VM PA (39%) (°)	$11.7 \pm 2.5$	$10.9 \pm 2.6$	0.128
VM FL (39%) (cm)	$10.4 \pm 1.5$	$9.2 \pm 1.4$	0.000

Abbreviations: MT, muscle thickness; PA, pennation angle; FL, fascicle length; GM, gastrocnemius medialis; GL, gastrocnemius lateralis; TA, tibialis anterior; BFlh, biceps femoris long head; ST, semitendinosus; RF, rectus femoris; VL, vastus lateralis; VM, vastus medialis.

	,		
	Males (n = 61)	Females (n = 40)	р
Proximal PT <sub>0.5cm</sub>	$0.41\pm0.08$	$0.34 \pm 0.06$	0.000
Proximal PT <sub>1cm</sub>	$0.39\pm0.06$	$0.32 \pm 0.04$	0.000
Distal PT <sub>0.5cm</sub>	$0.42\pm0.06$	$0.37\pm0.04$	0.000
Distal PT <sub>1cm</sub>	$0.39\pm0.04$	$0.33 \pm 0.04$	0.000
Medial PT <sub>proximal</sub> (cm)	$0.36\pm0.05$	$0.31 \pm 0.04$	0.000
Medial PT <sub>central</sub> (cm)	$0.36\pm0.05$	$0.3 \pm 0.04$	0.000
Medial PT <sub>distal</sub> (cm)	$0.36\pm0.05$	$0.3\pm0.03$	0.000
Soleus AT length (cm)	$4.8 \pm 1.2$	$4.0 \pm 1.1$	0.001
Gastrocnemius AT length (cm)	$19.8\pm2.1$	$17.9\pm2.0$	0.000
$MA_{AT}(cm)$	$3.5 \pm 0.4$	$3.3 \pm 0.2$	0.018
Forefoot length (cm)	$12.9\pm0.7$	$12.2 \pm 0.6$	0.000
Shank length (cm)	$37.4 \pm 2.0$	$35.0 \pm 1.6$	0.000
AT <sub>proximal</sub> (cm)	$0.41\pm0.05$	$0.36 \pm 0.05$	0.000
AT <sub>central</sub> (cm)	$0.42\pm0.05$	$0.37 \pm 0.05$	0.000
AT <sub>distal</sub> (cm)	$0.42\pm0.06$	$0.38 \pm 0.05$	0.000
AT CSA (mm <sup>2</sup> )	$0.54\pm0.06$	$0.45\pm0.07$	0.000

SUPPLEMENTARY TABLE 3. Tendon's morphology and leg segments (mean ± SD).

Abbreviations: PT, patellar tendon; AT, Achilles tendon;  $MA_{AT}$ , Achilles tendon moment arm; CSA, cross-sectional area. Details of each measurement are explained in the methodology section.

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