FEDERAL UNIVERSITY OF PAMPA

MULTICENTRIC GRADUATE PROGRAM IN PHYSIOLOGICAL SCIENCES

UNIVERSITAT DE VALÈNCIA

DOCTORAL PROGRAMME IN PHYSIOLOGY

INFRARED THERMOGRAPHY AS A TOOL FOR MONITORING DELAYED ONSET MUSCLE SORENESS, MUSCLE DAMAGE, AND RECOVERY IN SPORTS

Doctoral Thesis presented by

WILLIAN DA SILVA

Directed by:

Felipe Pivetta Carpes (University of Pampa, Brazil).

Jose Ignacio Priego-Quesada (Universitat de València, Spain).

Tutored by:

Mª Rosario Salvador Palmer (Universitat de València, Spain).

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DIRECTORS' REPORT FOR THE THESIS DEPOSIT

Felipe Pivetta Carpes, Ph.D. in Human Movement Sciences and Professor in the Faculty of Health Sciences from the University of Pampa, Brazil.

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CERTIFY

Directors and tutor from the doctoral thesis from Willian da Silva titled **STUDY OF INFRARED THERMOGRAPHY AS A TOOL FOR MONITORING DELAYED ONSET MUSCLE SORENESS, MUSCLE DAMAGE, AND RECOVERY IN SPORTS**, sent a **FAVORABLE** report for the deposit and defense of the thesis and gave their approval to the presented proposal.

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FEDERAL UNIVERSITY OF PAMPA

MULTICENTRIC GRADUATE PROGRAM IN PHYSIOLOGICAL SCIENCES

The examining committee, undersigned, approves the doctoral thesis.

STUDY OF INFRARED THERMOGRAPHY AS A TOOL FOR MONITORING DELAYED ONSET MUSCLE SORENESS, MUSCLE DAMAGE, AND RECOVERY IN SPORTS

Presented by

Willian da Silva

As a partial requirement for obtaining a

Ph.D. degree in Physiological Sciences

EXAMINING COMMITTEE

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Uruguaiana, RS, Brazil, 2023.

To God, for guiding my steps up to the present moment.

To my family, especially my parents, Isaías and Maria.

To my girlfriend and best friend, Tatiane.

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In this way, I move forward. I will continue to seek more daily to learn about the Biomechanics of human movement and human physiology to develop myself as a future professor and scientist.

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1 da Silva W, Machado ÁS, Kunzler MR, Perez I J, Calvo MG, Priego-Quesada JI, Carpes FP (2022). Reproducibility of skin temperature analyses by novice and experienced evaluators using infrared thermography. ***Journal of Thermal Biology***. Volume 110. doi: 10.1016/j.jtherbio.2022.103345.

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V. ABBREVIATIONS

ΔT: Difference between temperature immediately after the test and before.

Ach: Acetylcholine.

ΔT48: Difference between temperature 48 hours after the test and before.

ADP: Adenosine diphosphate.

ATP: Adenosine triphosphate.

CSA: Cross-sectional area.

COX: Cyclo-oxygenase.

CK: Creatine kinase.

DPMS: Descending pain modulatory system.

DOMS: Delayed onset muscle soreness.

HTM: Mechanosensitive receptors.

ICC: Intraclass correlation coefficient.

IL-6: Interleukin 6.

IL-10: Interleukin 10.

IRT: Infrared thermography.

LIPOX: Lipoxygenase.

LDH: Lactate dehydrogenase.

MB: Myoglobin pore.

mPTP: Mitochondrial permeability transition.

MSR: Maximum squat repetitions.

NGF: Nerve growth factor.

NPRS: Numeric rating pain scale.

PGE2: Prostaglandins E2.

PPT: Pressure pain threshold.

ROI: Regions of interest.

ROS: Oxygen-reactive species.

TNF-α: Tumor necrosis factor-alpha.

Tskin: Skin temperature reflected.

VAS: visual analog scale.

VI. ABSTRACT (ENGLISH)

The practice of physical exercise is an essential ally for maintaining health and quality of life. In this context, so that gains can be obtained in terms of performance and recreational level, the monitoring and control of training loads are essential to maximizing its gains. On the other hand, exercise without adequate planning can induce exercise-induced muscle damage and delayed onset muscle soreness (DOMS). DOMS is accomplished by several symptoms, such as strength loss, loss of range of motion, and local pain. As a result, it is interesting for coaches, athletes, and physiotherapists to understand better how to quantify this situation to limit its symptoms. Currently, different approaches have been explored to monitor muscle damage and DOMS. However, many of them are invasive, do not provide immediate feedback, also expensive, such as the quantification of blood markers. At the other extreme, we have behavioral measures that have a subjective character. Infrared thermography (IRT) appears as a possible alternative to monitor post-exercise recovery under the justification that changes in skin temperature is correlated with the inflammation process potentialized by muscle damage and DOMS. With the combination of experiments carried out over four years, the central objective of this Ph.D. thesis was to investigate whether the skin temperature, evaluated by IRT, under resting conditions and after the practice of the physical exercise, can be used to monitor exercise-induced muscle damage and DOMS in post-exercise recovery. Our main results indicated that: (1) When analyzes of thermal imagens are performed by evaluators with different levels of experience, the mean and maximum temperature should be prioritized due to better reproducibility, (2) Changes in skin temperature induced by exercise show a sex-dependent effect, (3) Changes in skin temperature after exercise did not relate to DOMS, but there was a positive relationship between maximum skin temperature and pressure pain threshold in the variation between pre-exercise and 48 h post-exercise, (4) Exercise-induced muscle damage did not reflect in changes in skin temperature rewarming after cold stress test applied 48h post-exercise. We consider that information in this thesis contributes to the formation of knowledge in the area; with an integrative approach of

concepts and experiments, we sought to understand better IRT's applicability to monitoring exercise-induced muscle damage, DOMS, and recovery status after physical exercise.

VII. ABSTRACT (PORTUGUÊS)

A prática de exercício físico é um aliado essencial para a manutenção da saúde e qualidade de vida. Neste contexto, para que se obtenham ganhos ao nível da performance e nível recreativo, a monitorização e controlo das cargas de treino são essenciais para maximizar os seus ganhos. Por outro lado, o exercício sem planejamento adequado pode induzir danos musculares induzidos pelo exercício e dor muscular de início tardio (DMIT). A DMIT é acompanhada por diversos sintomas, como perda de força, perda da amplitude de movimento e dor local. Por isso, é interessante que treinadores, atletas e fisioterapeutas entendam melhor como quantificar essa situação para limitar seus sintomas. Atualmente, diferentes abordagens têm sido exploradas para monitorar danos musculares e DMIT. No entanto, muitos deles são invasivos, não fornecem feedback imediato, também caros, como a quantificação de marcadores sanguíneos. No outro extremo, temos as medidas comportamentais que possuem um caráter subjetivo. A termografia infravermelha surge como uma possível alternativa para monitorar a recuperação pós-exercício sob a justificativa de que as mudanças na temperatura da pele estão correlacionadas com o processo inflamatório potencializado pelo dano muscular e DMIT. Com a combinação de experimentos realizados ao longo de quatro anos, o objetivo central deste doutorado foi investigar se a temperatura da pele, avaliada por termografia, em condições de repouso e após a prática do exercício físico, pode ser utilizada para monitorar o dano muscular induzido pelo exercício e DMIT na recuperação pós-exercício. Nossos principais resultados indicaram que: (1) Quando as análises de imagens térmicas são realizadas por avaliadores com diferentes níveis de experiência, a temperatura média e máxima deve ser priorizada devido à melhor reprodutibilidade, (2) mudanças na temperatura da pele induzidas pelo exercício mostram um efeito dependente do sexo, (3) Mudanças na temperatura da pele após o exercício não se relacionaram com DMIT, mas houve uma relação positiva entre a temperatura máxima da pele e o limiar de dor à pressão na variação entre o pré-exercício e 48 h pós-exercício, (4) O dano muscular induzido pelo exercício não refletiu em mudanças no reaquecimento da temperatura da pele após o teste de estresse ao frio aplicado 48h pós-exercício. Consideramos que as informações desta tese contribuem para a

formação do conhecimento na área; com uma abordagem integrativa de conceitos e experimentos, buscamos entender melhor a aplicabilidade da termografia infravermelha para monitorar o dano muscular induzido pelo exercício, DMIT e o estado de recuperação após o exercício físico.

VIII. ABSTRACT (SPANISH)

La práctica de ejercicio físico es un aliado imprescindible para mantener la salud y la calidad de vida. En este contexto, para que se puedan obtener ganancias, no solo en términos de rendimiento sino también a nivel recreativo, el seguimiento y control de las cargas de entrenamiento son fundamentales para maximizar sus ganancias. Por otro lado, el ejercicio sin una planificación adecuada puede inducir daño muscular inducido por el ejercicio y dolor muscular de aparición tardía (DMAT). DMAT se logra por varios síntomas, como pérdida de fuerza, pérdida de rango de movimiento y dolor local. Por lo tanto, es interesante que entrenadores, atletas y fisioterapeutas entiendan mejor cómo cuantificar esta situación para limitar sus síntomas. Actualmente, se han explorado diferentes enfoques para monitorear el daño muscular y DMAT. Sin embargo, muchas de ellas son invasivas, no generar un informe inmediato, además costosas, como la cuantificación de marcadores sanguíneos. En el otro extremo, tenemos medidas de comportamiento que tienen un carácter subjetivo. La termografía infrarroja (TRI) aparece como una posible alternativa para monitorear la recuperación después del ejercicio bajo la justificación de que los cambios en la temperatura de la piel están correlacionados con el proceso inflamatorio potencializado por el daño muscular y el DMAT. Con la combinación de experimentos realizados durante cuatro años, el objetivo central de esta tesis de doctorado fue investigar si la temperatura de la piel, evaluada por TRI, en condiciones de reposo y después de la práctica del ejercicio físico, puede ser utilizada para monitorear el daño muscular inducido por el ejercicio y el DMAT en la recuperación después del ejercicio. Nuestros principales resultados indicaron que: (1) Cuando los análisis de imágenes térmicas son realizados por evaluadores con diferentes niveles de experiencia, se debe priorizar la temperatura media y máxima debido a una mejor reproducibilidad, (2) Los cambios en la temperatura de la piel inducidos por el ejercicio muestran un efecto del sexo dependiente, (3) Los cambios en la temperatura de la piel después del ejercicio no se relacionaron con DMAT, pero hubo una relación positiva entre la temperatura máxima de la piel y el umbral de dolor por presión en la variación entre antes del ejercicio y 48 h después del ejercicio, (4) El daño muscular inducido por el ejercicio

no se reflejó en los cambios en el recalentamiento de la temperatura de la piel después de aplicar la prueba de estrés con frío 48 horas después del ejercicio. Consideramos que la información contenida en esta tesis contribuye a la formación de conocimiento en el área, con un enfoque integrador de conceptos y experimentos buscamos comprender mejor la aplicabilidad de TRI para monitorear el daño muscular inducido por el ejercicio, DMAT y el estado de recuperación después del ejercicio físico.

IX. EXTENDED ABSTRACT (SPANISH)

Introducción:

La práctica de ejercicio físico es un aliado imprescindible para mantener la salud y la calidad de vida. En este contexto, para que se puedan obtener y maximizar las ganancias, no solo en poblaciones de alto rendimiento sino también a nivel recreativo, el seguimiento y control de las cargas de entrenamiento es fundamental. Por otro lado, el ejercicio sin una planificación adecuada puede inducir daño muscular y dolor muscular de aparición tardía (DMAT). DMAT puede tener diferentes consecuencias, como pérdida de fuerza, pérdida de rango de movimiento y dolor local. Por lo tanto, es interesante que entrenadores, atletas y fisioterapeutas entiendan mejor cómo cuantificar esta situación para limitar sus síntomas.

La etiología de DMAT ha intrigado a los científicos a lo largo de los años. Actualmente se cree que la DMAT es causada no solo por uno sino por diferentes eventos correlacionados en respuesta al proceso inflamatorio desencadenado por el daño muscular. La respuesta inflamatoria tiene como objetivo eliminar los desechos y reparar el tejido dañado, sin embargo, activa la producción de varias proteasas, como la prostaglandina E2, que a su vez contribuye a aumentar la permeabilidad vascular y sensibilizar las fibras aferentes de tipo III y IV, lo que da como resultado el DMAT.

Actualmente, se han explorado diferentes enfoques para monitorear el daño muscular y el DMAT. Sin embargo, muchos de ellos son invasivas, y se necesita de tiempo de análisis (por ejemplo, en el caso los biomarcadores, en algunos casos necesitan ser analizados en un laboratorio). Además, en algunos casos son métodos costosos económicamente, como la biopsia muscular, la resonancia magnética o la cuantificación de marcadores sanguíneos como la creatina quinasa (CK). En el otro extremo, existen las medidas de percepción que tienen un carácter subjetivo como son las escalas análogas del dolor. La termografía infrarroja aparece como una posible alternativa para monitorizar la recuperación después del ejercicio bajo la justificación de que los cambios en la temperatura de la piel están correlacionados con el proceso inflamatorio

potencializado por el daño muscular y el DMAT. La termografía infrarroja es una técnica de imagen no invasiva capaz de determinar la temperatura de la superficie de un objeto o de la piel humana en función de la radiación infrarroja emitida. El coste relativamente bajo y su fácil manejo ha tenido como resultado que haya ganado popularidad en las ciencias del deporte. Sin embargo, hasta ahora, los estudios han mostrado resultados contradictorios sobre su posible uso en la determinación del daño muscular y el DMAT, y no hay evidencia que apoye el uso de la termografía infrarroja para monitorizar las adaptaciones agudas inducidas por el ejercicio.

Hipótesis y objetivos:

En este contexto, la presente tesis tiene la siguiente hipótesis general: La termografía infrarroja puede ser una herramienta útil para monitorizar las adaptaciones agudas inducidas por el ejercicio, como el daño muscular y el DMAT.

Para corroborar dicha hipótesis, se estableció como objetivo central de esta tesis de doctorado investigar si la temperatura de la piel, evaluada por termografía, en condiciones de reposo y después de la práctica del ejercicio físico, puede ser utilizada para monitorizar el daño muscular inducido por el ejercicio y el DMAT en la recuperación después del ejercicio. El objetivo central se dividió en tres objetivos específicos.

1- Determinar el efecto del nivel de experiencia del evaluador (experto vs. novato) sobre la reproducibilidad de las imágenes térmicas analizadas antes y después del ejercicio físico.
2- Determinar si el DMAT y los umbrales del dolor están relacionados con la temperatura de la piel medida mediante termografía infrarroja en hombres y mujeres.
3- Determinar el nivel de daño muscular y su asociación con los resultados de la evaluación del recalentamiento de la temperatura de la piel en las extremidades del ejercicio después de un protocolo de estrés por frío.

Para desarrollar estos objetivos se llevaron a cabo 3 experimentos originales a largo de los 4 años de esta tesis de doctorado. A continuación, se presenta un resumen de los métodos,

resultados y discusión de cada uno de ellos. Además, se presentan las conclusiones obtenidas con la realización de esta tesis doctoral.

Estudio experimental 1

Objetivos llevados a cabo:

Determinar el efecto del nivel de experiencia del evaluador (experto vs. novato) sobre la reproducibilidad de imágenes térmicas analizadas antes y después del ejercicio físico.

Publicaciones obtenidas:

da Silva W, Machado ÁS, Kunzler MR, Perez I J, Calvo MG, Priego-Quesada JI, Carpes FP (2022). Reproducibility of skin temperature analyses by novice and experienced evaluators using infrared thermography. ***Journal of Thermal Biology***. Volume 110. doi: 10.1016/j.jtherbio.2022.103345.

Método:

80 imágenes térmicas (40 imágenes de la región anterior del muslo y 40 imágenes de la región posterior de la pierna) fueron distribuidas entre dos grupos de evaluadores con diferentes niveles de experiencia. 4 evaluadores pertenecían al grupo experto (GE) y 4 evaluadores pertenecía al grupo sin experiencia previa (NG). Las imágenes fueran capturadas bajo dos condiciones (20 imágenes antes de ejercicio y 20 imágenes después del ejercicio, para las dos regiones). El ejercicio realizado consistió en repeticiones máximas de sentadillas y elevación de talones de pie hasta el agotamiento. Todas las imágenes fueron procesadas de forma aleatoria. Todos los evaluadores recibieron formación por parte de un miembro externo al grupo de los expertos que tenía una experiencia previa de 12 años en estudios de termografía (termografía de nivel I acreditada por el Infrared Training Center, y 32 manuscritos científicos sobre termografía publicados en los últimos 10 años antes de la formación). La formación se dividió en tres partes:

I) Introducción al software: En este paso, los participantes entendieron las características básicas y funciones principales del software.

II) Instrucción sobre la definición de las regiones de interés y la definición de las variables de interés: En este paso se determinaron las regiones de interés en base a referencias anatómicas de los segmentos corporales. Para la parte anterior del muslo, se instruyó a los evaluadores para que dibujaran una línea recta desde el área de la ingle hacia la parte externa del muslo y seleccionaran el área más grande posible delimitada por encima de la línea superior de la rótula. Para la parte posterior de la pierna, se instruyó a los evaluadores a trazar una línea recta desde la región más grande de los músculos de la pantorrilla hacia la parte externa y seleccionar hacia abajo el área más grande posible hasta el tobillo. Todos los evaluadores fueron instruidos a utilizar el mismo criterio de procesamiento en todas las imágenes.

III) Instrucción de adquisición de datos: Al final, se instruyó a los participantes sobre el proceso de adquisición de datos. La reproducibilidad inter examinador se determinó considerando las variables de temperatura media, máxima, mínima, desviación estándar y rango de temperatura de la piel.

Resultados y discusión:

Antes y después del ejercicio, la temperatura media mostró una excelente reproducibilidad para ambos grupos para la región del muslo (ICC > 0,98) y parte posterior de la pierna (ICC > 0,94), y la temperatura máxima mostró una excelente reproducibilidad para ambos grupos en la región posterior de la pierna (ICC >0,91). La influencia del nivel de experiencia no fue significativa considerando el muslo. De manera similar, el nivel de experiencia no afectó la temperatura media, máxima y de desviación estándar determinado para la región posterior de la pierna. Pero para la región posterior la pierna, la temperatura mínima presentó valores más bajos y el rango fue mayor entre los evaluadores novatos. La temperatura media mostró límites de

concordancia más estrechos de acuerdo con el análisis de Bland-Altman cuando fue comparado con las variables de temperatura mínima y máxima para ambas regiones y momentos.

Nuestros resultados están en línea con estudios previos que mostraron la temperatura media como el parámetro de temperatura de la piel más sólido. Por otro lado, se puede observar que la reproducibilidad de los datos térmicos cuando las imágenes son procesadas por evaluadores con diferentes niveles de experiencia depende de la elección de la variable. La mayor variabilidad de los datos presentados por el grupo novatos sugiere dificultad para seleccionar la región de interés, seleccionando áreas fuera del cuerpo humano, como los bordes de la imagen. La excelente y buena reproducibilidad observada para los datos de desviación estándar sugiere que este error fue común entre todos los evaluadores en el grupo sin experiencia. Esto a su vez influyó en los valores de temperatura mínima que tiene en cuenta un solo píxel con el valor más bajo. No obstante, Es importante tener en cuenta que la elección de la variable puede depender de la pregunta de investigación.

Sin embargo, sugerimos considerar la temperatura media y máxima cuando las imágenes térmicas sean procesadas por evaluadores con diferentes niveles de experiencia y que se consideren entrenamientos repetidos cuando la temperatura mínima y la amplitud se establezcan como variables de interés teniendo en cuenta la región posterior de la pierda. Este estudio presenta algunas limitaciones. No tenemos información sobre qué región de interés fue más costosa en términos de tiempo para analizar durante el procesamiento de imágenes. Por lo tanto, no es posible discutir si este fue un factor importante en los resultados. Además, incluimos 8 participantes en nuestros análisis, 4 evaluadores con experiencia y 4 evaluadores sin experiencia, y no está claro si los resultados podrían verse alterados al incluir una mayor cantidad de evaluadores.

Estudio experimental 2

Objetivos llevados a cabo:

Determinar si el DMAT y los umbrales del dolor por presión están relacionados con la temperatura de la piel medida mediante termografía infrarroja en hombres y mujeres.

Publicaciones obtenidas:

da Silva W, Machado ÁS, Lemos AL, de Andrade CF, Priego-Quesada JI, Carpes FP (2021). Relationship between exercise-induced muscle soreness, pain thresholds, and skin temperature in men and women. ***Journal of Thermal Biology***. Volume:100. doi: 10.1016/j.jtherbio.2021.103051.

Método:

22 participantes no entrenados (10 hombres y 12 mujeres) participaron en este estudio, evaluados en dos días de mediciones con un intervalo de 48 horas entre las mediciones. En el día 1, los participantes respondieron un cuestionario de anamnesis para recopilar información personal y se sometieron a una evaluación de caracterización. Poco después, todos los participantes fueron sometidos a un protocolo de máximas repeticiones voluntarias de sentadillas con el peso corporal para inducir el daño muscular y, en consecuencia, el DMAT. El protocolo de ejercicio implicó un número máximo de sentadillas a la mayor velocidad posible durante 1 minuto hasta el agotamiento. Entre cada serie, los participantes descansaron 15 segundos.

El protocolo se consideró terminado cuando los participantes ya no podían realizar el movimiento de sentadillas o cuando hubo una gran pérdida en la calidad del movimiento observado por el evaluador. Se tomaron medidas de termografía infrarroja antes e inmediatamente después del ejercicio, en la región del muslo y en la región del bíceps braquial como región de control.

La DMAT se evaluó con una escala numérica de dolor de 0 a 10, siendo cero sin dolor y diez la peor sensación de dolor jamás experimentada. El umbral del dolor a la presión se midió utilizando un algómetro de presión en la región proximal, medial y distal de los músculos recto femoral y vasto lateral. Las evaluaciones de DMAT, umbral de dolor a la presión, y termografía infrarroja se repitieron 48 h después del ejercicio (día 2).

Resultados y discusión:

La DMAT aumentó de manera similar en hombres y mujeres después del ejercicio (5,1 ± 2,2 puntos en comparación con 0,7 ± 1,1 antes del ejercicio, $p < 0,05$, ES = 2,3 [1,4, 3,3] y 48 h después del ejercicio (4,0 ± 2,9 puntos, $p < 0,001$, ES = 1,3 [0,7, 2,0] en comparación con antes del ejercicio. El umbral del dolor a la presión fue más bajo en las mujeres en comparación con los hombres la mayor parte del tiempo. El umbral del dolor a la presión disminuyó 48 h después del ejercicio para los hombres en comparación con los momentos antes del ejercicio e inmediatamente después ($p< 0,05$). Los valores antes del ejercicio para las mujeres no difirieron entre los otros dos momentos evaluados, pero 48h después fueron menores que después del ejercicio ($p< 0,05$).

Las respuestas de temperatura de la piel fueron dependientes del sexo. Las temperaturas media y máxima aumentaron después del ejercicio para los hombres, y la temperatura máxima se redujo 48 h después del ejercicio. En las mujeres, la temperatura mínima aumentó 48 h después del ejercicio. El DMAT no estuvo relacionado con la temperatura de la piel, pero sí que se observó una asociación directa entre la variación de la temperatura máxima de la piel y el umbral del dolor a la presión antes y 48 h después del ejercicio ($R^2 = 0,15$ $p = 0,01$).

Consideramos que nuestros resultados abren la puerta a debatir el efecto del género en el análisis de los cambios de temperatura de la piel en respuesta al ejercicio. Estudios previos han considerado principalmente a los hombres en su análisis y se supone que las inferencias son similares. Aquí demostramos que este puede no ser el caso. Las mujeres mostraron menores valores de temperatura en casi todos los momentos evaluados.

La inclusión de una medida mecánica del dolor como el umbral del dolor a la presión demostró ser muy eficaz, poniendo de manifiesto diferencias entre sexos con una reducción del umbral las 48 h del ejercicio para los hombres. Estudios previos destacan que los hombres pueden ser más susceptibles al daño muscular inducido por el ejercicio que las mujeres que muestran valores más altos de la creatina quinasa incluso cuando se normalizan por el área transversal del músculo cuádriceps. La magnitud del daño puede haber influido en la sensibilidad muscular y, por lo tanto, viéndose reflejado en valores del umbral del dolor a la presión más bajos para los hombres 48 horas después del ejercicio.

Cuando se analizan los cambios de temperatura, solo los datos de temperatura mínima de las mujeres aumentan 48 horas después del ejercicio, lo que sugiere la importancia de utilizar esta variable para detectar la hipertermia local. Además, la asociación entre la variación de temperatura y el umbral del dolor a la presión observada en nuestro estudio puede sugerir que dicho umbral y el nivel de vasoconstricción pueden estar directamente relacionados. La termografía infrarroja mide la temperatura de la piel y el musculo es un tejido profundo. Su aplicación en este contexto depende de los mecanismos de disipación del calor para la piel del musculo. El umbral del dolor a la presión tiene una directa relación con la sensibilidad muscular y por tanto con la inflamación.

Nuestro estudio tiene limitaciones. Aunque el daño muscular fue evidente, no evaluamos un marcador de daño muscular como la creatina quinasa. El daño puede considerarse presente debido a los valores de DMAT y umbral del dolor a la presión, pero los marcadores bioquímicos deben ser considerados en futuros estudios para mejorar la discusión sobre su relación con las medidas del umbral del dolor a la presión y temperatura de la piel en ambos sexos

En estudios anteriores, no se pudieron correlacionar los marcadores de daño y dolor con las mediciones de temperatura de la piel. Aquí consideramos que hemos avanzado al mostrar que la temperatura puede estar relacionada con los cambios en el umbral del dolor a la presión 48 h después del ejercicio.

Estudio experimental 3

Objetivos llevados a cabo:

Determinar el nivel de daño muscular y su asociación con los resultados de la evaluación del recalentamiento de la temperatura de la piel en las extremidades ejercitadas después de un protocolo de estrés por frío.

Publicaciones obtenidas: Under review.

da Silva W, Machado ÁS, Machado MS, Pereira MEF, Paz MM, Zacharias LMS, Morais ACL, Priego-Quesada JI, Carpes FP (2023). Preservation of baseline skin temperature despite

muscle damage and soreness: findings from cold-stress test and rewarming assessments. ***Journal of Thermal Biology***.

Método:

15 participantes no entrenados (8 hombres y 7 mujeres) participaron en este estudio. Todas las mujeres presentes en este estudio estaban en la fase lútea del ciclo menstrual. El protocolo consistió en tres días de visita al laboratorio el día 1, el día 2 y el día 4. El día 3 fue un día libre entre mediciones.

En el día 1 se tomaron medidas de termografía infrarroja al inicio y después de la aplicación de estrés por frío unilateral de la región posterior de la pierna. Además, se realizaron medidas de fuerza isométrica voluntaria máxima de los músculos flexores plantares para evaluar la fatiga.

En el día 2 se comenzó tomando medidas de extracción de sangre para determinar la actividad de la creatina quinasa, medidas de termografía infrarroja, de DMAT utilizando la escala numérica de dolor y de umbral del dolor a la presión con la utilización de un algómetro de presión. Después, los participantes fueron sometidos a un protocolo de máximas repeticiones voluntarias hasta la fatiga involucrando los músculos flexores plantares.

El protocolo consistió en un ejercicio de elevación del talón de pie con el peso corporal utilizando un metrónomo. Inicialmente, se instruyó a los participantes para que realizaran el máximo número de repeticiones voluntarias que pudieran en una serie. En base a este número de repeticiones, los participantes debieron realizar en la siguiente serie un número de repeticiones no superior al 75% respecto al número máximo de repeticiones previamente establecido. Cuando se alcanzaba este número, la serie se interrumpía y los participantes disponían de un intervalo correspondiente al tiempo de la serie anterior para iniciar la serie siguiente. El participante se consideró fatigado cuando no pudo realizar un número de repeticiones igual o superior al 50% con respecto al máximo número de repeticiones o cuando el evaluador pudo observar una pérdida considerable en la calidad y amplitud del movimiento.

Inmediatamente después del ejercicio los participantes fueron evaluados con termografía infrarroja tras el protocolo de estrés térmico de frío unilateral y se tomaron medidas de DMAT con las escalas y el umbral del dolor a la presión, así como medidas de fuerza isométrica voluntaria máxima.

Los participantes regresaron 48 horas después (día 4) para someterse nuevamente a extracciones de sangre para determinar la actividad de la creatina quinasa, evaluaciones del DMAT y el umbral del dolor a la presión, y tras esto someterse a mediciones de termografía infrarroja después del estrés por frío y la fuerza voluntaria máxima.

Resultados y discusión:

La actividad de la CK aumentó 48 horas después del ejercicio en comparación con los valores basales (742,1 ± 1474,5 vs. 6843,1 ± 1675,1 U/L, p<0,01, 95%CI de la diferencia [602, 4211 U/L], ESr = 0,7). Se puede observar un comportamiento similar para los datos de DMAT evaluados mediante la escala numérica, con valores elevados a las 48h después del ejercicio con respecto a los valores antes del ejercicio (0,2 ± 0,6 vs. 5,1 ± 2,2 puntos, p<0,001, 95%CI de la diferencia [4,0, 5,5 puntos], ESr = 0,9). El umbral del dolor a la presión no difirió entre los momentos evaluados, así como el promedio del pico de fuerza isométrica voluntaria máxima.

En relación a los datos de temperatura de la piel evaluados por termografía infrarroja, no se observaron diferencias en los valores de temperatura basales evaluados en los diferentes momentos. Además, tampoco se observó ninguna diferencia en los datos de temperatura obtenidos con la aplicación de estrés por frío. No hubo diferencia entre días para los coeficientes β_0 y β_1 obtenidos con la recta de regresión logarítmica obtenida con los valores de variación de la temperatura media de la piel tras el estrés térmico. Finalmente, el análisis de regresión lineal múltiple mostró que ninguna de las variables de estudio estuvo relacionada con los coeficientes obtenidos que se relacionan con la disminución de la temperatura de la piel y la tasa de recalentamiento después del estrés por frío.

Consideramos que nuestros resultados tienen importantes aplicaciones prácticas, ya que mostramos que tanto las mediciones de temperaturas basales como aquellas con la aplicación el

estrés térmico no refleja necesariamente la magnitud del daño muscular inducido por el ejercicio 48h después del ejercicio.

En este estudio, además de medir la temperatura basal de la piel, también utilizamos medidas de estrés térmico para analizar la tasa de recuperación. Esta estrategia se estructuró con el objetivo de generar un cambio en la actividad nerviosa simpática y sus mecanismos vasoconstrictores y así promover la vasoconstricción periférica. A su vez, tras la aplicación de estrés por frío, estos mecanismos se reducen con el fin de facilitar el riego sanguíneo y aumentar la temperatura de la piel. En este estudio nuestra hipótesis principal fue que la temperatura basal de la piel no mostraría cambios después del ejercicio, pero que la magnitud del daño muscular podría potenciar el enfriamiento de las extremidades o mejorar su tasa de recuperación. Sin embargo, nuestros resultados confirman parcialmente la hipótesis anterior. Estudios previos que exploraron la aplicabilidad del estrés térmico fueron más exitosos, por lo que algunas diferencias con nuestro estudio ayudan a comprender nuestros resultados.

Estudios previos observaron cambios en la tasa de recalentamiento inmediatamente después del ejercicio para inducir la fatiga muscular y 24 horas después de una maratón sin cambios observables 48 horas después. No podemos descartar que 48 horas después del ejercicio haya sido tiempo suficiente para que se restablecieran los cambios en los mecanismos de vasoconstricción impuestos por el daño muscular.

Además, las características de los participantes y la variabilidad de la actividad vasoconstrictora del nervio simpático de la piel podrían ser otros dos factores que nos ayuden a comprender nuestros resultados. Estudios previos han demostrado que los coeficientes obtenidos después del estrés por frío están relacionados con la masa magra y el porcentaje de grasa. En nuestro estudio, 4 participantes tenían un índice de masa corporal un poco más alto de lo esperado para su edad. Las personas con bajo porcentaje de grasa muestran una mayor tasa de recalentamiento en respuesta al ejercicio. Esta diferencia en la respuesta observada se debe en gran medida a la baja conductividad térmica del tejido adiposo. Estos factores también pueden haber influido en nuestros resultados.

Cuando analizamos los datos de temperatura basal, no se observan cambios 48 horas después, incluso con altos niveles de daño muscular cuantificados por los niveles de creatina quinasa. Es importante resaltar que la termografía mide la temperatura de la piel y el músculo es un tejido profundo. Estudios previos destacan que esta temperatura no refleja necesariamente la temperatura muscular o central. Los estudios futuros deben considerar participantes con diferentes características corporales y aptitud física para comprender mejor el posible efecto de estos factores de confusión en el recalentamiento de la temperatura de la piel después de la aplicación del estrés por frío. La posibilidad de monitorizar las variables de interés considerando una ventana de tiempo más corta, cada 12 o 24 h, por ejemplo, también ayudaría a comprender mejor la asociación en el curso temporal de los cambios de las diferentes respuestas fisiológicas involucradas en este fenómeno.

Conclusiones obtenidas en la tesis de doctoral:

Con la realización de esta tesis doctoral se obtuvieron diferentes conclusiones que se dividieron de acuerdo con los objetivos específicos planteados.

1. Reproducibilidad del análisis de imágenes térmicas

El análisis de las imágenes térmicas no se ve influenciado por el nivel de experiencia de diferentes evaluadores. La temperatura media de la piel es la variable térmica más robusta que muestra una excelente reproducibilidad para evaluadores con diferentes niveles de experiencia y ambas regiones analizadas. La reproducibilidad de los datos térmicos depende de la elección de la variable y de las regiones analizadas. Cuando se consideran la temperatura mínima de la piel y el rango, se deben considerar sesiones repetidas de entrenamiento cuando evaluadores con diferentes niveles de experiencia están involucrados en los análisis de las imágenes térmicas.

2. Relación entre el dolor muscular de aparición tardía, los umbrales del dolor y la temperatura de la piel en hombres y mujeres

En general, los cambios de temperatura de la piel después del ejercicio físico muestran un efecto dependiente del sexo. Además, los cambios en la temperatura de la piel no predicen la intensidad de la sensación de la DMAT evaluada 48 horas después del ejercicio en hombres y mujeres. La variación de la temperatura máxima de la piel está relacionada con una medida mecánica del dolor como el umbral del dolor a la presión 48h después del ejercicio. El umbral del dolor a la presión parece una medida más objetiva para cuantificar la sensación de dolor pudiendo detectar diferencias de sexo después el ejercicio físico, que no se observan con otros instrumentos de evaluación como escalas de percepción del dolor.

3. Relación entre cambios en la temperatura de la piel daño muscular y el DMAT considerando la aplicación del estrés térmico

Los cambios en la temperatura de la piel no están relacionados con el daño muscular inducido por el ejercicio cuantificado por la actividad de creatina quinasa y DMAT. La magnitud del daño muscular inducido por el ejercicio no influye en la tasa de recalentamiento de la temperatura de la piel 48 horas después del ejercicio considerando la aplicación del estrés por frío. Se recomienda precaución al suponer que los cambios en la temperatura de la piel reflejan la magnitud del daño muscular y el estado de recuperación.

X. PRESENTATION

This Ph.D. thesis was developed in collaboration between the University of Pampa (Unipampa) in Brazil and the Universitat de València (UV) in Spain in a cotutelle regime. This Ph.D. thesis is part of the Multicentric Graduate Program in Physiological Sciences from the Unipampa and the Doctoral Program in Physiology from the UV. Both universities' research groups are very interested in better understanding the possible applications of infrared thermography (IRT) in sporting Science and rehabilitation. This Ph.D. thesis was planned to explore the potential applicability of the IRT to monitoring acute adaptation induced by exercise, mainly exercise-induced muscle damage and muscle soreness (DOMS). The experiments listed here meet all the ethical and institutional requirements signed by both institutions in the cotutelle agreement.

In this context, strategies for monitoring sports performance have increasingly considered post-exercise recovery an essential phase to better manage training loads. Exercise-induced muscle damage and DOMS are important conditions to be considered. The DOMS can normally last from 24 to 7 days post-exercise and is associated with conditions of oxidative stress and inflammation of muscle tissue, generating swelling, changes in pain thresholds, and transient strength loss. It has been discussed that an inflammatory process can influence blood flow at the involved site, contributing to hyperthermia. Then comes the interest in employing a non-invasive technique to monitor these adaptations. Previous studies have investigated whether infrared thermography (IRT) can monitor acute adaptations to exercise, especially conditions of exercise-induced muscle damage and DOMS.

However, the results show significant variability. The IRT is a relatively easy-access and low-cost technique and has gained popularity among coaches and physiotherapists. Nevertheless, evidence is needed, as there is still no consensus on how measurements should be taken and how responses vary depending on the exercise, sex of participants, physical condition, and monitoring time. Therefore, the use of IRT to monitor acute adaptation is still based on a very limited theoretical framework. This Ph.D. thesis is organized into 3 phases that describe three original

experiments. Together, we hope that the experiments will help better understand how the thermography data should be evaluated and the relationships between changes in skin temperature, muscle damage, and post-exercise recovery. In the first phase, we determined the repeatability of skin temperature measurements before and after physical exercise using infrared thermography, considering different muscle groups and evaluators with different experience levels.

In the second phase, we determined the relationship between DOMS, pressure pain threshold, and skin temperature considering men and women. Finally, in the third phase, we determined the relationship between exercise-induced muscle damage, DOMS, and skin temperature, considering a cold stress protocol. In this document, we present state of the art from the literature regarding the main themes of this Ph.D. thesis. A justification of the thesis, the hypothesis and objectives, and the results of the three original manuscripts. Two manuscripts presented here are published in international journals with important relevance to the area, and one is in the submission phase. After that, we present a general discussion highlighting the main findings, conclusions, and future research perspectives regarding the topic addressed in this thesis.

Chapter I

State of art

1. CHAPTER I

1.1 STATE OF ART

1.1.1 THEORY OF MUSCLE CONTRACTION

Considering that exercise-induced muscle damage and DOMS occur mainly after unaccustomed eccentric actions, it is fundamental to review the primary mechanism that describes muscle contractions and the muscle structure to explain how muscle damage and DOMS occur and their consequences. The sliding filament theory of muscle contraction proposed by Andrew Huxley´s model (Huxley & Hanson 1954; Huxley & Niedergerke 1954) was the first mechanistic description of how a molecule can couple the energy released by a chemical reaction to produce work in a physiological cyclic process (Astumian 2015). The muscles are molecular structures present in the human body that can convert chemical energy in the production of force (Enoka 2015) and, therefore, have a significant influence on the execution of the movement of body segments.

The Sarcomere is the basic contractile unit of the muscle fiber striated and comprises and set of thin and thick contractile proteins (actin and myosin) (Sweeney & Hammers 2018). For the sliding filament theory, muscle contraction occurs by sliding two sets of filaments (Herzog 2009). This structure allows the connection between them for muscle contraction, generating force and contraction (Enoka 1994). The sarcomeres are organized in series to make up a myofibril and are defined as spanning from Z-line to Z-line (Sweeney & Hammers 2018). The myofilaments are distributed in different bands across the sarcomere; for instance, the thick filaments comprise the dark band called band A, and the thin filaments comprise the I band and also are attached to a central transverse band knowledge M and Z band, respectively (Figure 1) (Enoka 2015; Herzog 2009).

When the stimulus for force production occurs, the neurotransmitter acetylcholine (ACh) is released in the synaptic gap in response to the axonal action potential and will bind to the

postsynaptic nicotinic receptor resulting in the opening of specific channels, causing the influx of Na+ in the muscle fiber. This movement results in a synaptic potential in the sarcolemma. This potential propagates across the membrane until it reaches the T tubules, causing the release of Ca+2 ions from the smooth sarcoplasmic reticulum into the cytoplasm. Thus, Ca+2 will inhibit the regulatory action of troponin and tropomyosin, proteins present in the thin filaments, with the release of actin sites for binding by myosin, in the process called the cross-bridge cycle (Enoka 2015; Herzog 2009; Huxley & Hanson 1954; Huxley & Niedergerke 1954).

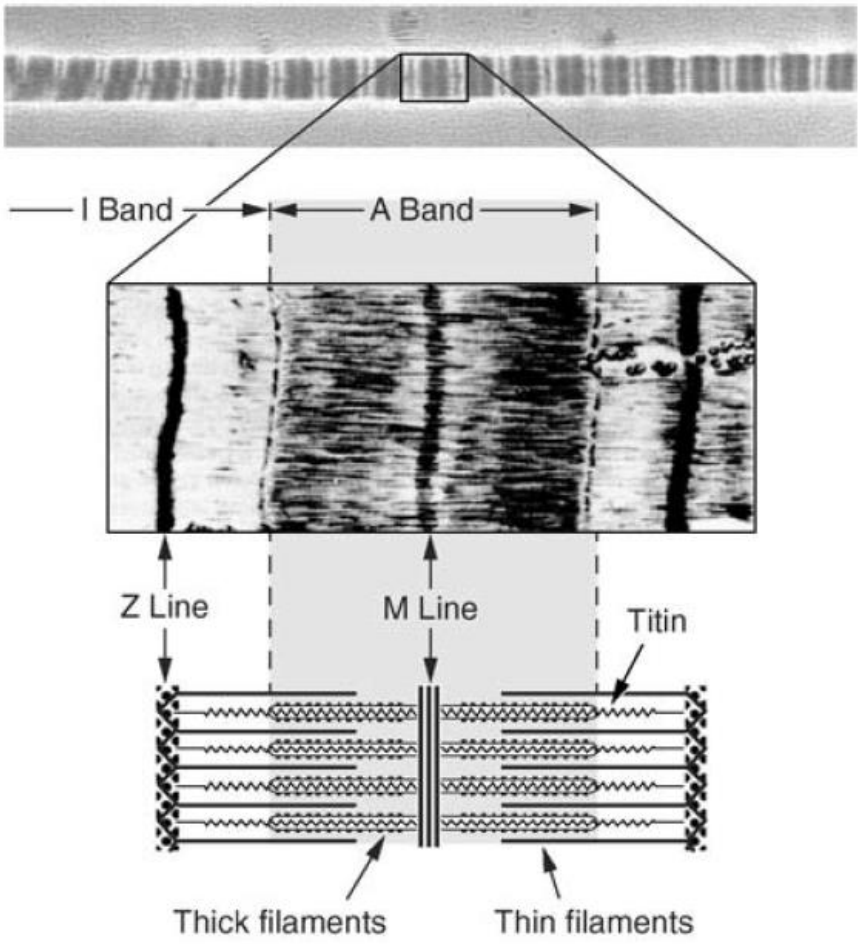

Figure 1.1 The sliding filament theory. Top panel: Micrograph of a series of sarcomeres from a single myofibril. Middle panel: An isolated sarcomere bordered by the z-lines containing myosin in the A-band region and actin in the I-band region. Bottom panel: A schematic representation of an isolated sarcomere with z-lines, thick filaments, thin filaments, and titin identified: Figure obtained from Herzog 2009.

In the cross-bridge cycle, the myosin head binds to specific sites on actin, and during this process, the hydrolysis of the adenosine triphosphate (ATP), molecule bound to the myosin head will occur. Consequently, there is a release of energy (power stroke) for the cross bridge to happen, promoting the sliding of the actin filaments toward the center of the sarcomere, and the result is sarcomere shortening, and the sum of several sarcomeres shortening results in muscle contraction (Enoka 2015). Each cross-bridge is associated with the hydrolysis of one ATP molecule (Herzog 2009).

Over the years, the proposed cross-bridge models have moved from two interaction stages (attached and detached) to a multistage model with, at a minimum, one detachment stage and two attachment stages (Herzog 2009). Moreover, conformational changes were proposed to occur in the light chain binding domain during cross-bridge rotation around a hinge in the myosin head (Herzog 2009; Rayment et al. 1993a,b). Also, it is essential to highlight the role of titin, a third filament that contributes actively to muscle contraction (Herzog 2014).

The process of muscle contraction can result in three different muscle actions: isometric, concentric, and eccentric. In an isometric muscle action, the torque produced by the muscle is equal to the external resistance torque, and the muscular contraction does not generate joint movement (Enoka 2015). However, if the torque produced by the muscle is greater than the resistance torque, muscle shortening causes movement overcoming the external load, which defines a concentric action (Enoka 2015). But, in eccentric actions, the torque produced by the muscle structure cannot overcome the torque produced by the external resistance. In this way, it is elongated while producing force (Enoka 2015). The eccentric actions are knowledge for producing a great amount of muscle strength and have great applicability in the training program and rehabilitation. However, the eccentric actions are knowledge for induced exercise-induced muscle damage and, consequently, DOMS (Hody et al. 2019). The process that describes exercise-induced muscle damage will be reviewed below.

1.1.2 EXERCISE-INDUCED MUSCLE DAMAGE: A SEQUENCE OF EVENTS

As well as the process of muscle contractions previously reviewed, exercise-induced muscle damage occurs in response to a complex chemical and mechanical interaction potentiated mainly after eccentric exercise. Performing an eccentric action with intensity or volume that an individual is not accustomed to can result in exercise-induced muscle damage (Markus et al. 2021).

Muscle damage can be initiated due to a lack of homogeneity in the sarcomere structure (Morgan 1990). The weakest sarcomeres will absorb most of the length changes, and depending on the length-tension ratio, they may be stretched beyond the point of myofilaments overlap, resulting in disrupted or "popped" sarcomeres (Morgan 1990; Peake et al. 2005). The structural disruption of the sarcomeres may damage adjacent areas in the muscle and lead to damage to the membranes of the sarcoplasmic reticulum, T tubules, or the sarcolemma (Peake et al. 2005). The process of exercise-induced muscle damage can be divided into two stages (figure 2): Initial mechanical damage and metabolic stress followed by the exercise and a secondary stage post-exercise that involves an inflammatory response (Hody et al. 2019; Markus et al. 2021).

During eccentric action, the muscle recruits a fewer number of motor units (Douglas et al. 2017), and as a result, a smaller cross-sectional area absorbs mechanical stress (Brown et al. 1997). This damage induced by the high mechanical stress affects several structures of the muscle resulting in disturbances in Ca^{+2} homeostasis that, once present in high concentration inside the muscle cells, can induce the activation of muscle proteases (calpains) (Hody et al. 2019). They are responsible for cleaving important structural proteins, such as desmin and alpha-actin, in charge of myofibril integrity (Hody et al. 2019). The activation of calpains may damage membrane constituents that also contribute to increasing calcium entry in the mitochondria, which in turn is associated with the opening of the mitochondrial permeability transition pore (mPTP), leading to the activation of cell death signaling, altering its function, and also leading to mitochondrial disfunction (Hody et al. 2019). These processes can produce oxygen-reactive species (ROS) and neutrophils and macrophages activation (Peake et al. 2005).

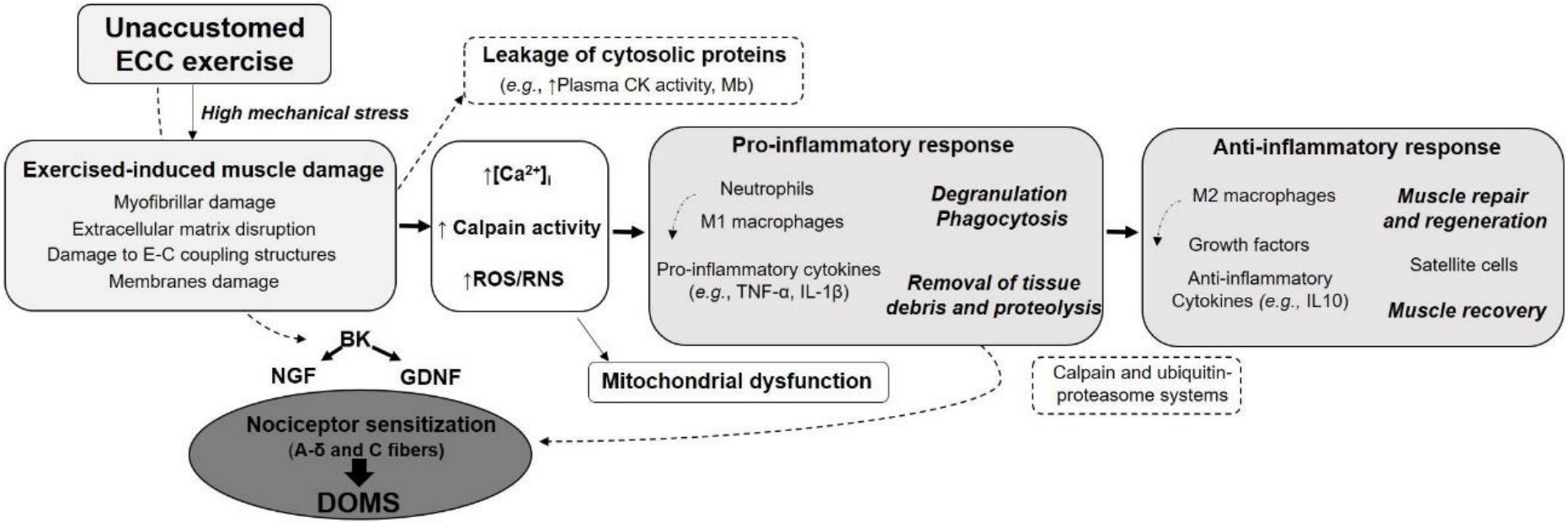

Figure 1.2 Summary of the main mechanisms induced by eccentric muscle action. BK: Bradykinin; Ca^{+2}: Calcio; CK: Creatine kinase; ECC: Eccentric exercise; GDNF: Glial cell line-derived neurotrophic factor; IL-10: Interleukin 10; IL-1β: Interleukin-1 beta; MB: Myoglobin; NGF: Nerve growth factor; RNS: Reactive nitrogen species; ROS: Reactive oxygen species. Figure obtained from Hody et al. 2019.

Proteins chemoattractant release in response to the alteration of membrane permeability are involved in the recruitment of circulating inflammatory cells that initiate the pro-inflammatory stage through phagocytosis and by releasing proteolytic enzymes and ROS (Hody et al. 2019). If the presence of ROS is greater than the available antioxidants can cause oxidative stress (Halliwell & Gutteridge 1984). The Oxidative stress can damage cellular structures, such as cell wall lipids, giving rise to a lipid peroxidation reaction and generating a cycle of new ROS in sequence, which can damage other cellular structures, even tissues, thus potentializing the inflammation (Urso & Clarkson 2003). The inflammatory process has two phases, including activating anti-inflammatory and pro-inflammatory mediators (Markus et al. 2021).

The accumulation of the pro-inflammatory macrophages (M1) contributes to the phagocytosis of tissue by secreting pro-inflammatory cytokines how TNF-a, IL-6, and IL-1b and secretory leukocyte protease inhibitors. Neutrophils and macrophages (M1) interact to regulate the pro-inflammatory response of muscle damage, and its influx inside the tissue may, paradoxically, exacerbate the cellular alteration (Hody et al. 2019).

In turn, macrophages (M2) will mediate the anti-inflammatory stage and muscle regeneration through the production of anti-inflammatory cytokines and molecular signaling that are involved in tissue regeneration. IL-6 seems to play an essential role by stimulating the production of anti-inflammatory cytokines, including IL-10, IL-1ra, and soluble TNF-α receptors (Petersen & Pedersen 2005). The transition between macrophages M1 to M2 is a stage that markets the changes between pro-inflammatory to anti-inflammatory stages, but many other cell types are essential in this complex process of inflammation and regeneration (Hody et al. 2019).

Exercise-induced muscle damage is a complex phenomenon; several factors, such as sex, age, nutrition, fitness level, and genetics, can influence the recovery time and the severity of its symptoms (Markus et al. 2021). Some symptoms are commonly associated with muscle damage, such as strength loss, increased muscle proteins in the circulation, which represent membrane damage, and delayed onset muscle soreness (DOMS) (Peake et al. 2005). In addition, it is essential to highlight that the inflammatory process is fundamental to remodeling and recovering tissue damage. Several studies have focused on strategies to monitor these adaptations and accelerate

the recovery period. DOMS is one of the main symptoms associated with muscle damage. The etiology of DOMS is reviewed below.

1.1.3 DELAYED ONSET MUSCLE SORENESS

Delayed onset muscle soreness (DOMS) is a sensation of discomfort often experienced by novice or elite athletes following intense or unaccustomed eccentric physical exercise (Cheung et al. 2003). The DOMS can be classified as a type I of muscle strain and is thought to be a consequence of the damage in the muscle structure induced by the exercise (Lewis et al. 2012; Safran et al. 1989). DOMS often presents peaks between 48h to 72h after the exercise and may last until seven days after the training session (Connolly et al. 2003). The typical symptoms related to DOMS are muscle tenderness, loss of range of motion, stiffness, swelling, and local pain (Lewis et al. 2012). DOMS can range from discomfort that can disappear during daily activities to severe debilitating pain that can restrict movement (Cheung et al. 2003). If we analyzed the timeline of each symptom isolated, the strength loss usually shows peaks immediately post-exercise or within the first 48 hours, with a return to baseline levels generally taking more than five days after. The pain and tenderness are usually concentrated in a distal portion of the muscle and show a peak between 1 to 3 days post-exercise and generally disappear within approximately seven days after (Connolly et al. 2003). Stiffness and swelling usually show peak between 3 and 4 days after exercise and typically resolve within ten days (Connolly et al. 2003).

The origin and progression of DOMS have intrigued scientists for many years. Different theories have been presented about its etiology, such as muscle spasm theory, lactic acid theory, connective tissue theory, ruptured tissue theory, enzyme influx theory, and fluid theory (Gulick & Kimura 1996).

The lactic acid theory, for instance, is based on the assumption that lactic acid would continue to be produced a long time after the exercise session ended and that the toxic metabolic products released potentiated the sensation of DOMS experienced 48 hours after (Armstrong 1984; Cheung et al. 2003; Francis & Hoobler 1987; Gulick & Kimura 1996). However, today it

is known that lactic acid returns to baseline values about one hour after exercise and that it is not related to DOMS but related to the acute pain experienced immediately after exercise in a condition of fatigue induced by exercise (Gulick & Kimura 1996). This theory has already been refuted.

On the other hand, the spasm theory was introduced based on the assumption that we have an increase in resting muscle activity after the exercise ended and that this increase would indicate the presence of a localized tonic spasm of the motor units, generating compression of the blood vessels and accumulation of substances that would induce the pain (Cheung et al. 2003; De Vries 1961). There is no evidence to support this theory, and it remains an object of debate.

The connective tissue theory was proposed based on the role of the connective tissue around the muscle fibers and its differences in composition and content. For this theory, fibers of type II have a less robust structure than fibers of type I, and these fibers would be more susceptible to injury by stretching of the connective tissue, which would eventually induce the DOMS sensation (Cheung et al. 2003; Hough 1902; Stauber 1989). Also, there is no evidence that supports this theory.

In general, one isolate theory cannot explain the etiology of DOMS, but a sequence of unique events is proposed integrating aspects addressed in several previous approaches to explain the etiology of DOMS (Cheung et al. 2003). The most supported theory is that biochemical, thermal, and mechanical changes associated with the inflammation process sensitize small muscles afferent to type III and IV, leading to the DOMS sensation (Figure 3) (Fridén & Lieber 1992). The release of Ca+2 into the cell after the cell membrane damage is responsible for activating several proteases, for instance, phospholipase A2 (Connolly et al. 2003). It can cleave arachidonic acid from the phospholipids of the cell membrane, resulting in free arachidonic acid, which in turn gives rise to inflammatory mediators how thromboxanes, prostaglandins, and leukotrienes (Connolly et al. 2003).

The metabolism of arachidonic acid can follow 1 or 2 pathways: The cyclo-oxygenase (COX) pathway, which leads to the production of prostaglandins E2 (PGE2) and thromboxanes or the lipoxygenase (LIPOX) pathway; that potentialize the production of leukotrienes (Connolly

et al. 2003). The PGE2 increases the vascular permeability and is responsible for the pain perception in the muscle by sensitizing the type III and IV afferent nerve fibers, whereas leukotrienes also increase vascular permeability and are responsible for attracting neutrophils to the site of structure damage potentializing oxidative damage (Connolly et al. 2003; Hertel 1997; Vane & Botting 1987).

Furthermore, the post-exercise chemical response is accompanied by intramuscular edema, and it is thought to be one of the mechanisms responsible for nociceptor activation and subsequent soreness sensations (Markus et al. 2021). Another pathway is proposed to be associated with DOMS (Markus et al. 2021). The pathway involves the activation of B2-bradykinin receptors which are released during exercise, and the nerve growth factor (NGF). For this theory, bradykinin triggers the process associated with mechanical hyperalgesia by the up-regulation of NGF through B2 receptors leading to DOMS (Hody et al. 2019; Paulsen et al. 2010). Also, the development of hyperalgesia may be associated with inflammation in the extracellular matrix (Peake et al. 2017).

In this complex etiology, the inflammation process plays an essential role in its development, although to date, the complete mechanism of delayed onset muscle soreness is not clearly understood (Markus et al. 2021). In addition, to several symptoms experienced in response to exercise-induced muscle damage and DOMS conditions, some strategies were developed to monitor these adaptations, contributing to better recovery and organization of training load. However, many of these strategies can be expensive or invasive, not allowing an immediate evaluation. In the next section, we will review the main tools for monitoring muscle damage and DOMS applied in sports science.

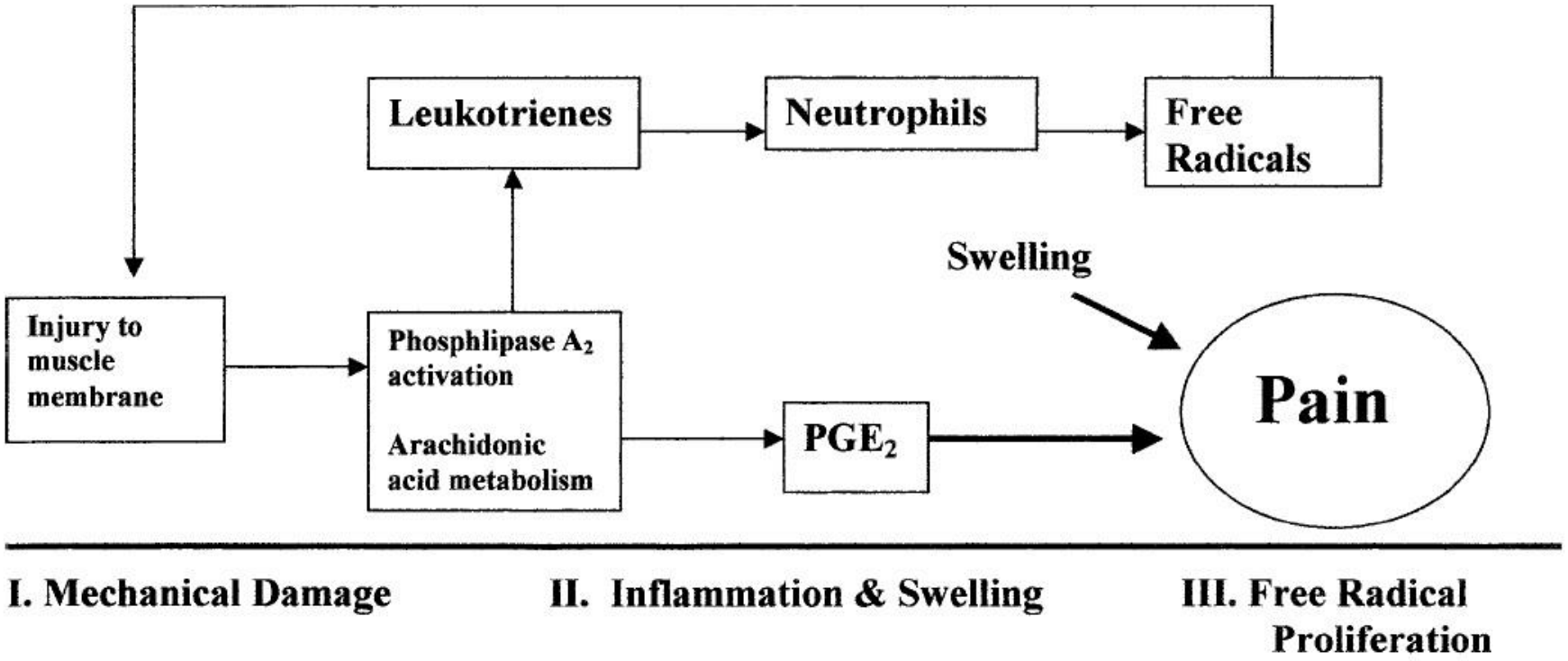

Figure 1.3 Proposed sequence of events from delayed onset muscle soreness (DOMS). PGE_2; Prostaglandin E2. Figure modified from Connolly et al. 2003.

1.1.4 STRATEGIES TO MONITOR EXERCISE-INDUCED MUSCLE DAMAGE AND DELAYED ONSET MUSCLE SORENESS

Over the years, several strategies have been developed to monitor recovery after physical exercise (Markus et al. 2021). In this section, we will focus on the main methods that were developed to monitor exercise-induced muscle damage and DOMS. The exercise-induced muscle damage result in an increase in muscle membrane permeability, causing leakage of different muscle proteins, for instance, creatine kinase (CK), lactate dehydrogenase (LDH), and myoglobin (MB) into the circulation, sugaring muscle fibers damaged (Brancaccio et al. 2010). For this reason, blood marker analysis is a useful tool for evaluating exercise-induced muscle damage, providing important information about recovery status (Markus et al. 2021). Perhaps, CK is the most used blood marker in the practical field to quantify muscle damage and, eventually, overtraining status (Lee et al. 2017). CK is a protein that catalyzes the exchange of high-energy phosphate bonds between phosphocreatine and adenosine diphosphate (ADP) (Kohlmeier 2015). At least three isoenzymes can be found (CK-MM, CK-BB, CK-MB) in the cytoplasm, each offering specific information about certain types of tissues(Brancaccio et al. 2010). The CK-MM is found in skeletal muscle, and its alteration suggests damaged muscle fibers; CK-MB is found in heart muscle and CK-BB from brain damage (Borrayo-Sánchez et al. 2006; Fe et al. 1983; Nigro et al. 1983). The CK usually shows a peak of serum concentration 24 to 48 h post-exercise, and its magnitude reflects the extent of muscle damage and cellular necrosis (Markus et al. 2021).

Another blood marker important in the context of monitoring exercise-induced muscle damage is the MB (Markus et al. 2021). Three MB isoforms are expressed in the human muscle, performing functions like transport and oxygen storage and nitric oxide (NO) regulation at the microvascular and tissue level (Jürgens et al. 2000; Plotnikov et al. 2009). Some aspects seem to differ between CK and MB as muscle damage markers. For instance, CK and MB show different timelines. MB is a smaller molecule that shows a peak of its concentration immediately post-exercise; changes in its concentration also indicate muscle fibers damaged (Pedersen & Fischer 2007).

Table 1.1 Methods to assess exercise-induced muscle damage and DOMS. Table modified from (Markus et al. 2021).

Parameter	Invasive	Non-invasive
Mechanical muscle damage	Muscle proteins (e.g., CK, LDH, MB).	Magnetic resonance imaging Ultrasound Edema Swelling
Inflammation	Pro and anti-inflammatory cytokines (e.g., IL-6, IL-8, TNF-α). White blood cells (e.g., neutrophils, macrophages) C-creative protein	
Delayed onset muscle soreness (DOMS)		Visual analog scales (VAS) and numeric rating pain scales (NPRS). Pressure pain threshold (PPT)
Performance and related measures		Muscle strength using maximal voluntary contraction and /or one maximum repetition. Vertical jump Economy/ efficiency
Muscle regeneration	Muscle biopsy	Magnetic resonance imaging Ultrasound

In turn, the LDH is a protein involved in the process that interconverts pyruvate and lactate and concomitant interconversion of NADH and NAD (Brancaccio et al. 2010). It is an enzyme described by having five forms isoforms, LDH1, LDH2, LDH3, LDH4, and LDH5, formed by the combinations of M-polypeptide and H-polypeptide that play different functions. The polymer M performs the activity of catalyzing the conversion of pyruvate to lactate, and the H monomer contributes positively to the increase in the aerobic oxidation of pyruvate (Brancaccio et al. 2010). The peak of its concentration variated depending on the intensity or duration of the effort. When resistance exercise (RE) and aerobic exercise (60 and 80% to VO2máx) were compared, the LDH showed a peak of its concentration immediately after exercise to RE and 24 hours after aerobic exercise (Callegari et al. 2017), changes in its concentration also indicate tissue muscle damage (Brancaccio et al. 2010; Callegari et al. 2017).

In addition, measures of muscle functionality like strength loss are a valid and important approach to monitoring exercise-induced muscle damage, and this process is often assessment by maximal voluntary isometric or concentric contractions (Markus et al. 2021). Depending on the exercise stimulus, the strength loss can be attributed to the damage to the sarcoplasmic reticulum and disturbances in the Ca^{2+} homeostasis in the muscle fibers or from compromised conduction velocity of action potentials across the sarcolemma and alteration in central nervous system activity and motor unit recruitment (Dartnall et al. 2008; Isner-Horobeti et al. 2013; Paulsen et al. 2012; Piitulainen et al. 2010; Prasartwuth et al. 2006).

When we talk about DOMS, in the absence of a gold standard for pain, behaviors measurements like scales of pain are reliable tools to monitor the pain sensation (Hawker et al. 2011). Generic unidimensional pain questionnaires like visual analog scales (VAS) and numeric rating pain scales (NPRS) are typically applied (Alburquerque Santana et al. 2022; Stewart et al. 2020). The VAS is a continuous scale comprised of a horizontal or vertical line, usually 10 centimeters (100 mm) in length, and traditionally anchored by two verbal descriptors: “no pain” refers to a score of 0, and “pain as bad as it could be or worst imaginable pain” to refer to score 100. It is a self-reported scale, and the participants are asked to place a line perpendicular to the VAS line at the point the better represent their pain intensity; the score is determined by the

distance between the word “no pain” and the participant`s marks providing a range of scores from 0-100 (Hawker et al. 2011).

Another scale widely applied to measure pain intensity is de NPRS. It is also a unidimensional measure of pain intensity usually formed by 11 items, and it is a numeric version of VAS. For this scale, the participants are asked to report the pain sensation based on values of 0 to 10. The value that better represents their pain sensation, higher scores represent greater pain intensity. The NPRS also is anchored by words that describe the pain severity, with 0 representing “no pain” and 10 representing “pain as bad as you can imagine” (Hawker et al. 2011).

The VAS and NPRS are unidimensional measures that consider pain intensity perception (Hawker et al. 2011; McCormack et al. 1988). However, DOMS is a complex phenom that is accomplished by several symptoms. In this context, a measurement like pressure pain threshold (PPT) could provide important information about DOMS once the PPT considers the muscle tenderness that is changed by the inflammation process (Fleckenstein et al. 2017). The PPT can be measured by a digital algometer, a tool that has been used in different adult populations in a condition of pain (Kelly-Martin et al. 2018; Park et al. 2011; Vučinić et al. 2018).

It is essential to highlight that all these methods used to quantify muscle damage or DOMS have limitations. When we talk about the analysis of blood markers of muscle damage, these analyzes require invasive approaches and trained professionals for blood collection, as well as being unable to provide immediate information about the magnitude of muscle damage since analyses take time and have a high economic cost. Also, the appearance of CK in serum after physical exercise with low or moderate intensity could represent a disturbance in the muscle energy processes and not represent muscle cell damage (Baird et al. 2012). Regarding force loss measurements, the post-processing of force data can be unintuitive.

On the other hand, pain scales do not provide an objective quantification of post-exercise recovery as seen after a rehabilitation program (Elfving et al. 2016). Older patients may have difficulty completing the VAS due to cognitive impairments or motor skill issues (Hawker et al. 2011). Moreover, the VAS and NPRS evaluate only the pain intensity and do not capture the complexity of the pain experience or improvements due to symptom fluctuations (Hawker et al.

2011). As a result, we highlight the importance of exploring new assessment methods that may allow rapid and objective quantification of exercise-induced muscle damage and DOMS, providing possible rapid feedback to the post-exercise recovery status.

1.1.5 INFRARED THERMOGRAPHY: WHY IT CAN BE AN ALTERNATIVE?

Infrared thermography (IRT) is an imaging technique that allows recording of the infrared radiation emission from a body and, based on this energy, estimates its surface temperature (Priego Quesada 2017). In this chapter, we will review the main historical milestones that have contributed to the evolution of IRT over the years, as well as basic concepts and physics principles of infrared thermography and the main physiological mechanisms that support the possible applicability of IRT to monitor exercise-induced muscle damage and DOMS.

Throughout history, several events contributed to the evolution of IRT to the technique that we know today. The discovered of Infrared radiation in the 1800s by Sir Frederick William Herschel is one of the most important historical landmarks (Herschel 1800; Priego Quesada 2017). Sir Frederick was born in 1738 in the city of Hannover in Germany, and he was interested in understanding how much heat passed through different colors by looking at sunscreens. He established this objective by noting that the amount of heat depended on the color, as he believed that the color could filter the amount of heat emitted. To answer this central question, Herschel directed sunlight through a glass prism creating a visible spectrum, a rainbow with different colors. In this way, he measured the temperature of this visible spectrum using mercury thermometers positioned in different colors and beyond the spectrum as a control condition. He observed that the amount of heat increased from the color violet to red (Herschel 1800). However, the temperature was even higher near the red spectrum, where there was no longer the presence of visible light. This experiment demonstrated for the first time the existence of energies that were not visible to the human eye. This energy came to be known later as infrared radiation.

In 1840 John Frederick William Herschel, son of Sir. Frederick repeated the historical experiments of his father, and by applying a method called evaporography, he was able to perform

the first thermogram in history using solar radiation (Ring 2006). Currently, this term is still used in studies involving IRT. After that, significant advances were observed in the development of infrared detectors. In this context, we can highlight Samuel Pierpont Langley, the inventor of the Langley Bolometer in 1880 (Rogalski 2002; Vardasca & Simoes 2013). The Langley Bolometer was a device capable of measuring electromagnetic radiation by increasing the resistance of an electrical conductor, potentiated for years after the creation of its prototype. The bolometer contributed to the development of current thermographic cameras (Priego Quesada 2017).

This technology continued to be potentiated throughout history with the development of modern infrared photon detectors at the end of World War II for military purposes (Ring 2006; Rogalski 2002). After the end of World War II, the technique was released for civilian use. With the discovery in 1934 that the human skin is an excellent emitter of infrared radiation (Hardy 1934), the IRT began to be explored as a possible medical diagnostic tool for several diseases or injuries (Lahiri et al. 2012). However, we have registered that the first human thermography was done in 1928 in Germany (Czerny 1929).

When we talk about IRT, any object with a temperature above absolute zero in the kelvin scale (0 K) emits electromagnetic energy depending on its temperature and can be a study object of IRT (Priego Quesada 2017). For this reason, IRT has been explored in different areas of knowledge. It has been applied in engineering to monitor buildings, military or surveillance sectors, and electrical installations to monitor the surface temperature from electrical components, veterinary, human medical science, and sport science (Priego Quesada 2017).

The first study published in sports science in 1975 by Keyl and Lenhart (Keyl & Lenhart 1975) was focused on sports medicine. This study, titled "Thermography in sports injuries and lesions of the locomotor system due to sport," assessed 82 patients and 52 athletes with different injuries and observed hyperthermia in the injury area (Keyl & Lenhart 1975). Another important study was published in 2010 by Merla and colleagues (Merla et al. 2010) titled "Thermal Imaging of Cutaneous Temperature Modifications in Runners During Graded Exercise." In this study, the authors assessed the skin temperature dynamic after running and incremental teste until to maximal heart rate. They showed that skin temperature decreases during all running test in

different body regions and return to the baseline levels in the recovery phase. This study is very important because it can be considered the first study that used infrared cameras with modern technology (Priego Quesada 2017).

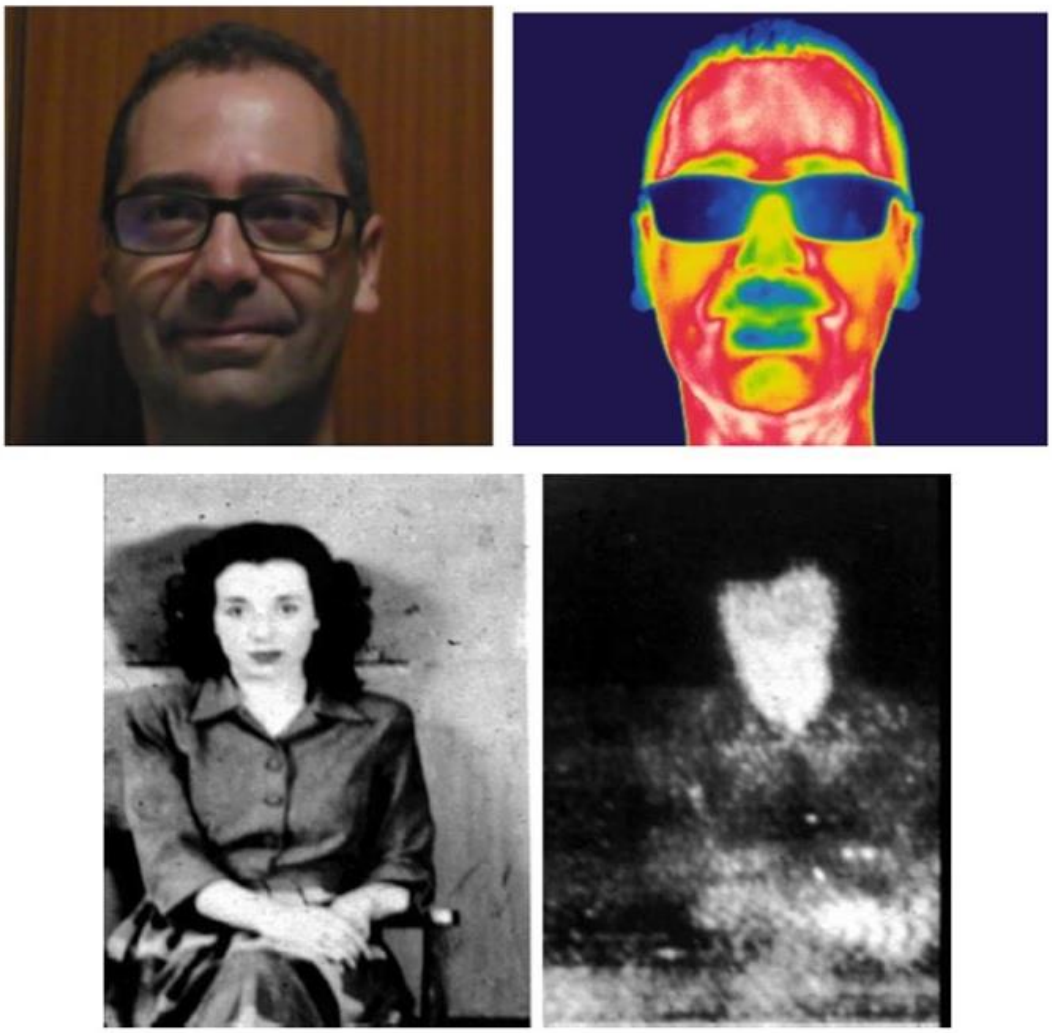

Figure 1.4 Evolution of infrared thermography images: Top of panel: A photography and infrared thermography of a man using current infrared thermography cameras. Bottom of panel: Photography and thermography of one woman taken in 1949. Figure modified from (Priego Quesada 2017; Ring 2006).

In addition to the important historical milestones, it is necessary to have a basic knowledge of concepts and physics principles related to IRT. These points will be reviewed below. Initially, we can highlight the concept of heat and temperature. Heat is energy in transit that passes from a hot body to a cold body, and its unit of measure is the joule (J) (Priego Quesada 2017). Temperature is the measure of the body's internal energy; a warm body decreases your internal energy, and a cold body increases your internal energy due to heat transfer (Priego Quesada 2017). Therefore, heat is the heat transfer that only occurs when we have two bodies with different temperatures (Priego Quesada 2017). This process always occurs following a gradient, from the hotter body to the cooler body, in a process called heat flow or heat flux (Priego Quesada 2017). The process of heat transfer is often explained by three mechanisms: Conduction, convection, and radiation.

Conduction is the heat transfer mechanism by contacting two solid bodies with different temperatures (Priego Quesada 2017). In the human body, this process takes place between the different structures of the body and depends mainly on the temperature gradient between the muscle and the skin and the conductivity characteristic of the muscle (González-Alonso 2012; Priego Quesada 2017). The temperature gradient can be increased during exercise, enhancing this process. But heat transfer by conduction is considered a slow and insignificant process in the human body. It is only significant when the human body is in contact with highly conductive surfaces for a long time, how to rest barefoot on a cold surface.

In contrast, convection is a heat transfer mechanism by contacting a solid body with a liquid or gaseous element like water or wind (Priego Quesada 2017). This heat transfer mechanism in the human body may explain heat dissipation via blood flow to the skin. Since the blood flow through the core is heated, it heats the skin as it passes in contact with it (Priego Quesada 2017).

Finally, radiation is the heat transfer of a body by the emission of electromagnetic radiation (Priego Quesada 2017). As we highlighted previously, according to the Stefan-Boltzmann Law, all bodies with a temperature above absolute zero (0K) emit electromagnetic radiation. The radiation is the energy detected by IRT. Infrared radiation is often called thermal

radiation because there is a relationship between temperature and infrared radiation. Temperature is associated with the movement of molecules. When we have more heat we have, consequently, we have more movement and kinetic energy (Priego Quesada 2017). Therefore, changes in the speed and agitation of the molecules from a body result in the emission of infrared radiation. This concept hopes us to understand how it is possible to determine the surface temperature of a body through the infrared radiation emitted (Priego Quesada 2017).

Another important concept to note is emissivity. The emissivity is a number that may vary between 0 and 1 and is the ratio of the actual amount of infrared energy emitted by a body when compared with the theoretically perfect amount that could be emitted (Bernard et al. 2013). The value of emissivity varies between different bodies. For this reason, knowing this concept is very important in studies with IRT. The human skin emissivity was determined with values between 0.97 and 0.99 ± 0.01 (Steketee 1973). This means that the human body is an excellent emitter of infrared radiation, which allows the estimate of its surface skin temperature with accuracy (Priego Quesada 2017).

The black body is an important concept to consider when the laws of physics that govern infrared radiation are studied (Priego Quesada 2017). The blackbody is the perfect emitter of infrared radiation because it absorbs all electromagnetic radiation emitted upon it, and its absorption is equal to its emission; no energy is reflected or passes through to the blackbody (Priego Quesada 2017; Vollmer & Möllmann 2018). The Stefan-Boltzmann's law (Figure 4) is the main law that governs the IRT, and it establishes that the total emissive power or energy radiated is proportional to the fourth power of its absolute temperature. In this way, we can observe that small changes in temperature values may result in significant changes in emissive power since the temperature is expressed as the fourth power (Priego Quesada 2017).

$$E = \sigma * T^4$$

Equation 1.1 Stefan-Boltzmann's law: E is the total emissive power. σ is the constant of Stefan-Boltzmann ($5.67 * 10^{-8}$ W/m^{-2} K^{-4}). T is the temperature in Kelvin. The Equation was obtained from (Priego Quesada 2017).

Perhaps the area of knowledge that most influenced sports science was medical science (Priego Quesada 2017). In medicine, IRT has been explored as a possible diagnostic tool for several pathologies, like a previous diagnostic for breast cancer, peripheral vascular diseases, diabetic neuropathies, and obstetrics and gynecology, among others (Ring & Ammer 2012). As a result, the use of IRT in sports science not only as a diagnostic tool but also as a prediction tool has gained popularity, mainly based on studies that seek to predict the risk of injury through thermal asymmetries (Gómez-Carmona et al. 2020).

These studies are based on the fact that human skin seeks to establish a constant thermal pattern over time, aiming to maintain the human body in a thermal balance and, thus, maintaining physiological functions within normal limits (Campbell 2008; Priego Quesada 2017). In this sense, IRT has been explored to detect possible thermal asymmetries potentiated for the presence, for instance, of a ligament injury (figure 5) or an inflammation process and consequently a risk of future injury (Gómez-Carmona et al. 2020; Hildebrandt et al. 2010). Considering the monitoring of injury risk, values higher than 0.8-1.0 ºC are usually considered a reference value to "prevention," and therefore, the monitoring by the athlete's medical team is suggested (Priego Quesada 2017). However, there is still no consensus about this applicability, which is still under debate.

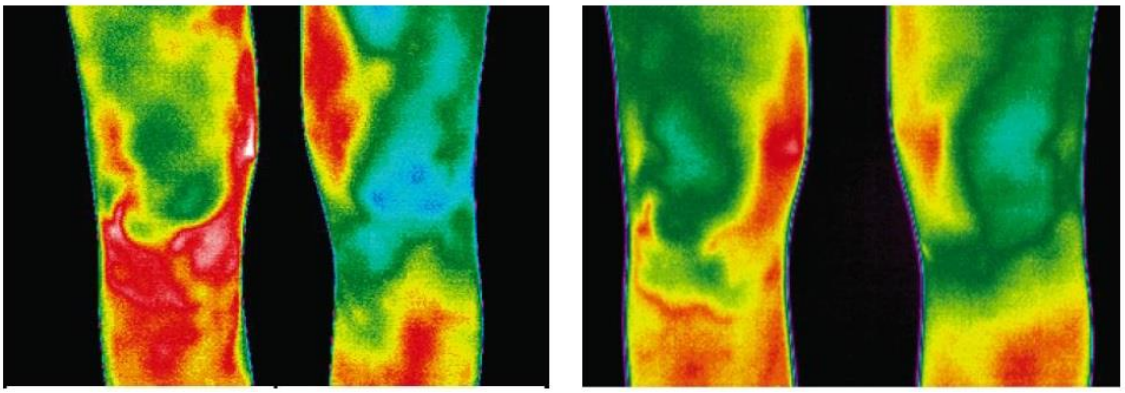

Figure 1.5 Infrared image of the anterior aspect of the knees (ACL rupture in the right knee). Left of the panel: The image was taken six weeks following an isolated ACL rupture of the right knee. Right of the panel: The image shows the same knee following six months of an extensive rehabilitation program. ACL: Anterior cruciate ligament. Figure obtained from (Hildebrandt et al. 2010).

When we talk about monitoring training loads, the main hypotheses that sustain the possible applicability of IRT to monitor exercise-induced muscle damage and DOMS is based on the fact that when the muscle damage occurs, an inflammatory process is instituted. With this process, various immune cells are recruited to the site of injury, resulting in muscle edema and an increase in muscle temperature (Markus et al. 2021). Also, the increase in temperature could activate nociceptors within muscle fibers and myotendinous junctions in a DOMS condition (Cheung et al. 2003). This process would reflect in the rise of skin surface temperature, in response to cutaneous vasodilatation, by the heat transfer from the heated blood flow to the skin by the convection, also influencing the heat transfer by radiation from the skin to the environment. From this, several authors have sought to understand the possible applicability of the technique to monitor exercise-induced muscle damage and DOMS. To date, there is no evidence to support this applicability, and further studies are needed.

Chapter II

Justification of this thesis

2. CHAPTER II

2.1 JUSTIFICATION OF THE THESIS

The regular practice of physical exercise is an essential ally for maintaining health and quality of life. In addition, it plays a vital role in the face of the decline of physiological functions over the years (McPhee et al. 2016). In general, the more physically active a person is, the better their physical capacity can be, which is directly related to positive adaptations of physiological systems such as the neuromuscular and cardiopulmonary systems (McPhee et al. 2016). In addition, there are a wide variety of benefits to practitioners from the regular practice of physical exercises, such as the prevention of cardiovascular and metabolic diseases, muscle weakness, osteoporosis, obesity, and mental health (Guthold et al. 2018; McPhee et al. 2016; Sharma 2006).

Although many countries have high levels of sedentary lifestyle (Guthold et al. 2018), a large part of the population have sought the benefits of practicing sports. This can be observed in the growing number of running, cycling, gym, and clubs in cities. In sports training, monitoring internal and external training loads in accordance with the overload principle is essential to enhance gains and lead to overcompensation. This principle is based on the fact that for gains to be obtained, the loads must progress to generate a planned overload (Hughes et al. 2018). On the other hand, an abrupt increase in training loads, or even poor planning of external loads, can potentiate situations of exercise-induced muscle damage, inflammation, and DOMS (Cheung et al. 2003).

DOMS is characterized as an acute and continuous sensation of discomfort, sensitive to movement and palpation. It usually reaches its peak 24 h to 48 hours post-exercise and disappears up to 7 days post-exercise (Connolly et al. 2003). DOMS responds to stimuli with specific characteristics and often occurs after the practice of exercise different from the usual in people who practice non-systematized exercises and exercises with a predominance of eccentric muscle actions (Cheung et al. 2003). During exercise, the neuromuscular system can be activated in different ways, which is why there are certain configurations that favor DOMS. Eccentric muscle actions, in which the torque produced by the muscle cannot overcome the resistance torque

(Enoka 2015), are identified as one of the factors that cause DOMS (Hody et al. 2019). During an eccentric action, the muscle is elongated while producing force, and although these actions are known to generate greater force, they also generate greater muscle damage and, consequently, DOMS (Cheung et al. 2003; Hody et al. 2019).

Eccentric muscle actions are present in several activities of daily living, such as deceleration actions of body segments or supporting the body's weight against gravity and absorbing shock (Hody et al. 2019). As we previously reviewed, it has been debated that DOMS is caused not by a specific factor but as a result of a sequence of correlated physiological and biochemical events, such as structural, and mechanical damage in the muscle that which in turn generates a physiological inflammatory process, oxidative stress and metabolic damage (Pereira Panza et al. 2015; Proske & Morgan 2001). This structural muscle damage is usually marked by the extravasation of internal enzymes that will appear increased in the blood flow, such as CK, LDH, and MB, among others (Stebbins et al. 2014).

The physiological inflammatory process, established locally, aims to remove debris (Clarkson & Hubal 2002). However, it also contributes to the production of ROS that potentiates muscle damage, causing a situation known as oxidative stress (Halliwell 2006; Peake et al. 2005). In response to tissue damage, chemicals released hypersensitize nociceptors to the locally felt pain sensation (Dee Unglaub Silverthorn 2017). It has been discussed that this inflammatory process can increase local temperature, influencing blood flow and leading to hyperthermia levels (Hildebrandt et al. 2010). This heat is conducted to the superficial layers of the skin by the blood through various compensatory mechanisms, such as temperature conduction of the blood flow to the skin in response to peripheral vasodilation (Priego Quesada 2017).

Several symptoms are presented in the DOMS condition, such as strength loss, loss of range of motion, swelling of the muscles affected by mechanical stress, the sensitivity of the tissue to touch, and pain (McHugh et al. 1999). The sensation can range from mild discomfort that disappears with daily activities to severe debilitating pain that can eventually restrict movement (Cheung et al. 2003). As a result of the various symptoms presented, it turns out to be a cause for concern among athletes, coaches, and physiotherapists, as it can impair neuromuscular

functionality and performance (Pearcey et al. 2015). To monitor these conditions, the most well-known indicators require invasive approaches, such as blood sample collection and analysis, which are also more expensive. Additionally, behavioral measures, such as the perceived pain scale, are usually applied but do not allow an objective quantification of the post-exercise condition.

In this sense, there is a growing interest in using IRT to monitor DOMS and physiological responses to exercise. This interest is justified by the fact that changes in skin temperature could be correlated with inflammatory reactions due to the previously mentioned mechanisms of heat dissipation (Hildebrandt et al. 2010). Furthermore, the accumulation of different metabolites in a DOMS condition and the increase in temperature could activate nociceptors within muscle fibers and myotendinous junction (Cheung et al. 2003), corroborating the possible relationship between temperature increase and pain sensation.

In fact, changes in skin surface temperature can be detected using IRT. This non-invasive technique employs an infrared sensor to measure the surface temperature of an object (Priego Quesada et al. 2017). Relatively easy to use, its use has gained prominence in sports, especially in sports such as running (Sanz-López et al. 2016), cycling (Priego Quesada et al. 2016), triathlon (Priego-Quesada et al. 2019) and soccer (Rodríguez-Sanz et al. 2017). This application of IRT would be of interest in many cases, as collecting and analyzing blood takes time and can be expensive, while measurements using IRT can be performed quickly, and once the camera is purchased, the cost of an evaluation is too low. However, in the context of monitoring exercise-induced muscle damage, DOMS, and recovery after exercise, the results of studies using IRT are pretty controversial.

A positive correlation was found between reflected skin temperature and DOMS 24 hours after a physical exercise involving elbow flexion in the biceps brachii muscle, in addition to a change in the muscle damage marker myoglobin 48 hours after exercise (Al-Nakhli et al. 2012). On the other hand, in soccer players, the activity of the biochemical marker of muscle damage CK and the skin temperature measured by IRT after two consecutive games showed a moderate correlation, although the skin temperature and CK showed an increase after the matches (de

Andrade Fernandes et al. 2017). This variability and contradiction in the results may be related to the type of exercise since, during soccer practice, different muscles act in different situations, and this variability in physiological demand and muscle activity may have influenced the magnitude of muscle damage produced and skin temperature.

The form of monitoring, parameters, and monitored variables can also influence the analysis results using thermography. In the study involving a specific muscle group (Al-Nakhli et al. 2012), the authors presented only absolute temperature data, which provides a limited view for assessing skin temperature in response to exercise. Once the basal and minimum temperatures are not considered, the range of variation is difficult. to be determined (Priego Quesada et al. 2017). Analyzing a smaller muscle group, it was observed that eccentric muscle actions of the plantar flexor muscles of the ankle, although resulting in DOMS, do not reflect a condition in which skin temperature is related to the reported DOMS sensation or CK enzyme immediately post and 48 hours post-exercise (da Silva et al. 2018). These measures may have been influenced by factors such as the participants' level of physical fitness since changes in skin temperature in untrained individuals manifest themselves more slowly than in trained individuals (Formenti et al. 2013). Furthermore, in measurements conducted after exercise in which sweat is negligible, the effect of the level of physical fitness on skin temperature response could be explained by the differences in the fat tissue proportion and skin blood flow (Neves et al. 2015; Simmons et al. 2011).

Establishing skin temperature responses to physical exercise, the influence of different forms of monitoring, monitoring time, data collection and analysis parameters, analyzed variables, and possible intervening factors are essential to guide future studies using IRT. The use of IRT in the sports context has enormous potential. However, evidence is needed before the popularization of IRT in monitoring physiological responses to exercise. Thus, this Ph.D. thesis seeks to answer the central question: Can infrared thermography (IRT) monitor exercise-induced muscle damage, delayed onset muscle soreness (DOMS), and physiological responses induced by the exercise?

Additionally, this central question is related to other relevant questions, such as: a) Do post-exercise IRT measurements present satisfactory repeatability? b) Is there an influence of the

evaluator's experience on the measures? c) Is there an influence of the gender of the participants on the behavior of the DOMS and skin temperature? d) Do active evaluation interventions affect temperature behavior in the post-exercise recovery phase? We believe that the answers to these questions can provide important evidence for the future use of IRT in sports science.

Chapter III

Hypothesis And Objectives

3. CHAPTER III

3.1 HYPOTHESIS AND OBJECTIVE

The general hypothesis of this Ph.D. thesis is that the IRT can be a valuable tool to monitor acute adaptations induced by exercises, like exercise-induced muscle damage and DOMS. This thesis has one general objective, which can be broken into three specific objectives. These objectives were developed in three original articles that make up this doctoral thesis.

3.2 General objective

To determine whether the skin temperature, evaluated by IRT, under resting conditions and after the practice of the physical exercise, can be used to monitor exercise-induced muscle damage and DOMS in post-exercise recovery.

3.3 Specific

- To determine the effect of evaluator experience level (experienced vs. novice) on the reproducibility of thermal images analyzed before and after physical exercise.
- To determine whether exercise-induced muscle soreness and pain thresholds are related to skin temperature measured using infrared thermography in men and women.
- To determine the level of muscle damage and its association with outcomes of the assessment of skin temperature rewarming in exercise limbs after a cold-stress protocol.

Chapter IV

Manuscript I

4. CHAPTER IV

4.1 MANUSCRIPT I

Publications obtained:

da Silva W, Machado ÀS, Kunzler MR, Perez I J, Calvo MG, Priego-Quesada JI, Carpes FP (2022). Reproducibility of skin temperature analyses by novice and experienced evaluators using infrared thermography. ***Journal of Thermal Biology***. Volume 110. doi: 10.1016/j.jtherbio.2022.103345.

REPRODUCIBILITY OF SKIN TEMPERATURE ANALYSES BY NOVICE AND EXPERIENCED EVALUATORS USING INFRARED THERMOGRAPHY

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4.1.1 Abstract

Infrared thermography (IRT) has become popular in several areas of knowledge. However, the analyses of thermal images often request manual actions, and little is known about the effect of the evaluator's experience on analysis thermal images. Here, we determine the reproducibility of IRT images analysis performed by evaluators with different levels of experience. Eight evaluators (GE, group experienced, n = 4; GN, group novice, n = 4) analyzed thermograms from 40 healthy participants recorded before and after exercise to determine the mean, minimum, maximum, standard deviation, and range of skin temperature in the anterior thigh and posterior leg. Before and after exercise, mean temperature showed excellent reproducibility for both groups for the anterior thigh (ICC > 0.98) and posterior leg (ICC > 0.94), and maximum temperature showed excellent reproducibility for both groups in the posterior leg (ICC > 0.91). The influence of experience level was not significant considering the anterior thigh. Similarly, experience level did not affect the mean, maximum, and standard deviation temperature determined for the posterior leg. For the posterior leg, minimum temperature presented lower values and the range was higher among novice evaluators. Mean skin temperature showed narrower 95% limits of agreement than minimum and maximum for both regions and moments. Caution is advised when temperature ranges and minimums are determined by different evaluators. We conclude that for IRT analysis by evaluators with different levels of experience, the mean and maximum temperatures should be prioritized due to their better reproducibility.

Keywords: Infrared camera; Mean skin temperature; Maximum skin temperature; Physical exercise; Thermal images.

4.1.2 Introduction

Infrared thermography (IRT) allows one to determine surface temperatures based on the infrared radiation emitted, which accounts for its increasing interest in areas related to human physiology. In medicine, IRT is a possible diagnostic tool that can be used to help screening for different conditions including breast cancer (Lozano et al. 2020) and peripheral vascular disease (Huang et al. 2011). The use of IRT is also being explored in sports and rehabilitation sciences with the aim of monitoring acute exercise-induced adaptations and injury risks (Gómez-Carmona et al. 2020). Whether and how a relationship between physiological markers of exercise adaptations and skin temperature responses exists is still not fully understood (Rojas-Valverde et al. 2021), but the increasing popularity of IRT and the availability of more affordable IRT cameras have allowed researchers and coaches to include this technique into their exercise monitoring routine (Hillen et al. 2020).

Despite the growth in the use of this technique and the establishment of increasingly rigorous and standardized protocols for taking images, there are still technical factors that can influence the results obtained (Moreira et al. 2017). In addition to the technical specifications of the cameras, which may affect outcomes when experienced evaluators analyze IRT images (Machado et al. 2021), the level of reproducibility is a crucial point to consider in clinical applications. For instance, different infrared camera models can have a significant impact on determining skin temperature data and influence comparisons different studies (Machado et al. 2021). Another matter of concern is the region of interest considered, as this can present a high inter-examiner reproducibility but with variable day-to-day reproducibility (Zaproudina et al. 2008). Additionally, while a high-resolution camera and application of automatic analyses of regions of interest (ROIs) result in low inter-image temperature variability (Tan et al. 2016; Requena-Bueno et al. 2020) when data from different researchers are compared, the outcomes may not be consistent enough to help in a clinical decision. An example of this was a qualitative study on the inter-observer reproducibility of mammary thermography. This involved 159 thermograms qualitatively analyzed in four analysis centers, which in the end were considered too poor and inadequate to support any therapeutic decision (Mustacchi et al. 1990). Although the

devices used in that study were limited, it is still a good example for the emergence for a more detailed care when performing IRT analyses.

To date, there is no consensus on how IRT analysis by different evaluators should be performed. Different recommendations for data acquisition have suggested that defining of ROIs must be rigorous (Moreira et al. 2017), but that is something that can be evaluator-dependent and affect reproducibility. Similar to the observed in ultrasonography image analysis (Tegnander & Eik-Nes 2006), the use of a software that requires manual actions and individual decisions can make IRT analysis very individual, and decision-making during this process may differ between evaluators, especially among novice evaluators. While the evaluator's experience can be controlled in a research setting, it may not be easy when the IRT is used in the field (e.g., clinics, hospitals, sports teams). Therefore, understanding the influence of evaluator experience on the reproducibility of IRT image analysis is fundamental, and has important practical implications. A previous study found that determining mean skin temperature presents higher reproducibility, even when experienced evaluators use different IRT cameras, especially for IRT measurements before physical exercise (Machado et al. 2021). However, it is not clear whether this remains true when evaluators with differing levels of experience are involved in the IRT data analysis routine.

The aim of the present study was to determine the effect of evaluator experience level (experienced vs. novice) on the reproducibility of thermal images analyzed before and after physical exercise. We hypothesized that analyses of thermal images by novice evaluators would present lower reproducibility than those of experienced evaluators, and specific recommendations could be provided not only for IRT analysis but also for evaluator training.

4.1.3 Materials and methods

Procedures and experimental design

In this study, we used the database from two previous studies (da Silva et al. 2018, 2021), approved by the local university ethics committee (IRB: 26037119.9.0000.5323), in which IRT measurements were taken from 40 healthy men and women (30 men: age 24 ± 5.7 years, body mass 75.7 ± 8.9 kg, height 1.75 ± 0.1 m, body max index: 24.0 ± 5.3 kg/m^2; and 10 women: age

25 ± 4.0 years, body mass 59.9 ± 9.2 kg, height 1.62 ± 0.6 m, body max index: 22.8 ± 3.1 kg/m^2). Eight evaluators (EG, experienced group, n = 4, with experience in research of IRT over the past 7 ± 1.8 years, and previous publications with IRT: 7.0 ± 4.9 manuscripts; and NG, novice group, n = 4, without previous experience of IRT research) processed 80 thermal images, from two different body regions (40 images from anterior thigh and 40 images from posterior leg) and recorded in two conditions (20 images from before and 20 images from after-exercise). The physical exercise performed involved maximal voluntary repetitions of squats or heel rise until exhaustion. All images were distributed between the two groups of evaluators. The images were numbered and processed in randomized order, so that evaluators were unaware of which image corresponded to each participant or condition. Thermacam Researcher Pro 2.10 software (FLIR, Wilsonville, OR, USA) was used to process all the images. Figure 1 depicts the experimental design.

All evaluators received online training conducted by an external experienced member who was not part of the EG (with experience in research of IRT over the previous 12 years, Level I thermographer accredited by the Infrared Training Center, and 32 scientific manuscripts published in the last 10 years prior to the training). This was divided into three stages:

I) Introduction to software analysis: to learn the basic characteristics and main functions of the software.

II) Instruction on ROI definition and variables of interest: the ROIs were determined based on anatomical references (Fig. 2). For the anterior thigh, evaluators were instructed to draw a straight line from the groin area towards the external portion of the thigh and select the largest area possible bounded above the upper line of the patella (da Silva et al. 2021). For the posterior leg, the evaluators were instructed to draw a straight line from the largest region of the calf muscles towards the external portion and select downward the largest possible area until the ankle (da Silva et al. 2018). Four ROIs were delimited: Two for the anterior thigh (right and left) and two for the posterior leg (right and left).

III) Instruction on data tabulation: skin temperature was quantified to determine inter-examiner reproducibility considering the mean, maximum, minimum, standard deviation, and range (max-min) of the skin temperature for each ROI.

From the online training to the end of the analysis, each evaluator spent around 20 days. After the images had been processed, the data were sent to an external member for statistical procedures.

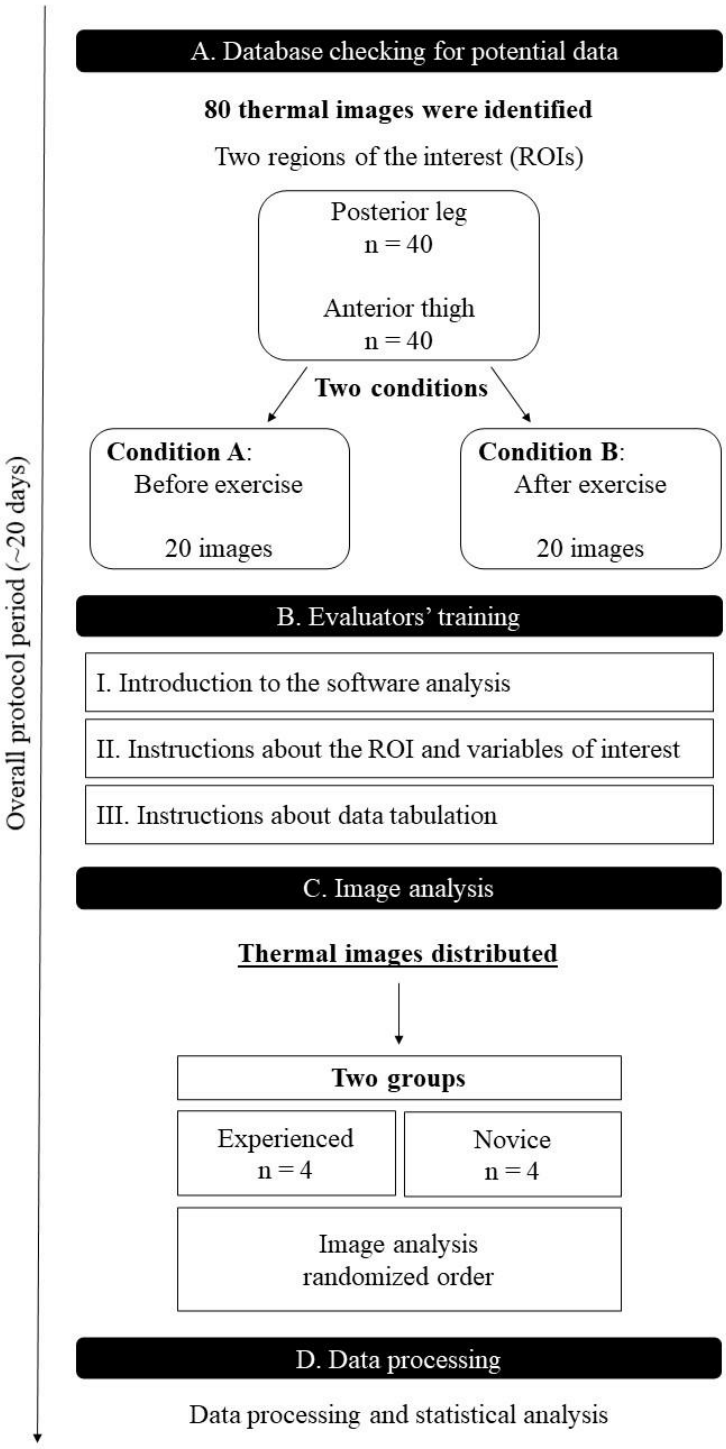

Figure 4.1 Experimental design. ROI: region of interest.

Skin temperature assessment

All thermal images were captured by the same evaluator using a thermal camera FLIR-E60 with a resolution of 320 x 240 pixels (FLIR model E-60, Flir Systems Inc., Wilsonville, Oregon, USA) with noise-equivalent temperature difference (NETD) < 0.05 °C, and measurement uncertainty of ± 2°C or 2%. All IRT assessments were taken in an air-conditioned and controlled environment: anterior thigh: room temperature: 24.4 ± 1.6 °C and air humidity: 46.8 ± 10.0% and posterior leg: room temperature: 23.1 ± 1.7 ºC and air humidity: 60.3 ± 8.4%. The camera was turned on at least 10 min before the evaluations. An anti-reflective panel was placed behind the participant to avoid interferences from radiation emitted by a non-neutral background. All images were captured at 1 meter from the ROI, with the camera lens perpendicular to the ROI. Moreover, all images were taken with participants positioned standing still with the musculature exposed for 10 min to ensure proper adaptation to the room temperature. All IRT procedures followed the Thermographic Imaging in Sports and Exercise Medicine checklist (Moreira et al. 2017). The participants were free of lower limb injury or any disease. To avoid the influence of external factors on skin temperature variability, the participants received instructions to avoid physical exercise on the day before testing and not use anti-inflammatory substances, avoid alcohol consumption, caffeinated drinks, cigarettes, hot baths, sunbathing, and creams and cosmetics 24 hours before the skin temperature assessment, and throughout participation in the study.

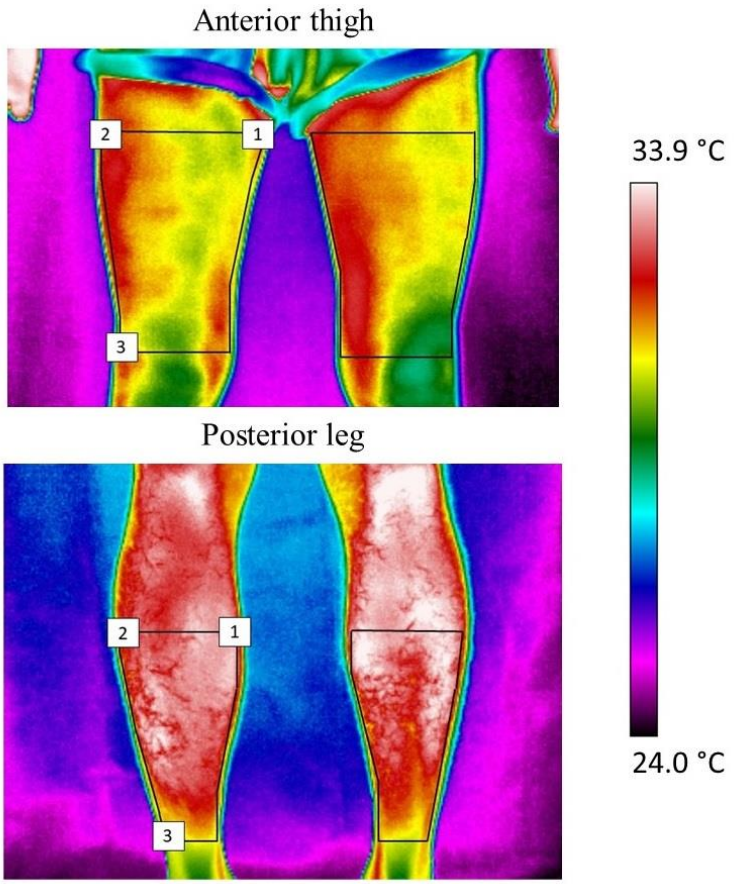

Figure 4.2 Representation of the four regions of interest (ROIs) for quantifying skin temperature. The image is representative of all participants. All anatomical references are represented by numbers. **Anterior thigh**: Groin area (1), External portion of the thigh (2), Line of the patella (3). **Posterior leg**: Larger region of the calf muscles (1), External portion of the leg (2), Ankle (3).

Statistical analyzes

Data are reported considering mean, standard deviation, and the 95% confidence intervals of the differences between comparisons (CI95%). The Shapiro-Wilk test and Levene tests were used to check the normality of data distribution and homogeneity of variances. Main effects and interactions were verified using two-way analysis of variance with Bonferroni post-hoc taking three factors into account: group (EG vs. NG), leg (right vs. left), and moment (before vs. after exercise), for mean, maximum, minimum, standard deviation, and range of skin temperature. The effect size index f (ESF) was determined and classified as large (ESF > 0.4), medium (ESF 0.26-0.4), and small (ESF 0.1-0.25) (Cohen 1988). For significant pair differences, Cohen's effect sizes

(*d*) were computed and classified as small (ES*d* 0.2–0.5), moderate (ES*d* 0.5–0.8), or large (ES*d* >0.8) (Cohen 1988). The agreement between the two groups (EG vs. NG) was analyzed using Bland–Altman plots.

The intraclass correlation coefficient (ICC), based on a single rater-measurement, absolute-agreement, and 2-way random-effects model, was calculated by the four evaluators in each group (inter-evaluator reproducibility), taking each measuring point (anterior thigh and posterior leg) and moment (before exercise and after exercise) into account for all variables of skin temperature. The ICC was classified an index from 1.00 to 0.81 being considered as excellent reproducibility, 0.80 to 0.61 as very good reproducibility, 0.60 to 0.41 as good reproducibility, 0.40 to 0.21 as reasonable reproducibility, and 0.20 to 0.00 as poor reproducibility (Weir 2005). Bland-Altman analyses were performed using GraphPad Prism (version 8.0.0 for Windows, GraphPad Software San Diego, California, USA). All other statistical analyses were performed using SPSS 21 (SPSS Inc., Chicago, USA). The significance level was established at $p<0.05$.

4.1.4 Results

The effect of the evaluator's experience on skin temperature measurements

Anterior thigh

The experience level of the evaluators did not affect the determination of the mean (before exercise: EG, right: 31.5 ± 1.0 °C vs. NG, right: 31.5 ± 0.9 °C and EG, left: 31.6 ± 1.0 °C vs. NG, left: 31.5 ± 1.0 °C; after exercise: EG, right: 32.2 ± 1.2 °C vs. NG, right: 32.3 ± 1.3 °C and EG, left: 32.2 ± 1.3 °C vs. NG, left: 32.3 ± 1.2; F = 0.06; p = 0.79; ESF = 0.00), maximum (before exercise: EG, right: 33.6 ± 1.3 °C vs. NG, right: 33.4 ± 1.2 °C and EG, left: 33.8 ± 11. 3 °C vs. NG, left: 33.4 ± 1.2 °C; after exercise: EG, right: 33.2 ± 1.2 °C vs. NG, right: 34.0 ± 1.1 °C and EG, left: 34.2 ± 1.2 °C vs. and NG, left: 34.1 ± 1.1 °C ; F = 2.01; p = 0.15; ESF = 0.05), and minimum skin temperature (before exercise: EG, right: 29.5 °C ± 1.2 vs. NG, right: 29.4 ± 1.1 °C and EG, left: 29.2 ± 1.2 °C vs. and NG, left: 29.2 ± 1.1 °C; after exercise: EG, right: 29.6 ± 1.4 °C vs. NG, right: 29.6 ± 1.5 °C and EG, left: 29.4 ± 1.4 °C vs. NG, left: 29.5 ± 1.4 °C; F = 0.01; p = 0.90; ESF = 0.00) in the anterior thigh.

A lack of effect for experience level was also observed for the range (before exercise: EG, right: 4.0 ± 1.3 °C vs. NG, right: 3.9 ± 1.4 °C and EG, left: 4.5 ± 1.4 °C vs. NG, left: 4.1 ± 1.3 °C; after exercise: EG, right: 4.3 ± 1.0 °C vs. NG, right: 4.3 ± 1.4 °C and EG, left: 4.6 ± 1.2 °C vs. NG, left: 4.5 ± 1.4 °C; $F = 1.99$; $p = 0.15$; ESF = 0.05) and standard deviation in skin temperature from anterior thigh (before exercise: EG, right: 0.5 °C ± 0.1 vs. NG, right: 0.5 ± 0.1 °C and EG, left: 0.6 °C ± 0.1 vs. NG, left: 0,6 ± 0.1 °C; after exercise: EG, right: 0.7 ± 0.1 °C vs. NG, right: 0.6 ± 0.1 °C and EG, left: 0.6 ± 0.1 °C vs. and NG, left: 0.7 ± 0.1 °C; $F = 0.19$; $p = 0.66$; ESF = 0.00).

Tests for the effect of leg and moment of measurement showed results similar to previous studies (da Silva et al. 2018, 2021), the most important result being the lack of interaction with the group factor. The full results of skin temperature are provided in the supplemental file (Table 2).

Bland-Altman plots (Fig. 3) showed narrower 95% limits of agreement for mean than minimum and maximum before exercise (bias mean: 0.0 ± 0.1 °C vs. minimum: 0.0 ± 0.3 °C vs. maximum: 0.3 ± 0.2 °C) and after exercise (bias mean: -0.1 ± 0.1 °C vs. minimum: -0.0 ± 0.5 °C maximum: 0.0 ± 0.2 °C).

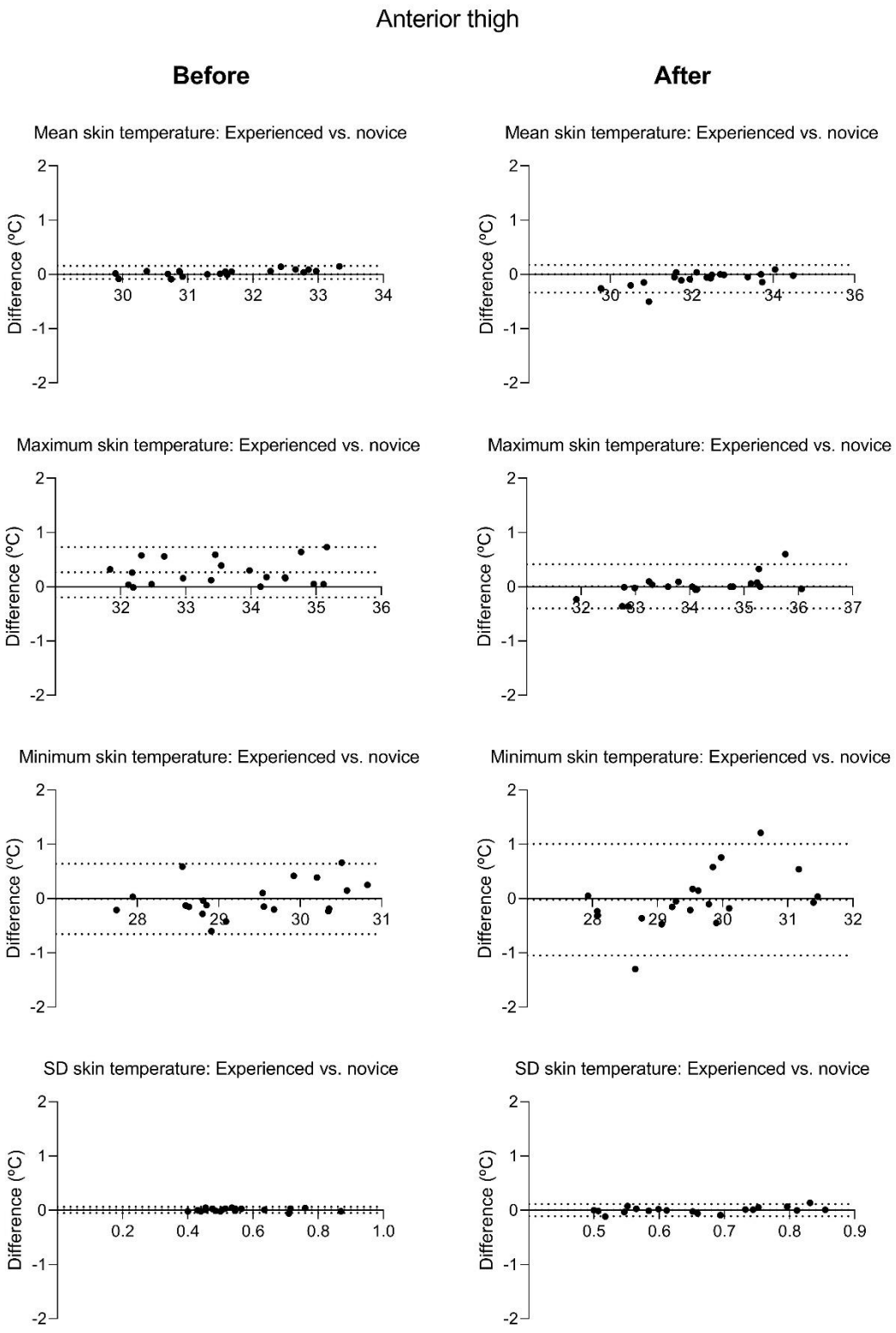

Figure 4.3 Bland-Altman plot with 95% limits of agreement illustrates the difference in mean, minimum, maximum and standard deviation of skin temperature measurements between values obtained by all evaluators from the two groups. SD: Standard deviation.

Posterior leg

Similar to that observed for anterior thigh, the experience level of the evaluators did not affect determining the mean (before exercise: EG, right: 32.1 ± 1.1 °C vs. NG, right: 32.0 ± 1.1 °C and EG, left: 32.1 ± 1.1 °C vs. NG, left: 32.0 ± 1.2 °C; after exercise: EG, right: 32.8 ± 1.0 °C vs. NG, right: 32.8 ± 1.1 °C and EG, left: 32.8 ± 0.1 °C vs. NG, left: 32.8 ± 0.1 °C; F= 0.13; p=0.71; ESF = 0.00), and maximum skin temperature (before exercise: EG, right: 33.4 ± 1.1 °C vs. NG, right: 33.4 ± 1.1 °C and EG, left: 33.2 ± 1.1 °C vs. NG, left: 33.2 ± 1.1 °C; after exercise: EG, right: 34.9 ± 1.1 °C vs. NG, right: 34.9 ± 1.1 °C and EG, left: 34.8 ± 1.2 °C vs. NG, left: 34.7 ± 1.1 °C; F = 0.06; p = 0.80; ESF = 0.00) in the posterior leg.

However, there was an effect of the experience level in determining the minimum (F= 10.52; p < 0.01; ESF = 0.12), and range (F = 12.26; p < 0.01; ESF = 0.13) of skin temperature in posterior leg. The minimum temperature was lower in NG than EG (before exercise: EG, right: 29.1 ± 1.9 °C vs. NG, right: 28.98 ± 2.1 °C and EG, left: 29.3 ± 1.7 °C vs. NG, left: 28.8 ± 2.2 °C; after exercise: EG, right: 29.8 ± 1.6 °C vs. NG, right: 29.3 ± 2.0 °C and EG, left: 30.1 ± 1.5 °C vs. NG, left: 29.5 ± 1.95 °C [$F_{(1,79)}$ = 4.47; p < 0.05; ES*d* = 0.36]).

The temperature range was higher in NG than EG (before exercise: EG, right: 4.2 ± 1.5 °C vs. NG, right: 4.5 ± 1.8 °C and EG, left: 3.9 ± 1.3 °C vs. NG, left: 4.4 ± 1.8 °C; after exercise: EG, right: 5.0 ± 1.5 °C vs. NG, right: 5.5 ± 1.9 °C and EG, left: 4.6 ± 1.4 °C; vs. vs. NG, left: 5.3 ± 1.9 °C [$F_{(1,79)}$ = 5.60; p < 0.05; ES*d* = 0.37]).

Although the ANOVA showed significant effect of experience level for standard deviation, the post hoc analysis did not identify paired differences (before exercise: EG, right: 0.6 ± 0.2 °C vs. NG, right: 0.6 ± 0.3 °C and EG, left: 0.5 ± 0.2 °C vs. NG, left: 0.6 ± 0.2 °C; after exercise: EG, right: 0.7 ± 0.2 °C vs. NG, right: 0.8 ± 0.3 °C and EG, left: 0.7 ± 0.2 °C vs. NG, left: 0.7 ± 0.2 °C; F = 4.22; p < 0.05; ESF = 0.08).

Tests for the effect of leg and moment of measurement showed results similar to previous studies (da Silva et al. 2018, 2021), the most important result being the lack of interaction with the group factor. However, we did find that in the posterior leg there was a group effect for minimum temperature, indicating the expected difference between before and after exercise for

right leg only for the data analyzed by the EG $p < 0.05$. The full results of skin temperature are provided in the supplemental file (Table 3).

Bland-Altman plots (Fig. 4) showed similar results to the anterior thigh, with narrower 95% limits of agreement for mean skin temperature than minimum and maximum before (bias mean: 0.0 ± 0.1 °C vs. minimum: 0.4 ± 0.5 °C vs. maximum: 0.0 ± 0.2 °C) and after exercise (bias mean: 0.0 ± 0.1°C vs. minimum: 0.6 ± 0.5 °C vs. maximum: 0.0 ± 0.2 °C).

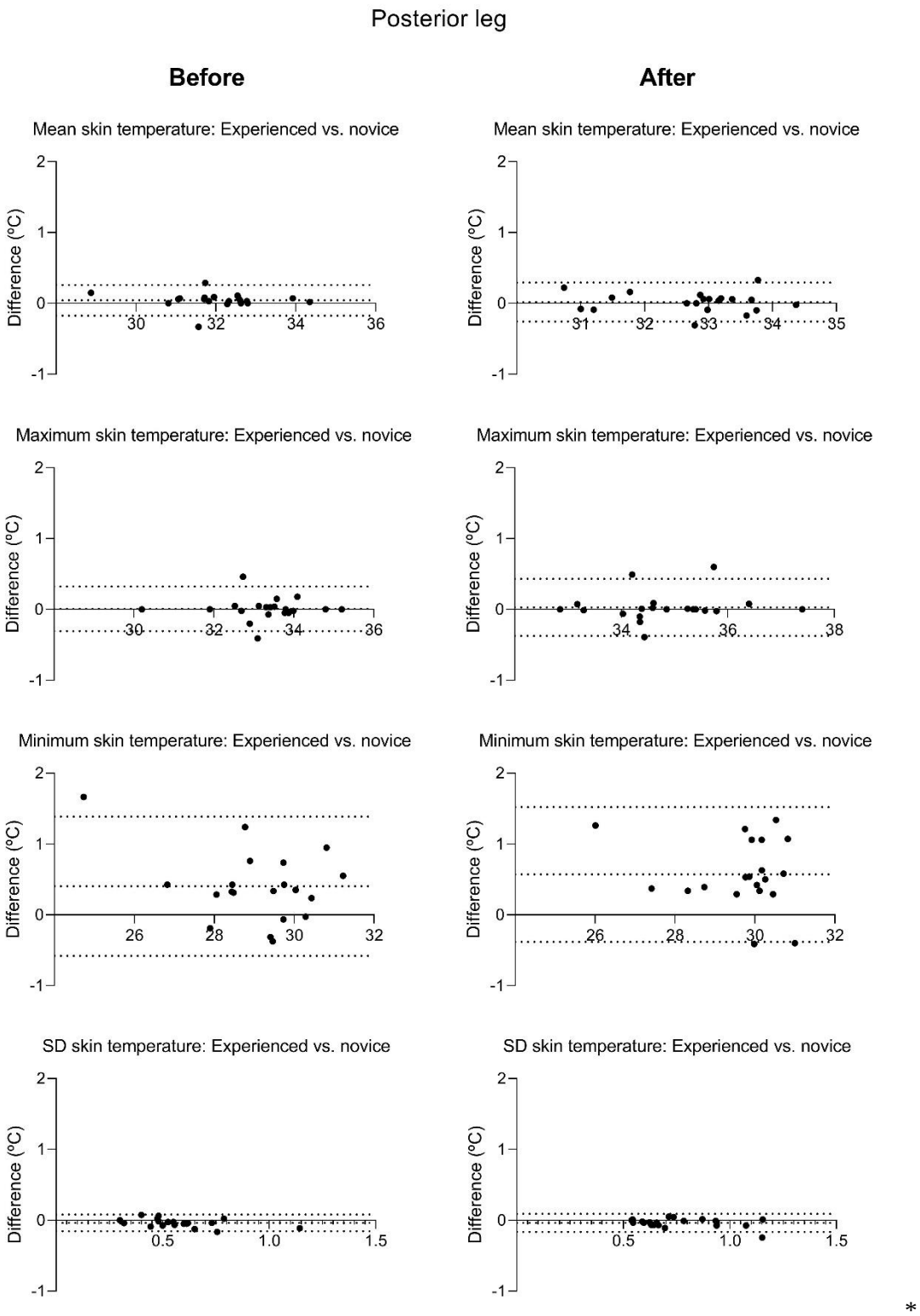

Figure 4.4 Bland-Altman plots with 95% limits of agreement illustrate the difference in mean, minimum, maximum and standard deviation of skin temperature measurements between values obtained by all evaluators from the two groups. SD: Standard deviation.

The effect of evaluators' experience on skin temperature measurement reproducibility

Table 1 shows inter-evaluator reproducibility for all variables of skin temperature considering the experienced and novice evaluators. Before and after exercise, the mean temperature from the anterior thigh showed excellent reproducibility for both groups (ICC values from 0.98 to 0.99). Still considering the anterior thigh, maximum skin temperature showed excellent reproducibility after exercise for both groups (ICC values from 0.92 to 0.93). Regarding the posterior leg, before and after exercise, mean (ICC values from 0.94 to 0.98) and maximum (ICC values from 0.91 to 0.97) temperature showed excellent reproducibility. The range of skin temperature showed poor reproducibility at almost all moments for all regions and groups.

Table 4.1 Mean [95% confidence intervals] intraclass correlation coefficients (ICC) determining inter-examinator reproducibility for the different regions of interest (anterior thigh and posterior leg) and moments (before and after exercise) considering the mean, minimum, maximum, max-min range, and standard deviation of skin temperature.

	Anterior thigh							
	Experienced				Novice			
	Before exercise		After exercise		Before exercise		After exercise	
	Right	Left	Right	Left	Right	Left	Right	Left
Mean	.99 [.98,.99]	.99 [.97,.99]	.99 [.99,.99]	.99 [.99,.99]	.99 [.97,.99]	.99 [.98,.99]	.98 [.97,.99]	.98 [.97,.99]
Minimum	.59 [.30,.80]	.44 [.11,.72]	.69 [.40,.86]	.49 [.18,.74]	.39 [.10,.67]	.49 [.22,.73]	.37 [.13,.64]	.25 [.04,.52]
Maximum	.64 [.40,.82]	.72 [.51,.86]	.92 [.86,.96]	.93 [.87,.97]	.82 [.65,.91]	.77 [.58,.89]	.93 [.86,.97]	.93 [.87,.97]
Max-min range	.23 [.03,.49]	.19 [.01,.45]	.29 [.06,.56]	.14 [.00,.37]	.30 [.05,.59]	.27 [.05,.54]	.18 [.01,.42]	.10 [.-01,.30]
Standard deviation	.67 [.46,.84]	.84 [.66,.93]	.91 [.84,.96]	.78 [.56,.90]	.64 [.34,.83]	.83 [.69,.92]	.67 [.44,.84]	.51 [.29,.73]
	Posterior leg							
	Experienced				Novice			
	Before exercise		After exercise		Before exercise		After exercise	
	Right	Left	Right	Left	Right	Left	Right	Left
Mean	.98 [.95,.99]	.98 [.97,.99]	.97 [.94,.98]	.97 [.94,.98]	.97 [.94,.98]	.97 [.94,.98]	.94 [.84,.98]	.94 [85,.98]
Minimum	.55 [.24,.77]	.49 [.16,.74]	.53 [.23,.76]	.49 [.16,.74]	.53 [.19,.77]	.37 [.06,.66]	.38 [07,.67]	.26 [.04,.54]
Maximum	.94 [.89,.97]	.97 [.94,.98]	.94 [.89,.97]	.92 [.86,.96]	.97 [.94,.98]	.94 [.89,.97]	.93 [.88,.97]	.91 [.83,.96]
Max-min range	.30 [.08,.57]	.15 [.00,.38]	.52 [.20,.76]	.39 [.10,.67]	.34 [.07,.62]	.15 [.00,.39]	.35 [.07,.63]	.26 [.04,.52]
Standard deviation	.77 [.55,.90]	.72 [.81,.48]	.84 [.65,.93]	.77 [.53,.90]	.68 [.29,.86]	.54 [.21,.78]	.75 [.48,.89]	.73 [.49,.87]

4.1.5 Discussion

Changes in skin temperature in response to interventions (for instance, physical exercise) are in general of small magnitude (e.g., 2ºC is a 6% of 33ºC) (Stewart et al. 2020). As a consequence, reproducibility and variability in skin temperature outcomes can lead to significant bias in the interpretation of these data. In this study, we determine the effect of evaluators' experience level on the reproducibility of thermal images analysis taking measurements conducted before and after physical exercise into account. We initially hypothesized that analyses by novice evaluators would show lower reproducibility, and therefore we could provide specific recommendations not only for IRT analysis but also for evaluator training. Our main result was that mean temperatures showed excellent reproducibility despite the evaluator's experience level for images from the anterior thigh and posterior leg. Also, maximum temperatures presented excellent reproducibility for both groups, especially for the posterior leg. However, analyses by novice evaluators presented higher range values than experienced evaluators and lower values for minimum temperature in the posterior leg. We consider these novel results can help to improve IRT routines for assessment in multicenter studies.

Since the thermal camera has a range of temperatures that influences its accuracy, our study evaluated the skin temperature at two moments: before and after exercise, to induce changes in skin temperature response to peripheral vasodilation and to better understand the influence of this physiological response on the thermal data analyses (Machado et al. 2021). The effects of physical exercise were not the focus of this research, and therefore our discussion is focused on the influence of different evaluators on data analysis.

Our results open up a discussion on recommendations regarding evaluator training in the use of IRT. One source of variation in the IRT analysis from humans is the selection of the regions of interest (Fernández-Cuevas et al. 2015). In the Bland Altman plots, we showed that the mean of skin temperature is in greater agreement between the two groups than the maximum and minimum for both regions before and after exercise. We, therefore, suggest that the use of well-defined anatomical criteria allows novice evaluators to improve the reliability of data when evaluating the anterior thigh and posterior leg, especially when considering the mean of skin

temperature. This supports the hypothesis of the mean skin temperature being the most robust thermal parameter for evaluation with IRT (Priego Quesada 2017). The use of mean skin temperature is also recommended when different camera models are used to collect IRT data (Machado et al. 2021). Maximum skin temperature is provided by most of the IRT software as the highest value of the ROI which can make it dependent on the position of the ROI and, therefore, on the evaluator. This issue was addressed by previous research by using the Tmax method, which consists of selecting an area of five by five pixels around each of the five warmest pixels, so determining the mean temperature across the 125 pixels included in the selection (Formenti et al. 2018). On the other hand, as standard deviation and the max-min range had a lower value than the other parameters, similar alterations are relatively larger, so decreasing their reproducibility (Machado et al. 2021).

The influence of different evaluators might differ from the influence of experience level. A previous study with IRT did not find significant differences in the interpretation of results depending on evaluator's experience (Zaproudina et al. 2008). However, the authors did not describe the level of experience of both evaluators, and there were only two evaluators. In our study, four evaluators with previous experience and four without previous experience processed all thermal images. Interestingly, data from different regions of interest show different outcomes for reliability such as maximum skin temperature that varied between excellent and very good reproducibility in the anterior thigh but presented excellent reproducibility in the posterior leg for both groups and moments. This is also observed with other imaging techniques. The evaluator's experience seems to influence the diagnosis of fetal heart structures and the prenatal detection rate of major heart diseases using obstetric ultrasound (Tegnander & Eik-Nes 2006). Furthermore, the reliability of measuring the length of ligament structures such as the anterior talofibular ligament using ultrasonography is also inversely influenced by the examiner's experience (Kristen et al. 2019). Therefore, finding strategies to minimize any influence of the evaluator are beneficial to improving the quality of the data.

We found that the choice of variable can also influence the reproducibility of analyses by experienced and novice evaluators, with mean temperature providing the most robust

measurement. There is much discussion about which thermal variable should be used depending on the purpose of assessment (Priego Quesada 2017). Although we recommend mean temperature to minimize the influence of evaluator experience, maximum skin temperature can be more sensitive to skin blood flow and inflammation, and minimum temperature to discuss outcomes related to vasoconstriction of skin blood flow (Formenti et al. 2018; Priego Quesada 2017). For these variables, some specific recommendations can be made. The analysis by novice evaluators presented lower minimum temperature and higher range values than those observed for experienced evaluators. The higher variability in novice evaluator analyses suggests difficulties in selecting the ROIs, especially in the posterior leg, and selecting areas outside the participant body (Machado et al. 2021). The very good and good reproducibility found in the standard deviation analysis shows that this error occurred in all the novice evaluators. While the mean value seems to have dissipated this effect, due to the large number of pixels considered, the influence of the evaluator in determining minimum temperature (which involves a single pixel with the lowest value) and the possible selection of areas outside the participant body (for instance, the edges of the image), may have influenced lower reproducibility for variables related to the variability of the measurement (Fernández-Cuevas et al. 2015). In addition to mean temperature, the use of maximum skin temperatures might be a solution, as previously suggested (Fernández-Cuevas et al. 2015).

There are different situations in which minimum and range of skin temperature are important variables to be considered. The range of skin temperature can be a useful measurement for exploring thermal gradients such as heat distribution on facial skin surface for monitoring microcirculatory dynamics and identifying anatomical areas that are expected to be symmetrical in normal conditions, but are affected by facial disease (Haddad et al. 2016). On the other hand, minimum temperature has been observed to be a promising measurement for monitoring acute adaptation induced by exercise in women 48 hours after exercise, possibly influenced by inflammation and muscle damage (da Silva et al. 2021). Moreover, when looking for asymmetries between muscles, the presence of varicose veins, lesions, or other abnormalities may indicate a false positive in the variables of maximum and mean temperature (Priego Quesada 2017).

Therefore, a standard deviation analysis needs to be undertaken to quantitatively assess points or outliers of each ROI. In addition, standard deviation can indicate whether any evaluators are incorporating regions outside the segment when drawing the ROI (Machado et al. 2021), as previously mentioned, so adding lower values to the final calculation and again producing false positives of asymmetry. In this study, we have shown that minimum and range of skin temperature can be influenced by the evaluator´s experience, so hindering objective conclusions.

We recommend that repeated training sessions are be necessary for novice evaluators to process IRT images from the posterior leg. Therefore, we urge caution when different evaluators determine minimum and temperature ranges.

Our study has limitations. We do not have information about which regions of interest were more exhaustive to determine, and therefore we cannot discuss whether this may be a factor of influence in the analysis. We included 8 examiners in our analysis, and it remains unclear if increasing the number of examiners would influence the results. We considered relatively large regions of interest considering the main aims of IRT studies in sports sciences and medicine, but future studies should also consider smaller regions of interest to verify if the results remain similar.

4.1.6. Conclusions

We conclude that when IRT analysis requires actions of evaluators with different levels of experience, mean and maximum temperatures should be prioritized due to better reproducibility. These results have important applications in IRT assessment routines in various contexts, not only those of sports sciences and medicine.

4.1.7. Conflict of interest statement

The authors declare that they have no financial or other interest concerning the content of this paper.

4.1.8 Acknowledgments

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4.1.9 References

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4.1.10 Supplementary material

Anterior thigh																				
Experienced																				
	Right										Left									
	Before exercise					After exercise					Before exercise					After exercise				
Evaluators	Minimum	Maximum	Range	Mean	Standard deviation	Minimum	Maximum	Range	Mean	Standard deviation	Minimum	Maximum	Range	Mean	Standard deviation	Minimum	Maximum	Range	Mean	Standard deviation
A	29.6 (1.1)	33.6 (1.3)	3.9 (1.4)	31.5 (1.0)	0.5 (0.1)	30.0 (1.1)	33.1 (1.2)	3.9 (0.8)	32.2 (1.2)	0.6 (0.2)	29.5 (0.1)	33.7 (1.2)	4.2 (1.2)	31.6 (1.0)	0.6 (0.2)	29.5 (1.1)	34.1 (1.2)	4.6 (1.1)	32.2 (1.3)	0.7 (0.1)
B	28.7 (1.4)	34.2 (1.4)	5.5 (1.3)	31.5 (1.0)	0.6 (0.1)	28.9 (1.4)	34.2 (1.4)	5.4 (1.1)	32.2 (1.2)	0.7 (0.2)	27.1 (1.4)	34.3 (1.6)	6.3 (1.3)	31.6 (1.1)	0.7 (0.2)	28.3 (1.2)	34.4 (1.3)	6.1 (1.2)	32.2 (1.3)	0.7 (0.1)
C	29.9 (1.1)	33.5 (1.3)	3.6 (0.7)	31.5 (1.1)	0.5 (0.1)	29.8 (1.6)	33.1 (1.2)	4.1 (0.8)	32.2 (1.3)	0.7 (0.2)	29.6 (1.0)	33.5 (1.2)	3.9 (0.8)	31.7 (1.1)	0.6 (0.2)	29.9 (1.5)	34.1 (1.3)	4.2 (0.9)	32.2 (1.3)	0.6 (0.1)
D	29.9 (1.0)	33.1 (1.1)	3.2 (0.6)	31.5 (1.1)	0.5 (0.1)	30.1 (1.3)	33.1 (1.2)	3.9 (0.8)	32.2 (1.3)	0.7 (0.2)	29.7 (0.9)	33.4 (1.2)	3.6 (0.7)	31.7 (1.1)	0.6 (0.2)	30.2 (1.4)	34.0 (1.2)	3.8 (0.8)	32.2 (1.3)	0.6 (0.1)
Novice																				
A	28.4 (0.9)	33.7 (1.2)	5.4 (0.1)	31.6 (1.1)	0.6 (0.1)	28.5 (1.8)	34.2 (1.0)	5.7 (1.7)	32.2 (1.2)	0.7 (0.2)	28.6 (1.3)	33.9 (1.3)	5.3 (1.2)	31.6 (1.1)	0.6 (0.2)	28.2 (1.3)	34.3 (1.1)	6.1 (1.4)	32.2 (1.3)	0.7 (0.1)
B	30.2 (0.9)	33.0 (1.1)	2.9 (0.7)	31.5 (1.0)	0.4 (0.1)	30.6 (1.1)	33.8 (1.2)	3.2 (0.9)	32.3 (1.2)	0.6 (0.2)	29.1 (0.9)	33.1 (1.1)	3.1 (0.7)	31.6 (1.0)	0.6 (0.1)	30.6 (1.2)	34.0 (1.2)	3.4 (0.8)	32.3 (1.2)	0.6 (0.1)
C	29.6 (1.0)	33.3 (1.1)	3.7 (1.2)	31.4 (0.1)	0.5 (0.1)	29.7 (1.2)	34.1 (1.1)	4.4 (1.1)	32.3 (1.1)	0.7 (0.2)	29.2 (1.2)	33.3 (1.1)	4.1 (1.2)	31.6 (1.0)	0.6 (0.2)	29.6 (1.1)	34.2 (1.0)	4.6 (0.1)	32.3 (1.2)	0.7 (0.1)
D	29.7 (1.1)	33.5 (1.4)	3.8 (1.4)	31.5 (0.1)	0.5 (0.1)	29.1 (1.1)	34.0 (1.2)	4.1 (0.1)	32.3 (1.2)	0.7 (0.2)	29.4 (0.9)	33.4 (1.4)	4.1 (1.2)	31.6 (1.0)	0.6 (0.2)	29.9 (1.1)	34.1 (1.2)	4.2 (0.1)	32.2 (1.2)	0.6 (0.1)

Table 4.2 Mean and standard deviation of skin temperature parameters from the anterior thigh. All evaluators are represented by letters (A, B, C, D).

Posterior leg																				
Experienced																				
Right											Left									
	Before exercise					After exercise					Before exercise					After exercise				
Evaluators	Minimum	Maximum	Range	Mean	Standard deviation	Minimum	Maximum	Range	Mean	Standard deviation	Minimum	Maximum	Range	Mean	Standard deviation	Minimum	Maximum	Range	Mean	Standard deviation
A	29.7 (1.7)	33.4 (1.1)	3.7 (1.4)	32.1 (1.2)	0.6 (0.2)	30.1 (1.4)	34.9 (1.1)	4.8 (1.3)	32.8 (1.1)	0.7 (0.2)	29.9 (1.4)	33.2 (1.1)	3.3 (0.9)	32.1 (1.2)	0.5 (0.2)	30.7 (1.2)	34.8 (1.2)	4.2 (1.0)	32.8 (0.1)	0.7 (0.2)
B	27.9 (1.9)	33.4 (1.1)	5.5 (1.5)	32.0 (1.2)	0.7 (0.2)	28.8 (1.7)	34.9 (1.1)	6.2 (1.7)	32.8 (1.0)	0.8 (0.2)	27.1 (1.8)	33.2 (1.1)	5.2 (1.2)	32.1 (1.2)	0.6 (0.2)	28.9 (1.7)	34.8 (1.1)	5.1 (1.8)	32.8 (0.9)	0.8 (0.2)
C	28.1 (1.6)	33.3 (1.1)	4.4 (1.0)	32.0 (1.2)	0.6 (0.2)	29.7 (1.3)	34.9 (1.1)	5.2 (1.2)	32.7 (1.1)	0.8 (0.2)	29.2 (1.4)	33.2 (1.1)	3.1 (0.1)	32.0 (1.2)	0.5 (0.2)	30.2 (1.1)	34.8 (1.2)	4.6 (0.9)	32.8 (0.1)	0.7 (0.2)
D	30.0 (1.6)	33.5 (1.1)	3.4 (1.1)	32.2 (1.1)	0.5 (0.2)	30.6 (1.4)	34.8 (1.3)	4.2 (1.2)	32.7 (1.1)	0.7 (0.2)	30.4 (1.4)	33.3 (1.1)	2.9 (0.7)	32.2 (1.2)	0.5 (0.1)	30.8 (1.1)	34.6 (1.3)	3.8 (0.9)	32.8 (1.0)	0.7 (0.2)
Novice																				
A	27.4 (1.7)	33.4 (1.1)	5.1 (1.6)	31.9 (1.2)	0.8 (0.3)	27.5 (1.8)	34.7 (1.1)	7.3 (1.9)	32.5 (1.1)	0.9 (0.3)	26.6 (1.1)	33.2 (1.1)	6.5 (1.4)	31.9 (1.2)	0.7 (0.2)	27.9 (1.1)	34.7 (1.3)	6.8 (1.2)	32.6 (1.1)	0.8 (0.2)
B	30.4 (1.6)	33.4 (1.1)	2.1 (1.0)	32.2 (1.1)	0.5 (0.2)	30.1 (1.6)	34.9 (1.1)	3.1 (1.1)	32.9 (1.0)	0.7 (0.2)	30.4 (1.5)	33.2 (1.1)	2.8 (0.1)	32.1 (1.2)	0.4 (0.2)	31.1 (1.3)	34.8 (1.1)	3.7 (1.1)	32.9 (0.1)	0.6 (0.2)
C	28.7 (2.3)	33.4 (1.1)	4.7 (1.8)	32.0 (1.2)	0.6 (0.3)	28.8 (1.7)	34.8 (1.2)	5.9 (1.8)	32.6 (1.0)	0.7 (0.2)	28.9 (2.1)	33.1 (1.2)	4.3 (1.5)	32.0 (1.2)	0.6 (0.2)	28.8 (2.2)	34.7 (1.2)	5.1 (2.2)	32.7 (1.0)	0.8 (0.2)
D	29.1 (1.9)	33.4 (1.1)	4.3 (1.4)	32.1 (1.2)	0.6 (0.2)	29.1 (1.4)	34.9 (1.1)	4.9 (1.3)	32.8 (1.0)	0.8 (0.2)	29.3 (1.4)	33.2 (1.1)	3.9 (0.1)	32.1 (1.2)	0.5 (0.2)	30.2 (1.3)	34.8 (1.1)	4.6 (1.2)	32.9 (0.1)	0.7 (0.2)

Table 4.3 Mean and standard deviation of the skin temperature parameters from the posterior leg. All evaluators are represented by letters (A, B, C, D).

Chapter V

Manuscript II

5. CHAPTER V

5.1 MANUSCRIPT II

Publications obtained:

da Silva W, Machado ÁS, Lemos AL, de Andrade CF, Priego-Quesada JI, Carpes FP (2021). Relationship between exercise-induced muscle soreness, pain thresholds, and skin temperature in men and women. ***Journal of Thermal Biology***. Volume:100. doi: 10.1016/j.jtherbio.2021.103051.

RELATIONSHIP BETWEEN EXERCISE-INDUCED MUSCLE SORENESS, PAIN THRESHOLDS, AND SKIN TEMPERATURE IN MEN AND WOMEN

Exercise-induced pain and skin temperature responses in men and women

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5.1.1 Abstract

Infrared thermography (IRT) has gained popularity in sports medicine for determining whether changes in skin temperature relate to pain and muscle damage. Such a relationship would support IRT as a non-invasive method to monitor these physiological responses. However, the literature remains controversial. Here, we determine the relationship between exercise-induced muscle soreness (DOMS), pain, and skin temperature in men and women before and after exercise. Twenty-two physically active adults (10 men and 12 women) completed a squat exercise protocol to induce muscle damage. Skin temperature, DOMS, and pressure pain threshold (PPT) were assessed in the quadriceps pre, post-exercise, and 48 h post-exercise. DOMS increased similarly in men and women post-exercise and 48 h post-exercise. PPT was lower in women compared to men. PPT decreased 48 h post-exercise for men but did not differ between the moments for women. Skin temperature responses were sex-dependent. Mean and maximum temperatures increased post-exercise for men, and maximum temperature reduced 48 h post-exercise. In women, the minimum temperature increased 48 h post-exercise. DOMS was not predicted by skin temperature but showed a direct association between pre and 48 h post-exercise variation of maximum skin temperature and PPT. We conclude that there is a sex-dependent effect in analyzing skin temperature changes in response to exercise, something that seems to not have been addressed in previous studies. To date, inferences are generally assumed as similar for both men and women, which we show may not be the case.

Keywords: muscle damage, physical exercise, muscle fatigue, exercise recovery, infrared thermography.

5.1.2 Introduction

Exercise-induced delayed onset muscle soreness (DOMS) commonly results from abrupt increases in exercise loads, the performance of unusual exercise gestures, and especially after the performance of exercises requiring eccentric actions (Cheung et al. 2003). Some of the mechanisms associated with DOMS include mechanical strains in muscle structures, overstretching sarcomeres, filament overlap and disruption (Peake et al. 2017), biochemical reactions affecting muscle contractile structures (Cheung et al. 2003), and inflammatory processes associated with oxidative stress and higher sensitivity of pain receptors (Peake et al. 2017). It results in transient strength loss, reduced joint range of motion, and generates exacerbated pain sensation (McHugh et al. 1999; Peake et al. 2017), which may last up to 72 hours after exercise (Connolly et al. 2003) and impairs performance of daily activities and sports (MacIntyre et al. 1995).

Local pain and DOMS are often assessed using subjective scales that provide quick feedback about muscle damage. However, pain perception may vary depending on participant familiarization with instructions and the pain sensation itself (Katz & Melzack 1999). Biochemical tests, on the other hand, may be more precise in quantifying damage associated with pain but are costly and time-consuming. There is an increasing interest in the use of infrared thermography (IRT) to estimate damage before, during, and after exercise sessions (Priego-Quesada et al. 2020). Different studies argue that the inflammation that occurs in the DOMS mechanism increases blood flow and local hyperthermia, both of these changes being detectable using IRT (Gómez-Carmona et al. 2020; Hildebrandt et al. 2010).

However, there is no concise evidence to support a relationship between DOMS and changes in skin temperature due to acute local inflammation and tissue adaptations. Higher DOMS and higher skin temperature were found up to 48 h post-exercise in the quadriceps of recreationally active men, but DOMS and skin temperature did not correlate (Stewart et al. 2020). DOMS, fatigue, and muscle damage were observed in trained men and women after a competitive half marathon or a marathon, but skin temperature did not increase and did not predict DOMS

(Pérez-Guarner et al. 2019; Priego-Quesada et al. 2020). Higher DOMS and creatine kinase levels were found after heel-rise exercise in untrained men, but correlations between biochemical markers of damage, inflammation, DOMS, and skin temperature were not significant (Alfonsin et al. 2019). On the other hand, a relationship between DOMS and skin temperature in the quadriceps was found in men triathletes undergoing a cumulative training load for three days under controlled conditions in a training camp (Priego-Quesada et al. 2019). These studies assessed DOMS through individual perception using information from a visual analog scale (VAS) but did not measure pressure pain thresholds (PPT). VAS considers an overall perception of pain intensity (MacIntyre et al. 1995), while PPT considers muscle tenderness in response to muscle damage and inflammation (Fleckenstein et al. 2017). Muscle nociception is boosted by the sensitization of several physiological structures such as C-fibers and Aδ-fibers, mediating the sensation of pain, and the high threshold mechanosensitive receptors HTM, which are pressure-dependent (Basbaum et al. 2009; Fleckenstein et al. 2017). In comparison to VAS, PPT could provide a more accurate measure of pain (Alfonsin et al. 2019).

Another factor of confusion in these studies is the differences between men and women. Women may be more sensitive to the experimental stimulus of pain than men (Dannecker et al. 2005), but this is not clear when DOMS is measured by VAS (Dannecker et al. 2003, 2005). In terms of clinical relevance, PPT has been shown to differ between men and women (Cámara et al. 2020), whereas DOMS has not been compared in previous studies. Sex differences were observed in the descending pain modulatory system (DPMS) and corticospinal motor pathway integrity test during a conditioned pain modulation (Gasparin et al. 2020).

Here, women presented a higher DPMS inhibitory function and a higher level of brain-derived neurotrophic factor level, an association with the DPMS function only being found in women (Gasparin et al. 2020). Therefore, neuroplasticity may be a crucial factor in explaining the difference in pain perception between men and women. Regarding acute adaptations, a different fatigue pattern could also be experienced depending on sex, such as immediately after the performance of eccentric exercise (Morawetz et al. 2020). Finally, women usually present

lower skin temperature than men due to a higher body fat proportion, lower body surface area, and lower peripheral blood flow (Barnes 2017; Jimenez-Perez et al. 2020).

Understanding whether the methods to indirectly quantify muscle damage influence the association of exercise-induced pain and skin temperature, as well as whether sex influences these parameters, can provide more conclusive information on applying IRT for monitoring the acute adaptations caused by physical exercise. This is of special interest due to the ever-increasing interest in applying IRT to the field (Gómez-Carmona et al. 2020; Priego-Quesada et al. 2020; Stewart et al. 2020). The aim of our study, therefore, was to determine whether exercise-induced muscle soreness and pain thresholds are related to skin temperature measured using infrared thermography in men and women. Taking into account that exercise would cause acute changes of small magnitude in skin temperature, we hypothesized that the changes in pain thresholds could more closely be related to skin temperature, given that those thresholds provide continuous measurements rather than the scalar measurements provided by scales. Furthermore, PPT provides an assessment of muscle soreness in the same sites where skin temperature is measured, unlike scales that provide an overall perception of pain. Bearing in mind that women present lower pain thresholds and skin temperature, we expected the outcomes to show a sex effect.

5.1.3 Material and methods

Participants and experimental design

A sample size of 20 untrained participants was estimated using the G * Power 3.1 software (Faul et al. 2007) (University of Düsseldorf, Düsseldorf, Germany) and considering a repeated measures ANOVA design, with 90% power, α value of 0.05, and an effect size of 0.26 for data on the amplitude of skin temperature (da Silva et al. 2018). To anticipate any participant dropout, 10% additional participants for both sexes were added. Two men participants were excluded because they did not show up at the time scheduled for the measurements. We included 22 participants (10 men: 23.5 ± 6.6 years old, body mass 72.7 ± 9.1, height 1.72 ± 0.1, body max index (BMI) 24.3 ± 2.2 kg/m^2, and 12 women: 27.0 ± 4.2 years old, body mass 59.2 ± 9.2, height

1.59 ± 0.1, body max index (BMI) 22.8 ± 3.2 kg/m^2). All participants signed a consent form agreeing to participate, and all procedures established in the Helsinki Declaration were respected. The University's local ethics committee approved this research (IRB: 26037119.9.0000.5323).

The participants were recruited from the university community. They had to be free of lower limb injury, could not have experienced DOMS in the past six months before the experiment, and could not be enrolled in any systematic physical training. In the days before the experiments, they were instructed to avoid physical exercise, not use anti-inflammatory substances, avoid alcohol consumption, caffeinated drinks, cigarettes, hot baths, sunbathing, and use of creams and cosmetics (Priego Quesada 2017). Data collections were undertaken over two days, with an interval of 48 h between them. On the first day, the participants answered an anamnesis questionnaire for personal information, were submitted to an anthropometrical assessment, and completed a squat exercise protocol to induce muscle damage in the quadriceps. Skin temperature, DOMS, and pain thresholds were evaluated pre-exercise and post-exercise on day 1 and repeated 48 h post-exercise. All tests were performed at the same time of the day. Figure 1 illustrates our experimental design.

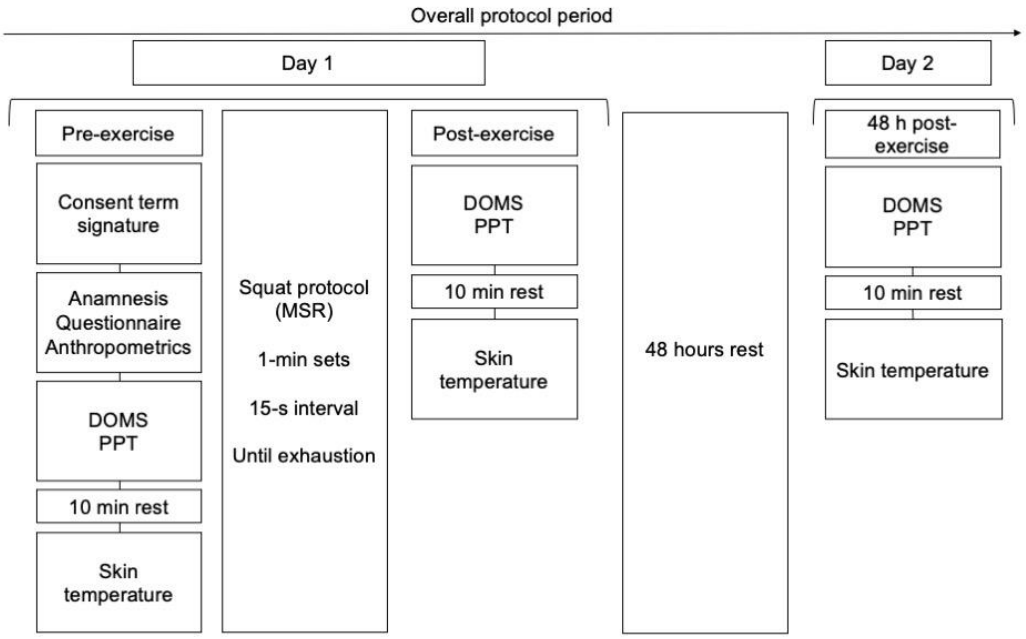

Figure 5.1 Experimental design. All measurements were undertaken in the same order and manner for all participants. A pre-exercise measurement was undertaken before the squat protocol. Post-exercise measurements were conducted immediately after the squat protocol, and 48 hours post-exercise. DOMS: delayed onset muscle soreness; PPT: pressure pain threshold; MSR: Maximum squat repetitions.

Exercise protocol

To induce DOMS in the quadriceps the participants completed an exercise protocol for maximum squat repetitions with their body weight as workload (Guo et al. 2017). The protocol started with a standardized warm-up on a motorized treadmill walking at the preferred speed for 5 min. After the warm-up, a researcher demonstrated how the squat movements should be performed in terms of amplitude and velocity. From the upright posture, the squat movement should be performed bending the knees to 90º in the eccentric phase followed by the concentric phase to the upright position again. Participants were instructed to avoid valgus movement or trunk projection in front of the knees, and that their feet and knees had to remain aligned with the shoulders. They had to perform the maximum number of squats possible at the highest velocity possible for each one-minute set. The one-minute sets were repeated with a 15-second interval in between until exhaustion. Exhaustion was defined by the participant being no longer able to

perform the squat movements or when movement technique deteriorated, for example, with loss of balance or a drop in movement velocity.

Muscle soreness and pain threshold assessments

DOMS was assessed using a numeric pain rating scale (NPRS) ranging from 0 to 10 (Hawker et al. 2011). The values on the scale of 0 (no pain) to 10 (extreme pain) were explained to the participants, and they had to report the corresponding pain sensation when asked. The pressure pain threshold (PPT) was measured in response to mechanical pressure stimuli (Kelly-Martin et al. 2018). PPT was assessed bilaterally in the proximal, medial, and distal portions of the rectus femoris (RF) and vastus lateralis (VL), and, in order to reduce sensitization by systemic factors, in a non-exercised body region (the medial portion of the right deltoid) using a digital algometer (Instrutherm DD-200) with a resolution of 0.01 Newton and a flat tip with 1 cm^2. The algometer was positioned perpendicular to the skin surface and pressed on the skin by an experienced researcher (Pöntinen 1998).

Participants were previously advised on how to report the moment when the sensation of pressure became an uncomfortable pain. The pain threshold was recorded in N/cm^2. PPT data were assessed using absolute and normalized values (difference between the exercised and non-exercise regions). To check the validity of normalized values, we assessed the measurement reproducibility (considering pre-exercise, post, and 48h post-exercise measures) of deltoid measurements (intraclass correlation coefficient [ICC], single rater-measurement, absolute-agreement, and 2-way random-effects model), observing an ICC of 0.89 ($p<0.001$). For both DOMS and PPT assessment, participants were supine, lying on a stretcher, and were asked to relax the muscles and not look at the algometer during the assessment. The same researcher performed all assessments.

Skin temperature assessment

Skin temperature was quantified using an infrared thermography camera with a resolution of 320 x 240 pixels (FLIR model E-60, Flir Systems Inc., Wilsonville, Oregon, USA) with noise-

equivalent temperature difference (NETD) <0.05 °C, and measurement uncertainty of ± 2°C or 2%. The camera was turned on at least 10 min before the evaluations to allow stabilization of the electronic components. All images were captured at a distance of 1 m from the region of interest (ROI), with a camera lens perpendicular to the ROI. Three ROIs were defined, the left and right anterior thigh (bilateral) and the right biceps brachii (unilateral) (Figure 2). The mean number of pixels of the ROIS were 17.639, 17.498, and 1.150 pixels for the left anterior thigh, right anterior thigh, and right biceps brachii, respectively. All images were recorded with participants positioned standing still with the musculature exposed 10 minutes after DOMS and PPT assessment to ensure proper adaptation to the room temperature.

The thermal images recorded post-exercise were captured 17.83 ± 5.9 min after the baseline measurement, and the images recorded 48 h post-exercise were captured 48 hours post to the baseline measurement. An anti-reflective panel was placed behind the participant to avoid interferences from radiation emitted by a non-neutral background. Skin temperature was determined considering the average, maximum, minimum, the post-exercise (ΔT0), and the 48 h post-exercise (ΔT48). The mean of skin temperature data was determined based on the average of the values for pixels inside ROIs. The maximum and minimum skin temperature data were determined based on the value of a single hottest and coldest pixel inside the ROIs. All thermal images were taken in an air-conditioned and controlled environment: room temperature 24.4 ± 1.6 ºC and air humidity 46.8 ± 10.0 %. All procedures followed the TISEM checklist (Moreira et al. 2017). The same researcher performed all image records. Infrared images were processed using commercial software (Thermacam Researcher Pro 2.10 software, FLIR, Wilsonville, OR, USA).

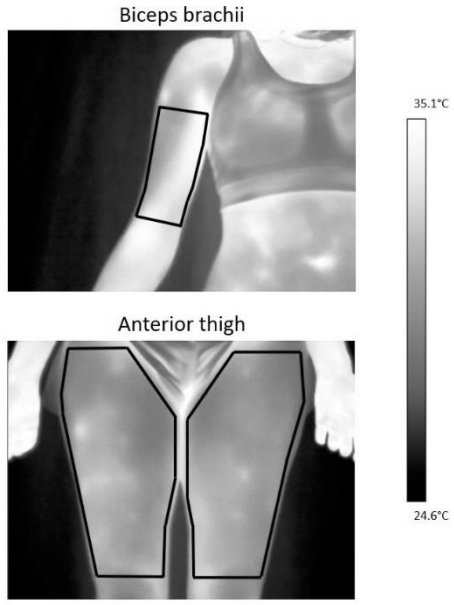

Figure 5.2 Definition of the regions of interest (ROI) for analysis of skin temperature. The image is representative for all participants.

Statistical analysis

Data are reported as mean and standard deviation. The Shapiro-Wilk test confirmed the normality of data distribution, except for DOMS. DOMS was compared between the moments using the Friedman test with Wilcoxon post-hoc, and differences between sexes were assessed using the U of Mann-Whitney. Repeated measures ANOVA with Bonferroni post-hoc were applied to compare PPT at each region of the vastus lateralis and rectus femoris with three intra-subject factors: moment (pre, post-exercise, and 48h post-exercise), muscle region (proximal, medial, and distal), and leg preference (preferred and non-preferred), and one inter-subject factor: sex (men and women). A similar approach was undertaken to analyze the normalized values of PPT and skin temperature. For significant pair differences, Hedge's effect sizes (ESg) were computed with paired correction (Gibbons et al. 1993; Hedges 1981) using RSTUDIO (v. 1.2.5033 and package effsize (Marco Torchiano 2020) and classified as small (ESg 0.2–0.5), moderate (ESg 0.5–0.8), or large (ESg>0.8) (Jacob Cohen 2013).

Mean with 95% confidence intervals of the ESg were provided. Finally, stepwise multiple linear regressions were performed using the variations of skin temperature (ΔT) parameters from

the anterior thigh as predicting variable. Inputs of the models were: moment (variation between post vs. pre or between 48h post-exercise vs. pre), sex, age, body mass index, variation of DOMS, and variation of PPT (absolute and normalized values) in the vastus lateralis and rectus femoris. For the models obtained, the coefficient of each variable of the equation, the percentage of the variance explained by the model (R^2), and the significance value of the model was provided. The significance level was set at 0.05 for all analyses using the SPSS version 26 (SPSS Inc., Chicago, IL).

5.1.4 Results

Muscle soreness and pressure pain threshold

DOMS increased immediately post-exercise (5.1 ± 2.2 points compared to 0.7 ± 1.1 pre-exercise, $p<0.001$, ES=2.3 [1.4, 3.3]) and remained higher 48 h post-exercise (4.0 ± 2.9 points, $p<0.001$, ES=1.3 [0.7, 2.0] compared to pre-exercise, and $p=0.12$ compared to immediately post-exercise), without differences between men and women ($p>0.42$).

The main effect of leg preference was not significant in PPT ANOVA ($p>0.05$) and neither was its interaction (that of leg preference) with the other factors. For this reason, this factor was not considered in the following results. PPT in the non-exercised muscle did not differ between the moments for men and women (men, average and SD pre-exercise: 48.9 ± 13.8, post-exercise: 49.9 ± 16.1, 48 h post-exercise: 48.3 ± 15.5; $p>0.05$; women, pre-exercise: 24.1 ± 6.1, post-exercise: 24.7 ± 9.9, 48 h post-exercise: 24.1 ± 6.4; $p>0.05$). PPT in the VL was lower in the distal compared to the medial and proximal portions (distal vs. medial: 27.0 ±14.2 vs. 29.2 ±14.5 N/cm^2, $p<0.01$, ES=0.2 [0.1, 0.2]; distal vs. proximal: 27.0 ±14.2 vs. 29.3 ±12.6 N/cm^2, $p=0.01$, ES=0.1 [0.1, 0.2]). In the RF, proximal portion showed lower PPT than the medial and distal (proximal vs. medial: 30.3 ±11.4 vs. 34.6 ±14.2 N/cm^2, $p<0.001$, ES=0.3 [0.1, 0.5]; proximal vs. distal: 30.3 ±11.4 vs. 36.1 ±15.2 N/cm^2, $p<0.001$, ES=0.4 [0.2, 0.6]).

The following results show the interaction between measurement moment and sex, as similar results were observed on assessing differences between moments and sex in proximal, medial, and distal portions. With regard to absolute and normalized data, PPT was lower in

women compared to men for most of the moments. For women, PPT pre-exercise magnitudes did not differ from the other two moments, but 48 h post-exercise PPT was lower than post-exercise (Figure 3). PPT increased in men post-exercise but was lower at 48 h post-exercise in both anterior thigh regions.

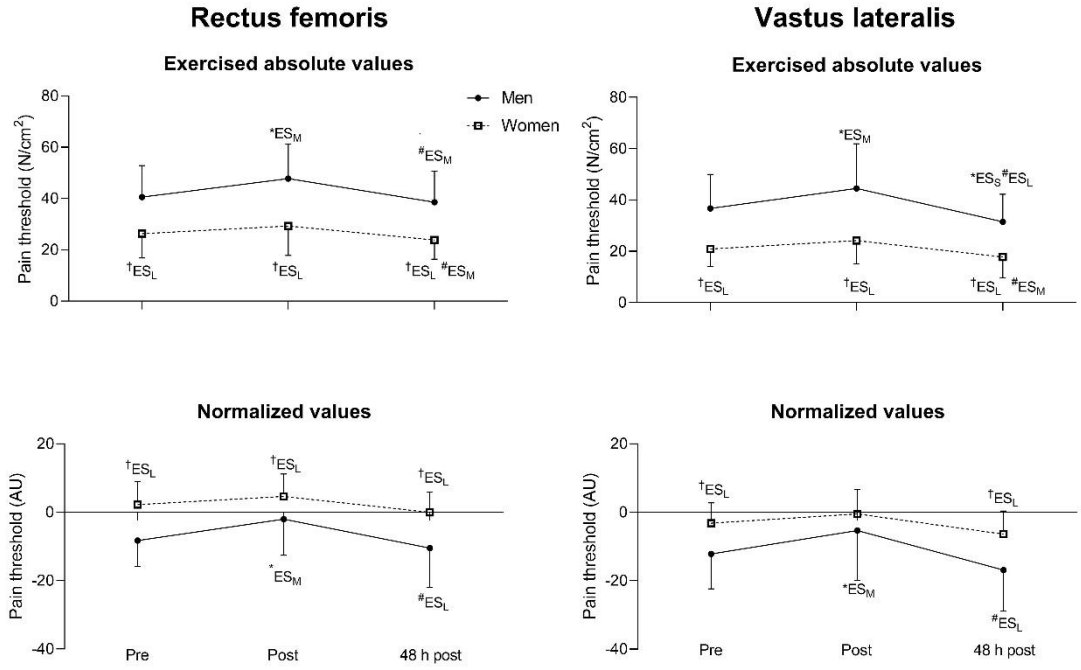

Figure 5.3 Mean (points) and standard deviation (vertical lines) of pain threshold. AU refers to arbitrary units (N/cm^2 - N/ cm^2), symbols identify differences between women and men ($^{\dagger}p < 0.05$), between the moments (different of pre-exercise: $^{*}p < 0.05$; different of post-exercise: $^{\#}p < 0.05$), and letters identify the magnitudes of Hedge's effect sizes (small effect size ES_S; moderate effect size ES_M; large effect size ES_L).

Skin temperature

The main effect of leg preference was not significant in the mean and minimum skin temperature ANOVAs ($p>0.05$), nor was its interaction with the other factors. The maximum temperature was slightly higher in the non-preferred thigh, but the effect size was smaller than small (33.8 ± 1.0ºC vs. 33.6 ± 1.0ºC, $p=0.02$, ES=0.1 [0.0, 0.2]). Therefore, this factor was not

considered in the following results. In the non-exercised region, the main effect of sex and interaction between sex and moment were not significant for any of the skin temperature parameters ($p>0.05$).

Mean and maximum skin temperature in the exercise region increased post-exercise, and the maximum temperature was lower 48 h post-exercise in men (Figure 4). Temperature pre-exercise and 48h post-exercise did not differ in the exercised region in men in any of the thermal parameters ($p>0.05$). In women, the mean and maximum skin temperature in the exercised region did not differ between pre-exercise and post-exercise ($p>0.05$), whereas the minimum temperature was higher 48 h post-exercise (pre-exercise 28.9 ± 1.1ºC vs. 48 h post-exercise 30.0 ± 0.8ºC, $p=0.03$, ES=1.1 [0.2, 2.0]). At all the moments the mean temperature of the exercised region and at some moments of the other thermal parameters, women present lower skin temperature than men (e.g., pre-exercise mean temperature: men 32.1 ± 1.1ºC vs. women 31.1 ± 0.7ºC, $p=0.02$, ES=1.1 [0.2, 2.0]; post-exercise mean temperature: men 33.0 ± 0.9ºC vs. women 31.4 ± 1.0ºC, $p<0.001$, ES=1.7 [0.7, 2.7]).

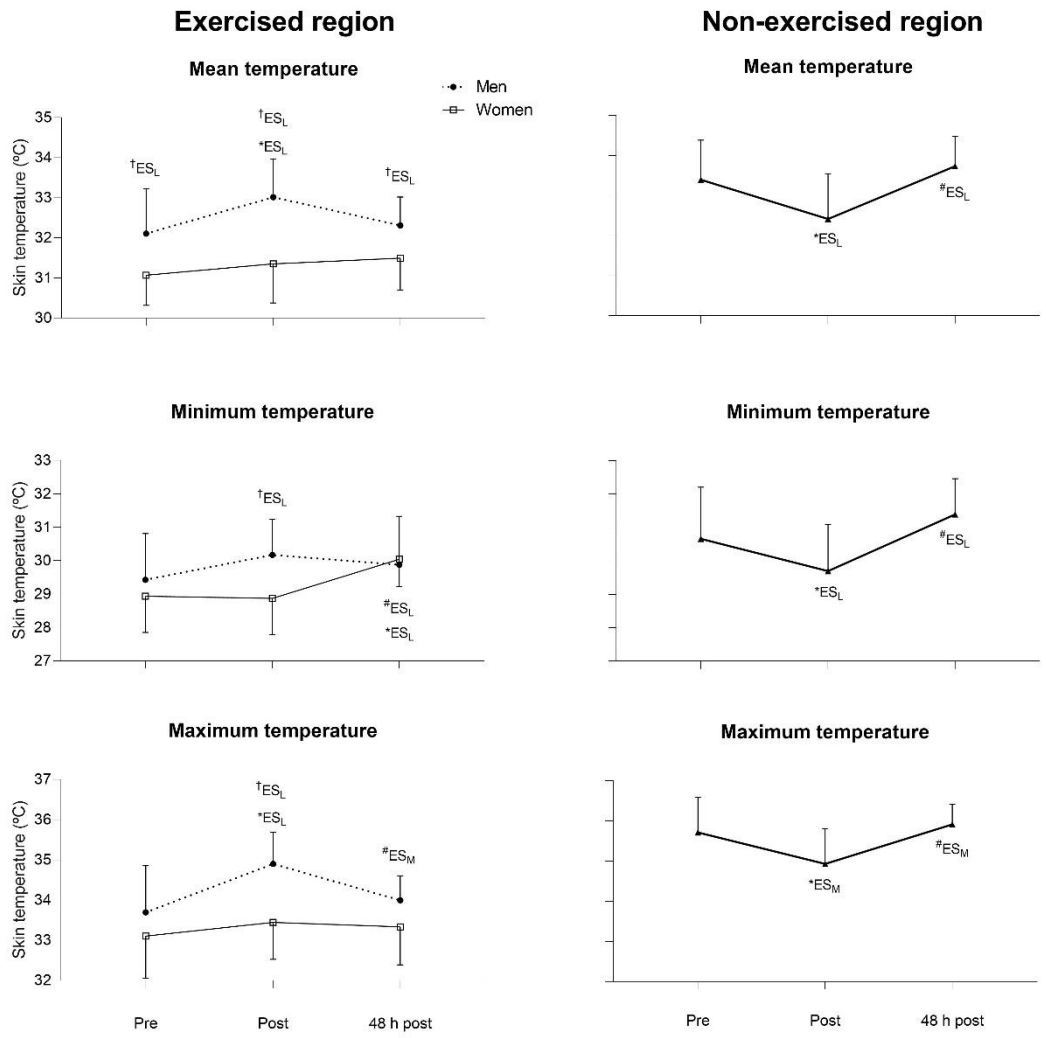

Figure 5.4 Mean (points) and standard deviation (vertical lines) of skin temperature parameters. Differences between men and women are identified by symbols ($^{\dagger}p < 0.05$), moments (different of pre-exercise: $^{*}p < 0.05$; different of post-exercise: $^{\#}p < 0.05$) and the Hedge's effect sizes with letters (small effect size ES_S; moderate effect size ES_M; large effect size ES_L).

Relationship between pain and skin temperature

No significant regression model was obtained for the association of DOMS, PPT, and variation of mean skin temperature (Table 1). The minimum temperature was inversely related to the measurement moment, and variation of maximum skin temperature from the anterior thigh showed a direct relationship with the absolute values of the Δ pain threshold of the rectus femoris.

Table 5.1 Regression models obtained by multivariate stepwise regression analyses using variations in skin temperature parameters of the anterior thigh (ΔT) and moment (variation between post vs. pre or between 48h post vs. pre), sex, age, BMI as inputs, and the corresponding variation of NPRS and pain threshold of absolute and normalized values of VL and RF.

	Regression models obtained		
Predicting variable	**Variable**	**Coefficient [CI95%]**	**R^2 (p-value)**
	ΔT		
Mean	No variable was included		
Minimum	Constant	4.01 [2.64, 5.38]	0.64 (<0.001)
	Moment*	-3.71 [-4.57, -2.84]	
Maximum	Constant	0.42 [0.15, 0.69]	0.15 (0.01)
	Δ absolute RF pain threshold	0.06 [0.02, 0.10]	

*Note: For the equation, variation between post-pre had the value of 1, and variation between post48h-pre the value of 2.

5.1.5 Discussion

Here we determine whether exercise-induced muscle soreness and pressure pain thresholds are related to changes in skin temperature differently in men and women. DOMS following exercise did not differ between sexes, but PPT was lower in women in all-time moments and reduced to a larger extent in men 48 h post-exercise. We also found that skin temperature was lower in women than men. A relationship between the variation of maximum skin temperature and variation of PPT 48 h post-exercise was found, explaining 15% of the variance.

We consider that our results open room for a discussion on a sex-dependent effect in analyzing skin temperature changes in response to exercise, something that seems to not have been addressed in previous studies. Previous studies mainly considered men as participants, or when including both men and women, did not provide a sex comparison. To date, inferences are generally assumed as similar for both men and women, which we show may not be the case.

The acute effect of the maximal squat exercise protocol was a reduction in the PPT 48 h post-exercise, especially in men, when DOMS was at the highest level. This result is similar to previous observations of men performing barbell back squats at 60% of individual 1 RM (Pearcey et al. 2015), and after maximal knee extensors eccentric exercise (Naderi et al. 2020). Men can be more susceptible to exercise-induced muscle damage than women (Morawetz et al. 2020), even when CK is normalized by the cross-sectional area (CSA) in the quadriceps muscle (Morawetz et al. 2020). Furthermore, DOMS shows a moderate correlation ($r = 0.56$) with CK in men, but not significant for women after eccentric exercise (Sewright et al. 2008). The magnitude of muscle damage may reflect a lower PPT in men 48 h post-exercise, and it could help to explain the difference observed in our results for both men and women.

Moreover, it is important to consider where the PPT is measured. We assessed the rectus femoris and vastus lateralis muscles considering three different regions and found PPT varying between the regions. We consider that the differences between the muscle regions may be a result of the exercise configuration affecting muscle recruitment, as for each angulation, a different portion of the muscles can be more or less activated (Priego Quesada et al. 2015). Also, muscle tenderness is usually concentrated in the distal portion of the muscle due to a higher concentration

of pain receptors (Cheung et al. 2003). On the other hand, PPT is lower in women and stays stable after exercise, despite the presence of high variability. The women included in our study were at different menstrual cycle phases (7 in luteal, and 5 in the follicular phase). This hormonal variability can increase the variability of the PPT. A previous study showed that hormone fluctuations throughout the menstrual cycle phase, such as the estrogen level, affect exercise-induced muscle damage in DOMS and strength loss (Romero-Parra et al. 2021). This behavior is not clear when considering well-trained women submitted to eccentric squat exercise. However, women in the luteal phase do show a higher interleukin-6 acute response (Romero-Parra et al. 2020). We did not include the effect of menstrual cycle phases in the results section due to the low statistical power provided by the low number of participants in each cycle phase. However, in our study women show a lower PPT post-exercise in the luteal phase. Although the menstrual cycle phase can influence body temperature and consequently influence skin temperature (Steward & Raja 2022), in an exploratory analysis of our data, we did not observe any effect of these different phases on skin temperature.

Men showed higher mean and maximal skin temperature post-exercise than women, but temperature returned to baseline values 48 h post-exercise. Interestingly, skin temperature in the women was altered only when considering the minimum temperature, which increased 48 h post-exercise. This result may suggest the desirability of using this thermal parameter to detect local hyperthermia. PPT, NPRS, or VAS are different ways of quantifying DOMS. The PPT measurement is common in DOMS assessment, but few studies have explored its relationship with skin temperature post-exercise. We found changes in maximum skin temperature showing a direct relationship with changes in the absolute PPT in the rectus femoris. An inverse and weak to moderate association was observed between PPT and skin temperature in men lumbar pain patients ($r = -0.36$ and $r = -0.49$), who also showed higher skin temperature than controls without pain (Alfieri et al. 2019). This association and the relationships observed in the present study may indicate that the level of skin vasoconstriction (which inversely affects skin temperature) and PPT could be directly related, in line with the hypothesis of a previous study (Priego-Quesada et al. 2020). However, it is important to consider that the skin temperature variance explained by PPT

was of 15%. This percentage can rely on the multifactorial dependence of skin temperature, which increases the variability of the results (Fernández-Cuevas et al. 2015; Priego-Quesada et al. 2019). In men triathletes submitted to cumulative training load, this relationship between DOMS and skin temperature may have arisen from the high volume and intensity of exercise administrated over three days of exercise, with athletes being submitted to the same daily routine in a training camp (Priego-Quesada et al. 2019). Moreover, although sex was not observed as an influenced factor in the regression analysis, it might contribute to increase the data variability. As women can be more sensitive to loss of strength after eccentric exercise (Morawetz et al. 2020), and the fact that a negative correlation between the maximum change in maximum isometric voluntary force (MIVF) and PPT was observed ($r = - 0.48$, $p = 0.03$) (Fleckenstein et al. 2017), it is possible that muscle strength plays a role in damage responses and, therefore, pain outcomes. Also, it is important to take into account that skin temperature may change less and more slowly in untrained participants compared to trained participants (Formenti et al. 2013).

We hypothesized that women would show lower skin temperature values than men due to differences in physiological characteristics (Barnes 2017; Jimenez-Perez et al. 2020). Women presented lower skin temperature than men for almost all moments, which agrees with a previous study (Jimenez-Perez et al. 2020). Nevertheless, we found this difference remained after exercise. IRT measures skin temperature, but muscle tissue is deeper. Its application depends on the relationship between heat dissipation of the muscle to skin by mechanisms of vasodilatation and blood flow. PPT is important in the assessment of exercise-induced muscle soreness because it has a direct relationship with muscle tenderness, and consequently, with inflammation and muscle damage, especially after eccentric actions (Fleckenstein et al. 2017). In a previous study, we showed that muscle damage markers such as CK, LDH, and DOMS intensity measured using VAS did not correlate with skin temperature in men pre-exercise, post-exercise, and 48 h post-exercise (da Silva et al. 2018). In this study we have replicated these results for men by analyzing the quadriceps exercise but have not found the same in women. Hence, in this study, we have made progress on this issue by showing that skin temperature may be related to PPT 48 h post-exercise.

There is no consensus about the application of the different thermal parameters in sports medicine and sports sciences (Priego Quesada & Vardasca 2017). For this reason, we decided to analyze different thermal parameters. Considering that mean skin temperature is a robust measure, maximum temperature could be more sensitive to increases in skin blood flow and inflammation, and minimum skin temperature could relate with the vasoconstriction of skin blood flow (Formenti et al. 2018; Priego Quesada & Vardasca 2017). The fact that we have observed changes only in the minimum temperature of women and not in the other parameters, or that the relationship obtained with the PPT regarded the maximum temperature, may suggest the need to use different thermal parameters in research studies. We also consider this can be a limitation because the maximum and minimum temperatures consider a single pixel value, also due to limitations in the common software used to determine the IRT metrics. This question was addressed by other studies in which a maximum temperature was obtained from the average of the 5 hottest regions composed each one by 25 pixels (Formenti et al. 2018).

Our study has limitations. Although the muscle damage was evident, we did not evaluate a muscle damage marker like creatine kinase (CK). The damage can be considered present due to changes in DOMS measured by NPRS and PPT, but biochemical markers should be considered in future studies to improve the discussion about its relationship with PPT and IRT measures in both sexes. Future studies could consider additional measurements of DOMS and skin temperature for the days before and after exercise, which could help to better understand the behavior and fluctuations in skin temperature and DOMS symptoms within a longer recovery period. As estrogen may play a protective but not well-understood role against muscle damage and inflammation in women (Kendall & Eston 2002), its quantification would help to improve future experiments. Finally, we evaluated physically active people, and the proportion of subcutaneous fat can differ between men and women with effects on skin temperature (Neves et al. 2015).

Our study has practical application. Skin temperature measure shows a sex-dependent effect, and caution is need when interpreting evidence from studies analyzing only men participants or not consider sex comparisons. When assessing DOMS, the analog scales do not

detect the difference between men and women, which are observed for PPT measures. Therefore, the better elucidation of any relationship between PPT and skin temperature opens room for the potential use of these measurements in the physical exercise routine, load control, and recovery.

5.1.6 Conclusion

Skin temperature changes in response to exercise showed a sex-dependent effect. The changes in skin temperature after exercise did not relate to DOMS, but there was a positive relationship between maximum skin temperature and PPT in the variation between pre-exercise and 48 h post-exercise. Investigating the relationship between DOMS and skin temperature, sex, and pain thresholds are crucial factors that must be considered in studies aiming to apply IRT measured in training load control and recovery assessment.

5.1.7 Conflict of interest statement

The authors declare that they have no financial or other interest concerning the content of this paper.

5.1.8 Acknowledgments

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Chapter VI

Manuscript III

6. CHAPTER VI

6.1 MANUSCRIPT III

Publications obtained: Under review.

da Silva W, Machado ÁS, Machado MS, Pereira MEF, Paz MM, Zacharias LMS, Morais ACL, Priego-Quesada JI, Carpes FP (2023). Preservation of baseline skin temperature despite muscle damage and soreness: findings from cold-stress test and rewarming assessments. ***Journal of Thermal Biology***.

PRESERVATION OF BASELINE SKIN TEMPERATURE DESPITE MUSCLE DAMAGE AND SORENESS: FINDINGS FROM COLD-STRESS TEST AND REWARMING ASSESSMENTS

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6.1.1 Abstract

Changes in skin temperature measured using thermography are speculated as responsive to the presence of muscle damage following exercise. However, the results of previous studies are controversial. We hypothesize that due to the influence of blood flow and vasoconstriction on local skin temperatures, using a cold-stress test could improve the measures of infrared thermography in the condition of muscle damage. To determine whether the assessment of skin temperature rewarming after a cold-stress protocol is influenced by the presence of muscle damage. 15 untrained participants (8 men and 7 women) completed a protocol of maximal voluntary heel rise repetitions to induce delayed onset muscle soreness (DOMS) of calf muscles. Infrared thermography camera images were recorded to determine baseline skin temperature and the rate of rewarming curve after a cold-stress test (30, 60, 120, and 180 seconds after finishing cooling), obtaining the β_0 and $\beta 1$ coefficients with the equation: Δ skin temperature variation = $\beta_0 + \beta 1 * \ln$ (Time in sec). Thermal images were recorded 24 h before, post-exercise, and 48 h post-exercise. Pain perception, pressure pain threshold, CK activity, and maximal voluntary isometric strength were determined pre-exercise and 48 h post-exercise. We found higher DOMS and CK activity 48 h post-exercise than pre-exercise ($p<0.01$). Baseline skin temperature and skin temperature following rewarming did not differ between days on β_0 ($p=0.7$) and $\beta 1$ ($p=0.8$). No associations were found for CK activity and DOMS 48h post-exercise with Δ skin temperature variation. Despite significant muscle damage and soreness 48 h post-exercise, changes in skin temperature over the affected muscle did not differ from those measured in the baseline, even when the measurements considered a cold-stress test and rewarming assessment.

Keywords: Dynamic infrared thermography; Thermal image Muscle fatigue; Physical exercise; Injury.

6.1.2 Introduction

Exercise-induced muscle damage is a physiological and transient phenomenon leading to transitory motor performance losses (Markus et al. 2021) and potentially increasing the injury risk (Proske & Morgan 2001). A condition of muscle damage may involve ruptures and disorganization of the muscle fiber contractile units, generating a loss of sarcomere efficiency and triggering oxidative and inflammatory processes (Clarkson & Hubal 2002). Inflammation activates several proteases, such as prostaglandins E2 (PGE2), which increase vascular permeability and sensitize type III and IV afferent fibers resulting in the so-called delayed onset muscle soreness (DOMS). In sports, DOMS is a reference for investigating the physiological situation of athletes, mainly to prevent damage from evolving into macroscopic ruptures with more severe repercussions on performance (Brancaccio et al. 2008; Geneva et al. 2019). A better understanding of the individual responses to exercise, including muscle damage, DOMS, and biomarkers, stimulates researchers to find strategies to monitor these acute adaptations indirectly.

Some alternatives to measure muscle damage include quantifying creatine kinase (CK), collecting muscle tissue samples (biopsies), and performing magnetic resonance imaging (MRI) assessment (Clarkson & Hubal 2002; Markus et al. 2021). An increased CK activity after exercise reflects the magnitude of muscle damage and cellular necrosis (Markus et al. 2021). In turn, biopsies allow us to analyze adaptations at the tissue level, like myofibrillar disturbance and Z-line streaming (Clarkson & Hubal 2002). Finally, changes in the intensity of the T2 relaxation time signal in MRI indicate edema (Clarkson & Hubal 2002). However, these analyses have broader application limitations due to costs, technical demands, and invasive nature (Stožer et al. 2020). Additionally, as in the case of biopsy, only a small sample is analyzed, and inferences are made for the entire muscle (Clarkson & Hubal 2002).

The physiological processes behind the presence of DOMS and the limitations of these technics motivate researchers to explore applications of infrared thermography (IRT), which is a non-contact and non-invasive technique to measure surface temperatures (Priego Quesada 2017), and therefore to measure skin temperature aiming to monitor muscle damage. The rationale for using IRT to monitor muscle damage is that blood circulation and possible inflammatory

processes could affect muscle temperature (de Andrade Fernandes et al. 2017), transmitting heat to the skin (Eddy et al. 2001). However, previous studies investigating muscle damage through skin temperature found contradictory results (Al-Nakhli et al. 2012; da Silva et al. 2018; Stewart et al. 2020). Despite Andrade Fernandes et al. found a moderate correlation between skin temperature and CK in soccer players, the level of muscle damage to which they were subjected is ambiguous due to the variability in the physical demands produced by the match. Moreover, although some studies found an increase in skin temperature 24 or 48 h after exercise, there was no correlation with pain or CK levels (Rojas-Valverde et al. 2021).

An increase in muscle temperature can be caused by local inflammation, this aggression to the muscle would also generate peripheral vasoconstriction, which can reduce the heat transference to the skin (Fagius et al. 1989). If that is the case, the measure of basal temperature may not be enough to detect the typical small magnitudes of change that will occur in skin temperature. Therefore, more than just measuring baseline skin temperature, inducing changes in the skin, such as by thermal stress and rewarming, could be an alternative (Priego-Quesada et al. 2022). This process is known as dynamic thermography (Burkes et al. 2016). Dynamic thermography has been explored in medical sciences to evaluate different pathologies, for instance, diabetes (Zeng et al. 2016) and breast cancer (Gonzalez-Hernandez et al. 2019). Little is known about its applicability to monitoring acute physical exercise-induced adaptations. The vasoconstriction due to muscle damage would be responsible either for intensifying the cooling of the limb or for delaying the normalization of the skin after the end of the cold stress compared to a non-exercised region (Priego-Quesada et al. 2020).

A previous Investigation found no differences in the baseline skin temperature measured 24 h and 48 h after a marathon but described lower skin temperature after cooling and a higher rewarming rate in the exercise leg 24 h post-marathon (Priego-Quesada et al. 2020). Although this study provided important information about the skin temperature rewarming rate after the induction of muscle damage, it is challenging to control the magnitude of exercise-induced muscle damage after a marathon competition in which different participants may perform at different levels. The measure under a more controlled condition in which there is an evident induction of

muscle damage would contribute to understanding the behavior of skin temperature rewarming and the applicability of this protocol to monitor acute adaptations induced by physical exercise. Therefore, it is worth noting whether the assessment of skin temperature rewarming after a cold-stress protocol is influenced by the presence of muscle damage.

In this study, we set out to determine the level of muscle damage and its association with outcomes of the assessment of skin temperature rewarming in exercised limbs after a cold-stress protocol. We hypothesized that the baseline skin temperature would not change 48 h after the induction of muscle damage, but a higher rewarming rate would be observed in the involved limb following a cold-stress test.

6.1.3 Material and methods

Participants and experimental design

A sample size of 15 participants was included in this study (8 men: 26 ± 7 years old, body mass 73.6 ± 9.3 kg, height 1.78 ± 0.06 m, BMI–- body max index 23.3 ± 3.1 kg/m^2, and 7 women: 23 ± 3 years old, body mass 68.0 ± 17.3 kg, height 1.68 ± 0.04 m, BMI 23.9 ± 5.2 kg/m^2). All women participants reported being in the luteal phase of the menstrual cycle while enrolled in the protocol. All participants signed a consent form agreeing to participate. The local University Ethics Committee approved the study (IRB: 26037119.9.0000.5323), and all procedures established in the Helsinki Declaration were respected.

The protocol consisted of 3 visits to the laboratory (days 1, 2, and 4 because day 3 was a rest day), always in the afternoon (between 02:00 and 07:00 PM). On day 1, IRT measurements were performed at baseline and after unilateral cold stress. Moreover, to verify muscle fatigue, we measured bilaterally maximal voluntary isometric plantar flexion contraction. On day 2, we started collecting blood samples for muscle damage evaluation, recorded IRT images, and evaluated DOMS and pressure pain threshold (PPT) bilaterally with an algometer. Next, participants performed a calf raise exercise bilaterally until exhaustion to induce muscle damage. After fatigue, IRT (post-exercise and after the cold stress test), DOMS, PPT, and plantar flexion

strength were evaluated. Finally, on day 4, we collected blood samples and recorded IRT images in the baseline condition and after the cold-stress test. Next, we ended the protocol by evaluating DOMS, PPT, and maximum plantar flexion strength. The experimental design is summarized in figure 1.

The participants received instructions to avoid physical exercise from one week before until the end of the protocol, not to use anti-inflammatory substances, avoid alcohol consumption, as well as caffeinated drinks, cigarettes, hot baths, sunbathing, and creams and cosmetics on days before the skin temperature assessment to avoid the influence of external factors in skin temperature variability.

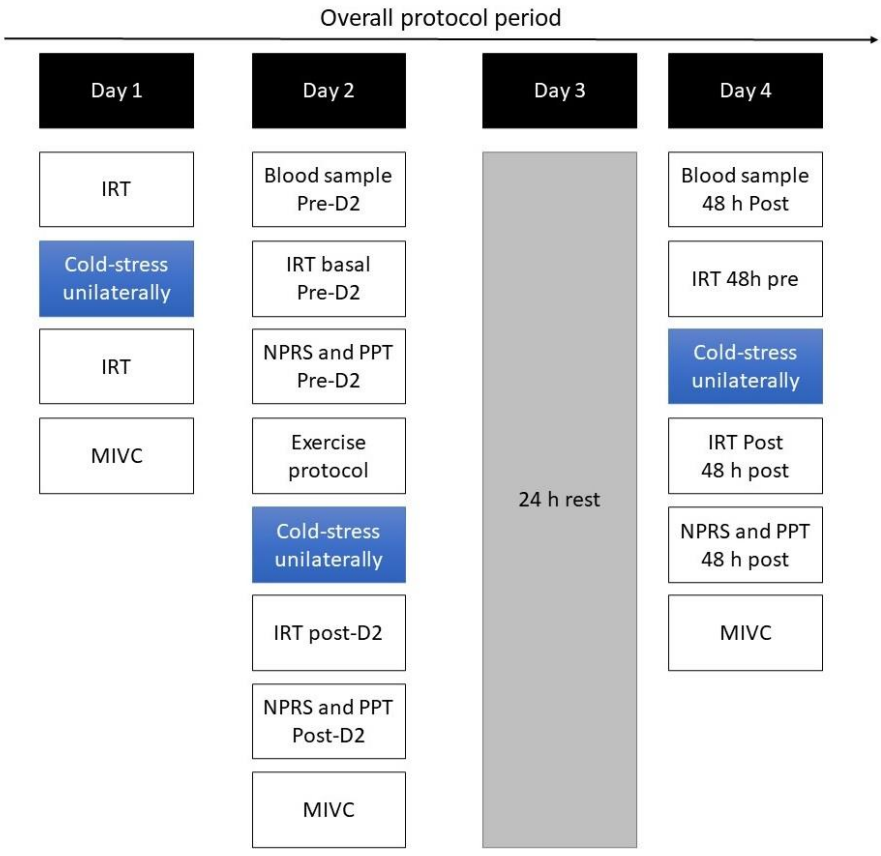

Figure 6.1. Experimental design indicating the three measurements days (1, 2, and 4) and the rest day (day 3). IRT: Infrared thermography; MIVC: Maximum isometric voluntary contraction; NPRS: Numeric pain rating scale; PPT: Pressure pain threshold.

Cold-stress protocol

The cold-stress protocol was conducted by immersing the leg up to the height of the popliteal fossa in a bucket containing water and ice. Half the participants immersed their preferred leg while the remaining immersed their non-preferred. The amount of ice was controlled to ensure a target temperature of 3°C, with a mean temperature among participants of 3.5 ± 1.4 °C at the end of the study. Water temperature was continuously measured using a digital electronic thermometer with a waterproof probe (temperature range: -50 to 110º C, temperature display resolution: 0.1, temperature measurement accuracy: ± 1°C). The participant immersed the leg for a continuous period of 3 min (Priego-Quesada et al. 2022). Since the water can affect IRT measurements, the leg immersed was protected by an impermeable plastic bag as an interface between the skin and the water, allowing cooling without wetting the skin. At the end of the cooling period, the evaluators helped the participant to remove the leg from the bucket. They removed the plastic bag, taking care not to promote friction on the participant's skin and not to accidentally wet the skin.

Exercise-induced muscle damage protocol

The induction of muscle damage was performed through a bilateral heel rise exercise to fatigue calf muscles (Thedon et al. 2011). The participants were instructed to stand on a step, supporting body weight only by the toes up to the metatarsal heads, leaving the midfoot and rearfoot suspended. Then, a series of plantar and dorsal flexions at the maximum range of motion was performed under a rhythm controlled by the sound signal of a metronome (40 Hz). An evaluator counted the number of repetitions, and then the next series with repetitions between 50 and 75% of the repetition number in the first series were performed. The interval between the series was defined by the total duration time of the previous set. When the participant could no longer perform at least 50% of the repetitions from the initial series, the participant was considered fatigued, and the protocol ended.

Infrared thermography

IRT was recorded using a camera FLIR-E60 (Flir Systems Inc., Wilsonville, Oregon, USA) with a focal plane array of 320 x 240 pixels, with noise-equivalent temperature difference (NETD) <0.05 °C, and measurement uncertainty of ± 2°C or 2%. TISEM (Thermographic Imaging in Sports and Exercise Medicine) checklist was followed to ensure that important methodological aspects were controlled (Moreira et al. 2017). The environmental temperature of the laboratory was controlled using an air conditioning system, resulting in a mean ± standard deviation temperature of 22.2 ± 3.3 °C and atmospheric humidity of 56 ± 14 %. IRT images from the calf muscles were taken for both limbs simultaneously, being the camera 1 m far from the region of interest (ROI) and with the lens perpendicular to the plane of measure. Baseline images were obtained for all participants after 10 min of resting with the ROIs exposed for room adaptation. The IRT images were taken 30, 60, 120, and 180 seconds after removing the limb immersed in the bucket to assess the rewarming of skin temperature after cold stress (Priego-Quesada et al. 2022).

The same researcher performed all image processing for IRT data processing using commercial software (Thermacam Researcher Pro 2.10 software, FLIR, Wilsonville, OR, USA). The regions of interest (ROIs, Figure 2) were defined as a line from the largest region of the calf muscles towards the external portion and downward to the largest possible area until the ankle (Da Silva et al. 2022). Two ROIs were delimited considering the posterior leg (right and left leg). Then, each ROI's maximum, minimum, standard deviation (SD), and mean temperature data were extracted using an emissivity of 0.98 (Moreira et al. 2017). A logarithmic equation (equation 1) was adjusted to describe the variation of mean skin temperature for each ROI, aiming to assess changes in skin temperature due to rewarming after the cold-stress test. Two coefficients were obtained, being β_0 relative to the decrease of skin temperature after cooling and $\beta 1$, relative to the rate of skin temperature rewarming:

$$\Delta \text{ skin temperature variation} = \beta_0 + \beta 1 * \ln (T) \quad (1)$$

Equation 6.1 The logarithmic equation to describe skin temperature after the cold-stress test: The β_0 and $\beta 1$ are the constant and slope of the equation, respectively; T is the time elapsed (in seconds) after the test; Δ skin temperature variation is the difference between the skin temperature at T compared with the pre-test (Priego-Quesada et al. 2020).

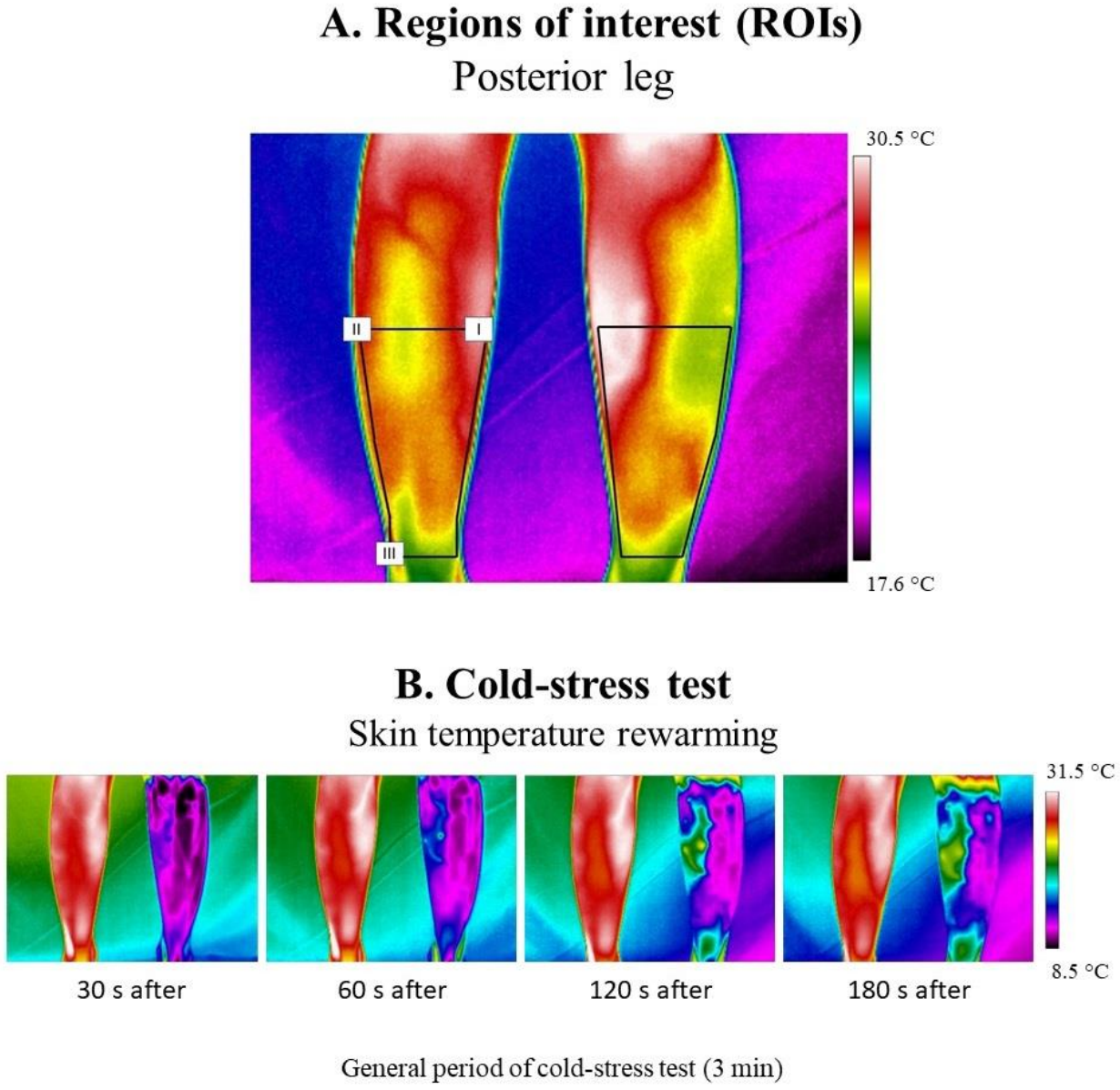

Figure 6.2 Representation of the regions of interest for quantifying skin temperature and skin temperature rewarming after the cold-stress test. The image is representative of all participants. **Panel A:** The regions of interest were determined by the larger region of the calf muscles (I), External portion of the leg (II), and Ankle (III). **Panel B:** Skin temperature rewarming considering 30s, 60s, 120s, and 180s after the cold-stress test. (ROIs): Regions of interest.

Plantar flexion strength

We evaluated maximum isometric voluntary contraction (MIVC) for plantar flexion using a force plate (OR6 2000, Advanced Mechanical Technology, Inc., Watertown, MA, USA) embedded at the floor level and sampling data at 50 Hz. For this evaluation, the participants were seated in front of the platform and placed one foot (forefoot region) over the force plate while the contralateral foot was placed on the floor. The posture during the test involved the ankle joint at 90º respect the lower leg, knee, and hip at 90°, the tibia at a vertical orientation, the trunk erect, and arms crossed in front of the chest. The foot was positioned so that the toes and metatarsal heads rested on the surface of the platform, keeping midfoot and rearfoot suspended. The participants should perform the plantar flexion while resistance against the movement was provided by an inextensible adjustable strap attached to the ground and crossing over the knee to restrict movements. Before the measurement, three familiarization trials were allowed. To register, the MIVC participants were requested to perform the plantar flexion aiming for the highest and fastest force possible, sustaining the contraction for 5 seconds (Guadagnin et al. 2019). The first leg to be evaluated was randomized among the participants. Three measures were performed for each leg with a 60-second rest between repetitions. The average peak force for the three measures was normalized to the individual body mass for statistical analyses.

Muscle soreness and pain pressure threshold assessment

Delayed onset muscle soreness was assessed using a numeric pain rating scale (NPRS) (Hawker et al. 2011). The participants should respond to their pain level in each calf muscle evaluated, considering a range from 0 (no pain) to 10 (maximal pain). Subsequently, PPT was evaluated with an algometer (Digital Dynamometer Model DD-200, with 0.05 Newton resolution, Instrutherm, SP, Brazil), in which the participant was positioned on a stretcher in a prone position. The evaluator progressively pressed the gastrocnemius muscle belly perpendicularly with the algometer with a flat tip of 1 cm². The participant had to refer to the moment when the stimulus became painful (Fleckenstein et al. 2017). At that moment, the value in N/cm² was recorded, and the pain threshold was defined. The same evaluator always performed DOMS evaluations.

Blood analyses

Blood analyses were conducted using 10 mL of blood samples collected from the ulnar vein and stored in heparin tubes. All samples were centrifuged for 8 min at 8000 rpm to extract the serum. The samples were used to determine creatine kinase (CK) activity by applying an enzymatic method using an A25 BioSystems analyzer (BioSystems Inc., PE, Brazil).

Statistical analysis

Data are reported with the differences' mean, standard deviation and 95% confidence interval (95%CI). The normality of data distribution was confirmed with the Shapiro-Wilk test ($p > 0.05$). CK and DOMS data showed a non-parametric distribution and were compared between the moments using the Wilcoxon test with Bonferroni *post-hoc*. Repeated measures ANOVAs with Bonferroni *post-hoc* were applied to all baseline thermal variables considering the moment (three levels: 24 h pre-exercise, pre-exercise, and 48 h post-exercise), and the leg condition (two levels: intervention [immersed during cold-stress test] or control [no-immersed]). The same approach was applied to the value obtained from PPT. To study the differences between moments on logarithmic coefficients in the intervention leg, the ANOVAs only were performed with the factor moment. For parametric data, Cohen's effect sizes (*d*) were calculated and classified as small (ES*d* 0.2–0.5), moderate (ES*d* 0.5–0.8), or large (ES*d* >0.8) (Cohen 1988). For non-parametric data, the Wilcoxon effect size was calculated (ESr) and classified: as 0.10 - < 0.3 (small effect), 0.30 - < 0.5 (moderate effect), and >= 0.5 (large effect) (Coolican 2009). Finally, stepwise multiple linear regressions were performed to assess whether other factors could influence the variation (post 24 h – pre 24 h) of β_0 and $\beta 1$. The inputs of the models were: age, BMI, the total number of repetitions during the exercise protocol, and perceptual variations (48 h post - pre) of CK, DOMS, PPT, and mean peak force. Finally, models were adjusted to retain only those variables yielding p-values < 0.05. All statistical analyzes were performed using RSTUDIO (version 2023.03.0). The level of significance was established in $p < 0.05$ for all analyses.

6.1.4 Results

Participants completed 95 ± 44 repetitions in the heel rise exercise protocol to induce muscle damage. CK increased 48 h post-exercise compared to the pre-value (742.1 ± 1474.5 vs. 6843.1 ± 1675.1 U/L, $p<0.01$, 95%CI of the difference [602, 4211 U/L], ESr = 0.7). DOMS estimated by the NPRS did not differ between pre-exercise and post-exercise on the first day when the exercise protocol was performed ($p = 0.13$), but NPRS was higher 48 h post-exercise when compared with pre-exercise (0.2 ± 0.6 vs. 5.1 ± 2.2 points, $p<0.001$, 95%CI of the difference [4.0, 5.5 points], ESr = 0.9) and post-exercise (0.9 ± 1.8 vs. 5.1 ± 2.2 points, $p < 0.001$, 95%CI of the difference [3.5, 5.0 points], ESr = 0.9). PPT did not differ between measurement moments (42.2 ± 15.3 vs. 45.7 ± 20.3 vs. 39.5 ± 17.7 N/cm^2, $p = 0.41$), similarly as mean peak isometric force (11.9 ± 2.9 vs. 12.6 ± 3.6 vs. 10.9 ± 2.7 N/kg, $p = 0.12$).

Figure 3 shows the baseline results of thermal variables. No differences were observed between days, conditions, and interactions between days and conditions in any of the thermal variables. Moreover, when we considered the skin temperature data obtained with the application of the cold-stress test (Figure 4), no differences were observed between days for β_0 ($p = 0.70$) and β_1 ($p = 0.80$). In addition, the multiple linear regression analysis showed that any of the variables of the study were related to the coefficients of the logarithmic equation (β_0 and β_1).

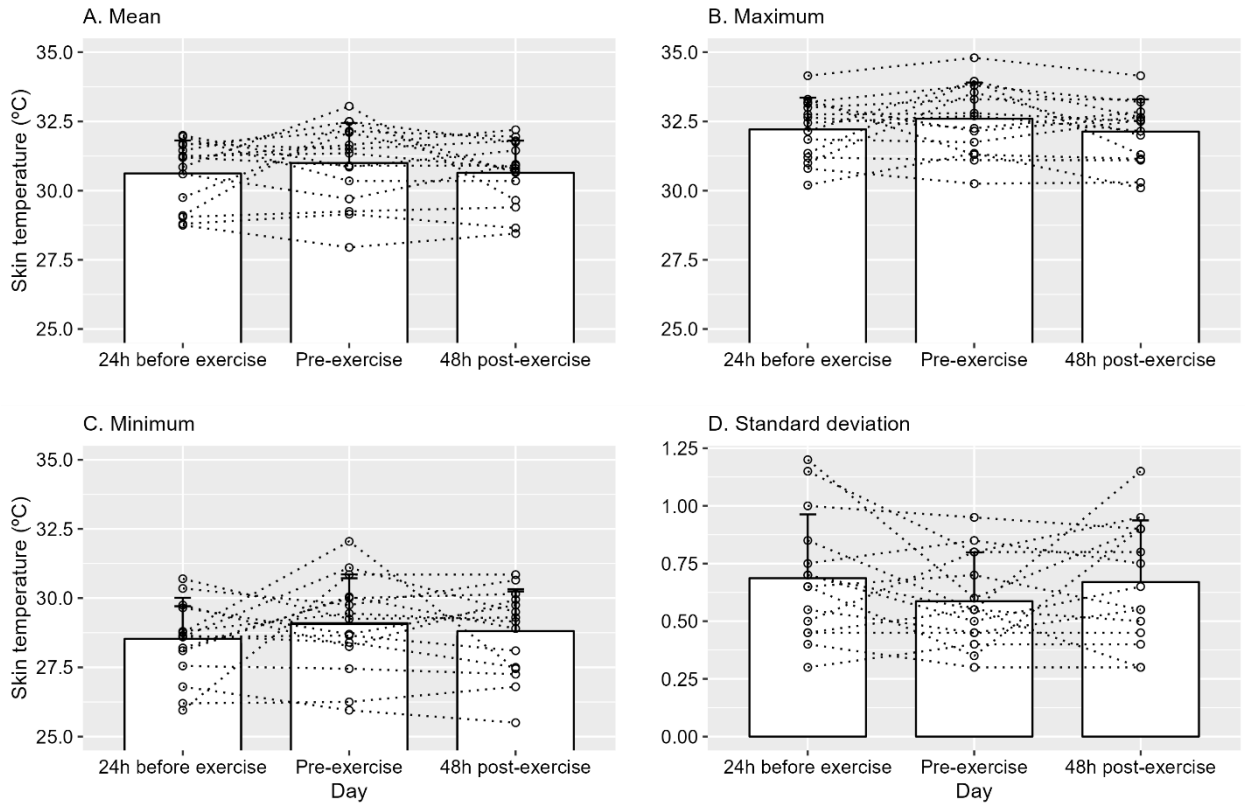

Figure 6.3 Mean and standard deviation (SD) and individual data (points connected by dotted lines) of baseline skin temperature measurements 24 h before exercise, pre-exercise and 48 h post-exercise.

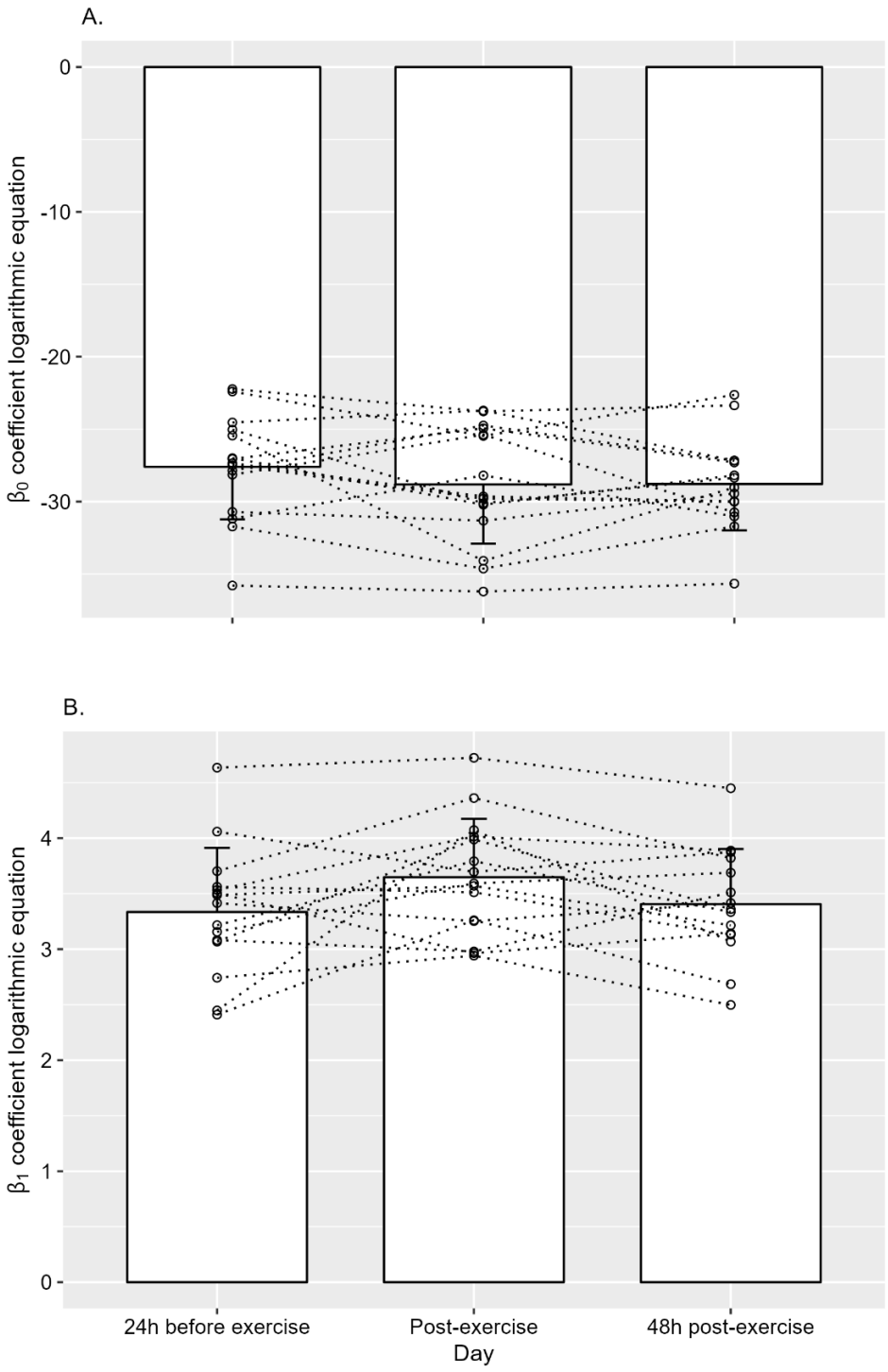

Figure 6.4 Mean and standard deviation (SD) and individual data (points connected by dotted lines) of the coefficients of the logarithmic equations obtained with the cold-stress test. The coefficients were obtained considering the values obtained 24 h before exercise, post-exercise, and 48 h post-exercise.

6.1.5 Discussion

This study aimed to determine whether exercise-induced muscle damage changes baseline skin temperature and skin temperature rewarming after a cold-stress protocol. Our main results showed that 48 h after a protocol to induce muscle damage of the calf muscles, changes in skin temperature were not significant and did not associate with makers of the magnitude of muscle damage. Neither baseline nor cold stress nor rewarming rate elicited significant changes in skin temperature in response to the exercise protocol. One novelty in our study was to address the temperature variations quantified by logarithmic coefficients (β_0 and $\beta 1$) obtained in the time course of the muscle damage process. Our initial hypothesis is partially supported because changes in baseline skin temperatures were not expected, but we did expect them for rewarming rate after the cold-stress protocol. Our results have important implications for the field as we demonstrate that common measures of skin temperature may not be enough to monitor muscle damage.

Unlike previous studies, we also used thermal stress in addition to skin temperature changes during baseline conditions. The application of the cold-stress test was performed to induce an activation of the sympathetic nervous activity, which in turn should promote peripheral vasoconstriction (Sawasaki et al. 2001). Vasoconstriction is reduced after the end of the protocol aiming to facilitate blood flow and increase skin temperature. We have applied the cold-stress to perturb this vascularization response (Priego-Quesada et al. 2020) and found no alteration in the coefficients of the logarithmic regression proposed to characterize the rewarming rate during recovery. Although previous studies have obtained promising results with this approach (Muñoz-Alcamí et al. 2021; Priego-Quesada et al. 2020), some differences between those studies and ours may explain the controversial results. A lower β_0 and a higher $\beta 1$ were observed post-exercise after a protocol for fatigue of knee extensors (Muñoz-Alcamí et al. 2021) and 24 h but not 48 h after a marathon (Priego-Quesada et al. 2020). It cannot be excluded the hypothesis about changes in vasoconstriction being restored 48 h after exercise explains our results. Unfortunately, we did not apply the cold stress 24 h after the damage protocol, which was needed to avoid confusion in the blood markers and DOMS during recovery. Therefore, further studies should evaluate the

evolution of rewarming rate during the posterior hours after the induction of muscle damage to determine whether there is an appropriate time window to the measure and whether it would provide better relationships with the late DOMS makers of muscle damage. Finally, a previous study reported that logarithmic coefficients were not altered 24 h after 10 km of running under the supposition that the exercise intensity performed was not enough to induce muscle damage and pain sensation (Priego-Quesada et al. 2022). The lower level of muscle damage might not be the case in our experiment due to the higher CK activity, and DOMS reported 48 h post-exercise.

The characteristics of the participants and the variability in the vasoconstrictor skin sympathetic nerve activity (SSNA) could be other important factors to explain our results. Regression models obtained from previous studies showed that the variation of both logarithmic coefficients has a direct relationship with muscle mass (Muñoz-Alcamí et al. 2021) and an inverse relationship with body fat percentage (Priego-Quesada et al. 2020). In our study, all participants were young adults and untrained, four participants had a BMI slightly above expected values for the age, and the percentage of muscle mass was not quantified. Participants with lower subcutaneous fat layers show higher skin temperature rate variation in response to physical exercise (Neves et al. 2015). The subcutaneous fat layer has a lower thermal conductivity capacity (Chudecka et al. 2014), playing a role in thermal isolation and impairing heat exchange. This factor may have influenced the skin temperature response after the cold-stress test, although it remains a speculation. Furthermore, the SSNA shows variability between humans in response to cold exposition (Sawasaki et al. 2001). This is an important factor because this variability considering the SSNA vasoconstrictor component could influence the skin temperature rewarming after the end of the cold-stress test, increasing the variability of our data and explaining the lack of differences.

Besides CK and DOMS outcomes, mean peak force and PPT did not differ in response to muscle damage. Although having DOMS without strength loss can be considered an unexpected result, the relationship between both outcomes is not contemplated in the literature. A previous study showed that DOMS and muscle performance evaluated by MIVC and single-leg forward jump did not correlate in untrained participants (Ma et al. 2022), while others suggest

isometric strength taking up to 3 days to recover after split squats (Dabbs & Chander 2018). Therefore, it is possible that the period of 48 h after the exercise was enough time for the recovery of the MIVC in our participants. Force sharing and differences in the participation of the muscles may also explain the MICV outcomes, as the soleus contribution for the plantar flexion with concurrent knee extensor activity (Suzuki et al. 2014), as well as the activation of different muscle fibers of the gastrocnemius during the isometric strength evaluation with knee and hip at 90° may have influenced the strength loss values during the MIVC evaluation. On the other hand, we showed no difference in PPT between moments. The PPT considers muscle tenderness that is associated with DOMS, muscle damage, and inflammation (Fleckenstein et al. 2017). The muscle tenders usually are concentrated in a distal portion of the muscle due to a higher concentration of pain receptors (Cheung et al. 2003). We performed the measure considering only the muscle belly, and this fact may have influenced our results. These results reinforce the importance of considering different markers of exercise recovery since we found altered CK and DOMS, which confirms the presence of muscle damage.

Finally, despite muscle damage and DOMS, baseline skin temperature parameters did not differ 48 h after exercise. These results align with previous studies considering different stimulus types, such as muscle damage protocol by a great presence of eccentric components on the calf (da Silva et al. 2018) or a soccer national championship (de Carvalho et al. 2021). The possible explanation for the lack of evidence that supports this relationship could be that muscle damage and, consequently, inflammation is a complex process that does not occur near the skin surface (Peake et al. 2017), and the IRT is a technique that measures the skin surface temperature, and its temperature is not usually related to muscle or core temperature (Fernandes et al. 2016; Priego Quesada et al. 2016).

Our results have important practical applications. Both changes in baseline skin temperature and skin temperature rewarming will not necessarily reflect the magnitude of the exercise-induced muscle damage and recovery status. Future studies should consider participants with different body characteristics and physical fitness to better understand the possible effect of these confounding factors on the skin temperature rewarming after the cold-stress test. The

possibility of monitoring the variables of interest considering a shorter time window, every 12 or 24 h, for instance, would be also helpful in better understanding the association in the time course of changes of the different physiological responses involved in this phenomenon.

6.1.6 Conclusion

The presence of exercise-induced muscle damage and delayed onset muscle soreness was not associated with changes in different parameters of skin temperature responses in the 48 h post-exercise based on both measures of baseline and cold-stress test and rewarming assessments.

6.1.7 References

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Chapter VII

General Discussion

7. CHAPTER VII

7.1 GENERAL DISCUSSION

In this section, we discuss all results of this Ph.D. thesis following the same order of the specific objectives proposed. This section is divided into three sub-sections involving discussions that address the following topics: ***analyses, applicability, and dynamic thermography***. Through this Ph.D. thesis, we presented different concepts related to exercise-induced muscle damage and delayed onset muscle soreness (DOMS). We described the complex etiology proposed and its possible implications for human performance and recovery status after physical exercise. Moreover, we presented different strategies to monitor and quantify muscle damage and DOMS and their limitations.

Infrared thermography (IRT) is one of the central topics explored. The IRT is a non-invasive image technique that provides information about an object's surface temperature and is applicable to quantify human skin temperature (Priego Quesada 2017). IRT has gained popularity in sports science and rehabilitation studies, mainly because skin temperature changes can relate to internal training loads and injury risk. We understand that IRT has well-established applications in health science, such as medical science (Ring & Ammer 2012).

However, in the context of physical exercise, despite the important advances related to the IRT, the technique is still relatively new when compared to other techniques that have several standard protocols for evaluation and established procedures for signal analysis like surface electromyography (EMG) or dynamometry, for example. For this reason, we highlighted the importance of exploring the potential of IRT to monitor acute adaptations induced by physical exercise in a controlled laboratory environment to provide more conclusive information.

7.1.1 ANALYSES

Before we explore its potential in the laboratory, it was necessary to standardize clear criteria for processing thermal images to obtain reliable results. Since one important source of

variation in IRT analysis in humans is the ROI selection (Fernández-Cuevas et al. 2015). In the first study, we determine the reproducibility of IRT image analysis performed by evaluators with different levels of experience, considering well-defined anatomical criteria to establish the ROI and ROIs that are usually explored in sports science like anterior thigh and posterior leg. We showed that the mean of skin temperature is the variable that shows excellent reproducibility despite the evaluator's experience with images of the anterior thigh and posterior leg. Similar results were obtained considering the posterior leg for maximums skin temperature. However, the minimum skin temperature and range (max-min) are variables that suffer the influence of the experience level of the evaluator.

Our finds are similar to the previous studies that highlighted the mean skin temperature as the most robust thermal variable (Machado et al. 2021; Priego Quesada 2017). Differing from these previous studies, we considered here the factor level of experience and observed similar behavior. Also, we showed that the choice of variable and the ROI can influence the reproducibility of the measure and that this is dependent on the evaluators' experience. In addition, in this article, we showed a detailed analysis of all thermal variables that are usually explored in studies involving IRT, the level of reproductivity before and after exercise, and the 95% limits of the agreement provided by the Bland-Altman plots analyses. This study has an important practical application since the level of reproducibility is a crucial point in clinical applications.

In a practical field, the analyses of thermal images can be performed by a multidisciplinary team such as coaches, doctors, and physiotherapists with different levels of experience. From our results, we were able to recommend that repeated training sessions are necessary for novice evaluators to process IRT images from ROIs like the posterior leg. However, we know that the choice of a thermal variable can depend on the research question and that there is no consensus on which variable should be considered. We reiterate that when a team performs IRT analyses with different levels of experience, the mean and maximum temperatures should be prioritized due to their better reproducibility.

In addition to exploring thermal image analyses, we explored different strategies of thermal data analysis. These strategies were addressed in another two studies that make up this

Ph.D. thesis. In study two, we included a non-exercised region with a control ROI, and in the third study, we applied a logarithmic coefficients equation to determine the level of skin temperature decrease and rate of skin temperature rewarming after the cold-stress test. It was important because the establishment of the control ROI, for instance, allows us to better understand the possible systemic effect of exercise-induced muscle damage and DOMS and the possible effect of external factors in the skin temperature data. In turn, the logarithmic equation applied in the study of dynamic thermography allows us to analyze the behavior of data over time, providing important information like the decrease of skin temperature in response to the test and its recovery. These approaches allow us to understand better the physiological phenomenon involved in the human body's answer to physical exercise and IRT's applicability to these acute adaptations.

7.1.2 APPLICABILITY

Once the analysis of thermal images was standardized, the next step was to verify in the laboratory the potential of the IRT to monitor exercise-induced muscle damage and DOMS. The second study considered men and women who submitted to the squat physical exercise until fatigue. This study showed that the skin temperature response after exercise presents a sex-dependent effect. Also, we showed that DOMS sensation is not predicted by skin temperature variation as we hypothesized previously. However, a mechanical measure of pain, like pressure pain threshold (PPT), is an important measure to consider. We showed a direct relationship between maximum skin temperature variation pre- and 48h post-exercise and PPT. This study has an important practical application since most previous studies did not consider men and women evaluated in response to physical exercise, and inferences are assumed to be similar. However, we showed in practice that this may not be the case. Moreover, a mechanical measure of pain like PPT seems to be a more objective measure to quantify the complex phenomenon of pain, able to detect sex differences that were not observed with the analog scale. This measure should be considered in future research with IRT.

Curiously, to date, the central hypothesis that supports the application of IRT as a possible diagnostical tool to monitor muscle damage and DOMS is the possible relationship between the inflammation process induced by the structural damage, which in turn produces heat that is conducted to the skin surface by different heat transfer mechanism like peripherical vasodilatation and therefore evaluated by IRT (de Andrade Fernandes et al. 2017; Hildebrandt et al. 2010). However, as highlighted in this Ph.D. thesis, several studies have failed to relate changes in skin temperature and muscle damage markers after physical exercise. Also, in study two, this hypothesis has not been confirmed.

Therefore, we hypothesize that exercise-induced muscle damage and DOMS can, in turn, generate peripheral vasoconstriction and do not reflect on observable changes when evaluating skin temperature with IRT in the presence of DOMS. This hypothesis contradicts the previous one; a third and the last original experiment was conducted to test it.

7.1.3 DYNAMIC THERMOGRAPHY

We set out to evaluate the applicability of IRT to monitor exercise-induced muscle damage and DOMS considering a cold-stress protocol. We hypothesized that the presence of muscle damage and DOMS would not influence changes in baseline skin temperature after exercise, as we observed in previous studies, but would be able to intensify the cooling of the limb and influence the rate of skin temperature rewarming after the cold-stress test. For this, all participants were submitted to a protocol of maximum voluntary repetitions involving the plantar flexor muscles until fatigue, and a decrease in skin temperature, and the rate of skin temperature rewarming was evaluated considering a cold-stress protocol applied 48h post-exercise. In this study, we use CK activity, a biochemical measure explored to quantify muscle damage. Also, we evaluated the muscle efficiency through maximum isometric voluntary contraction (MIVC) to better understand the relationship between muscle damage and loss of muscle performance, considering untrained participants.

When we executed this protocol, we observed higher activity of CK and DOMS sensation 48h post-exercise compared to pre-exercise values. The magnitude of muscle damage and DOMS did not reflect in baseline skin temperature changes as we hypothesized. However, the exercise-induction muscle damage and DOMS did not influence the rate of skin temperature rewarming as we expected 48h post-exercise.

Here we considered that the characteristic of our participants and the variability in the vasoconstrictor skin sympathetic nerve activity (SSNA) could be two important factors that influenced our thermal results (Muñoz-Alcamí et al. 2021; Sawasaki et al. 2001).

In our study, we considered untrained participants, and four had a BMI above the normal values; the fat layer's lower thermal conductivity capacity and its thermal insulating capacity can have influenced our thermal data (Chudecka et al. 2014). Also, previous studies were able to observe changes in the rate of skin temperature rewarming immediately after exercise and 24h after (Muñoz-Alcamí et al. 2021; Priego-Quesada et al. 2020). We take measures in the different time windows, and it is possible that changes in vasoconstriction may be restored at this time point, explaining our results. Our results do not support that changes in vasoconstriction 48h after exercise are influenced by the magnitude of exercise-induced muscle damage and DOMS. This study has important practical applications. We showed that both changes in baseline skin temperature and rate of skin temperature rewarming do not reflect the magnitude of exercise-induced muscle damage and DOMS 48h post-exercise.

In short, the analysis of the IRT images shows excellent reproducibility despite of experience of the evaluator. However, repeat training sessions can be recommended depending on the variable choice, the ROI considered, and the evaluators' experience. The mean of skin temperature is the variable more robust and can be considered when we have evaluators with different experience levels. Also, the skin temperature changes after exercise are sex-dependent when we have a sample of women in different phases of the menstrual cycle. Also, a mechanical measure of pain, like PPT, can be considered in future studies. Finally, changes in skin temperature do not reflect the magnitude of exercise-induced muscle damage and DOMS and do not influence in rate of skin temperature rearming 48h post-exercise. Therefore, we recommend

caution in the use of IRT to quantify exercise-induced muscle damage, DOMS, and recovery status after exercise.

Chapter VIII

Conclusions

8. CHAPTER VIII

8.1 CONCLUSIONS

In this section, we present the conclusions of the studies that make up this Ph.D. thesis in accordance with the aims proposed (sections 1.3.1 and 1.3.2).

8.1.1 REPRODUCIBILITY OF THERMAL IMAGES ANALYSIS

The analysis of thermal images from the anterior thing does not suffer influence by the level of experience from different evaluators. The mean of skin temperature is the most robust thermal variable showing excellent reproducibility for evaluators with different levels of experience and both ROIs analyzed. The reproducibility of thermal data depends on the variable choice and the ROIs analyzed. When the minimum skin temperature and range are considered, repeat training sessions must be considered when evaluators with different experience levels are involved in the thermal image analysis.

8.1.2 RELATIONSHIP BETWEEN EXERCISE-INDUCED MUSCLE SORENESS, PAIN THRESHOLDS, AND SKIN TEMPERATURE IN MEN AND WOMEN

In general, skin temperature changes after physical exercise shows a sex-dependent effect. Also, changes in skin temperature do not predict the intensity of DOMS sensation evaluated 48h post-exercise in men and women. The variation of maximum skin temperature is related to a mechanical measure of pain like PPT 48h post-exercise. The PPT appears to be a more objective measure to quantify pain sensation, being able to detect sex differences after physical exercise, which is not observed with other evaluation instruments like analog scales.

8.1.3 RELATIONSHIP BETWEEN CHANGES IN SKIN TEMPERATURE MUSCLE DAMAGE AND DOMS CONSIDERING COLD-STRESS TEST

Changes in skin temperature are not related to exercise-induced muscle damage quantified by the CK activity and DOMS. The magnitude of exercise-induced muscle damage does not influence the rate of skin temperature rewarming 48h post-exercise considering the cold-stress test. Caution is recommended to assume that changes in skin temperature reflect the magnitude of muscle damage and recovery status.

Chapter IX

Future research

9. CHAPTER IX

9.1 FUTURE RESEARCH

With the development of this Ph.D. thesis, several questions and hypotheses have aroused for future research. Below we enumerate some potential topics for future research on this topic.

- To explore measures in smaller regions of interest aiming to verify whether the results for the reproducibility and concordance analyses remain similar to the larger regions explored here.

- To explore the evolution of skin temperature along various measurements taken during the day in a 48 h of intervals after exercise to verify whether and when the peak of skin temperature in response to muscle damage can occur and establish the best time to perform thermal measurements.

- To explore the applicability of cold-stress test 24 h post-exercise to determine the exercise-induced muscle damage in participants with different body characteristics to better understand the possible effect of these confusing factors in the rate of skin temperature rewarming and its relationship with muscle damage.

- To explore the effect of repeat sessions of exercise on consecutive days to better understand the cumulative effects of the exercise on muscle damage and its relationship with skin temperature monitored by IRT.

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