VNIVERSITAT DE VALÈNCIA

Facultat de Ciències de l'Activitat Física i l'Esport

Doctoral Program in Physical Activity and Sport

Department of Physical Education

Faculty of Physical Education

Effects of phenylcapsaicin supplementation on physical performance and mechanical, metabolic, biochemical, perceptual and electrophysiological fatigue

International Doctoral Thesis presented by:

D. Pablo Jiménez Martínez

Directed by:

Dr. Juan Carlos Colado Sánchez

Valencia, October 2023

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Dr. Juan Carlos Colado Sánchez, full chair professor at the Department of Physical Education and Sport, Faculty of Physical Activity and Sport Sciences, University of Valencia (Spain).

Certify that:

> The present dissertation, entitled **"Effects of phenylcapsaicin supplementation on physical performance and mechanical, metabolic, biochemical, perceptual and electrophysiological fatigue"** has been written under his supervision by D. Pablo Jiménez Martínez. This manuscript corresponds to the Doctoral Program with International Mention in Physical Activity and Sport of the University of Valencia.

In recognition whereof, we sign the present certificate in Valencia, October 2023.

Dr. Juan Carlos Colado Sánchez

International mention:

Friedrich-Alexander-Universität
Technische Fakultät

FAU Erlangen-Nürnberg I Henkestr. 91, 91052 Erlangen

To
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Erlangen, den 03.07.2023

Stay at Friedrich-Alexander-University Nürnberg-Erlangen for researching purpose of PhD student Pablo Jiménez Martínez. Verification document:

The purpose of this document is to verify the stay conducted by the doctoral student Pablo Jiménez Martínez. The rationale of this stay was to acquire some corticospinal signals as well as dynamic and isometric forces with the use of quattrocento devices from the tibialis anterior and quadriceps muscles. For this reason, the doctoral student has successfully learned to use high-density electromyography with the quattrocento software and hardware. This stay was conducted between the **20/03/23** and **03/07/23**

Of note, research about the impact of mental fatigue on connectivity signals and motor units' behavior during a fatiguing physical exercise was also performed. Furthermore, the doctoral student was imbedded in an international project in which the effects of different joint angles in the knee and the ankle were evaluated on different neurophysiological outcomes, such as the recruitment threshold and the discharge rate.

As a result of this stay, the doctoral student has participated in the following scientific items:
Articles pending to be published from the conducted research during the stay:

- "Resting time influences the rate of force development and maximal isometric force in young subjects: a randomized trial".
- "Effects of different contraction configurations on electrophysiological outcomes in young active subjects".
- "Mental fatigue impairs physical performance but not the neural drive to the muscle"
- "Foot and hip positions influence the neural drive to the quadriceps muscles in Olympic athletes".

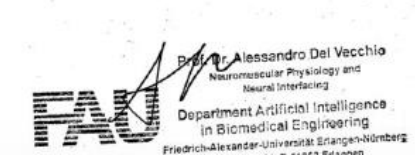
Poster and oral presentations:

- "Effects of mental fatigue on motor units' behavior during a fatiguing task". XII Congreso Internacional de la Asociación Española de Ciencias del Deporte, Madrid (UAM) 2023.

- Tutor in Spain: Dr. Juan Carlos Colado Sánchez
- Tutor in Germany and lab-head: Prof. Dr. Alessandro Del Vecchio

Yours sincerely,

Prof. Dr. Alessandro Del Vecchio



Acknowledgments

"I would like to dedicate some verses to you,
That they were worth what your light is worth,
But there's no room in a page for the universe,
Nor can my gratitude fit in a sentence."

Sharif Fernández

The doctoral thesis is the end of one stage and the exciting beginning of another. The defense of a doctoral thesis means saying goodbye to the past and opening your arms to the future; it is the step from child to adult in the academic world. Possibly, the defense of a doctoral thesis is the most important day in the life of any scientist. In short, the doctoral thesis is an example of life itself: Sweat, effort and sacrifice. Forgiveness and gratitude.

For many years I have dreamed with this document, with this moment, knowing and feeling that this was what I deserved, what my future brought me; a constant and continuous goal since moments that I do not even know how to remember. In this long time that began far before I enrolled in the PhD programme, I have learned a lot, many things that go beyond academic scientism, to a large extent I have learned the best and the worst of human beings.

Focusing on the positive side, being a very self-taught person, I could never have imagined that my professional and academic development would bring me so much personal growth, and the most important thing about a travel is not the destination but with whom you walk beside you. It is very difficult to thank all the people who have brought light into my life during this time, but here I will make an attempt to do so:

First of all, from the bottom of my heart I would like to thank Dr. Juan Carlos Colado for his kind hand for 5 years now. Thank you for your trust since the first day, for guiding me when I have needed it in a selfless way, thinking on my wellbeing and my future. Few people have the capacity to inject the ambition and adrenaline necessary to face all the challenges that arise along the way. This is only the beginning.

In relation to the above, a special mention should be made for the Prevention and Health in Exercise and Sport (PHES) group from the University of Valencia. The personal and professional relationship we have forged is of incalculable value. From these lines: One for all and all for one.

I would also like to thank Dr. Fernando Pareja Blanco and my colleagues from the Pablo de Olavide University, as well as Dr. Amador García Ramos and my colleagues from the University of Granada for everything that they have done for me in order to complete this doctoral thesis. I am enormously grateful to them for opening the doors of "their home" to me,

welcoming me as one of their own and allowing me to learn and work with them in the development of this doctoral thesis.

Without detriment, to Dr. Alessandro del Vecchio and the N-Squared group of the Friedrich-Alexander University or Erlangen (Germany) for giving me a home during my international doctoral stay.

For their interest in science, to Lucas Altepost and the whole team of Axichem AB, as well as the whole team of INDIEX and ICEN.

With affection to my working partners, I consider myself very fortunate to be able to work with my best friends and some of the people I admire the most. In turn, with special affection to Alejandro Monedero for changing my life, teaching me everything I know about the business world, trusting me blindly when I was only a kid and for reinforcing me ethically in what is right. In addition to this, another special dedication to my work partner, the person who is present 24 hours a day in my WhatsApp chat and one of the people without whom my life would not be the same, Carlos Alix. The future belongs to those who create it and between the 3 of us we have no limits.

From the deepest part of me, I am grateful to my uncles Javier and Pedro, for being the best people who have appeared in my life, for bringing me out of the darkness, giving me a future and making me the man I am today. Without the opportunities you gave me, this doctoral thesis would probably not exist. Everything I have today has a part of you. I can never be grateful enough for "adopting" me, for treating me as your son and for bringing me up through thick, thin and thinner.

Likewise, to my parents, brother, godmother and the rest of my family for all their warmth, effort and dedication during so many years that have nurtured my educational, personal and professional growth. Thank you for helping me to overcome all, today I can say that I am no longer a child.

Of course, to all the people who have been directly involved in this doctoral thesis or who have influenced my personal life along the way. Because, although they are no longer part of it, I cannot forget their contributions and selfless affection. The future rests on a chest of memories that is present, even if it is closed.

Finally, to the eyes that guide me like a lighthouse in the darkness. For appearing in a dreary moment, for rescuing me from my own shadows, for making me a better person. Thank you for patching up a broken and devastated heart. For giving me back the meaning of the word hope.

Thank you all. Wild hearts can’t be tamed.

Agradecimientos

"Quisiera dedicaros unos versos,
Que valieran lo que vale vuestra luz,
Pero no cabe en un folio el universo,
Tampoco en una frase cabe mi gratitud."

Sharif Fernández

La tesis doctoral es el final de una etapa y el ilusionante inicio de otra. La defensa de una tesis doctoral es decir adiós al pasado abriendo los brazos al futuro, es el paso de niño a adulto en el mundo académico. Posiblemente, la defensa de una tesis doctoral sea el día más importante de la vida de cualquier científico. En resumen, la tesis doctoral es un ejemplo de la vida en sí misma: Sudor, esfuerzo y sacrificio. Perdonar y agradecer.

Durante muchos años he soñado con este documento, con este momento, sabiendo y sintiendo que esto era lo que merecía, lo que deparaba a mi futuro; un objetivo constante y continuo desde momentos que no sé ni recordar. En este largo camino que empezó mucho antes de matricularme en el programa de doctorado he aprendido mucho, muchas cosas que van más allá del cientifismo académico, en gran medida he aprendido lo mejor y lo peor del ser humano.

Centrándonos en lo positivo, siendo una persona muy autodidacta jamás pude imaginar que mi desarrollo laboral y académico me aportarían un crecimiento personal tan grande, y es que lo más importante del viaje no es el destino sino con quien caminas a tu lado. Es muy difícil agradecer a todas las personas que han aportado luz en mi vida durante este tiempo, pero a continuación haré un intento de ello:

En primer lugar, desde el fondo más absoluto de mi corazón quiero agradecer al Dr. Juan Carlos Colado su mano amiga durante ya 5 años. Gracias por tu confianza desde el primer día, por guiarme cuando lo he necesitado de forma desinteresada pensando en mi bienestar y mi futuro. Pocas personas tienen la capacidad de inyectar la ambición y adrenalina necesaria para afrontar todos los retos que surgen en el camino. Esto sólo es el principio.

En relación a lo anterior, cabe dedicar una especial mención al grupo Prevention *and Health in Exercise and Sport* (PHES) de la Universidad de Valencia. La relación personal y profesional que hemos forjado tiene un valor incalculable. Desde estas líneas: Uno para todos y todos para uno.

No menos importante agradecer al Dr. Fernando Pareja Blanco y a los compañeros de la Universidad Pablo de Olavide, así como al Dr. Amador García Ramos y a los compañeros de la Universidad de Granada por todo lo que han hecho por mi para la consecución de esta tesis doctoral. Agradezco enormemente que me abrieran las puertas de "su casa", me acogieran

como a uno más y me permitieran aprender y trabajar junto a ellos en la consecución de esta tesis doctoral.

Sin menoscabo, al Dr. Alessandro del Vecchio y al grupo N-Squared de la Universidad Friedrich-Alexander de Erlangen (Alemania) por darme un hogar durante la estancia internacional doctoral.

Por su interés en la ciencia, a Lucas Altepost y todo el equipo de Axichem AB, así como a todo el equipo de INDIEX e ICEN.

Con cariño a mis compañeros de trabajo, me considero muy afortunado de poder trabajar con mis mejores amigos y algunas de las personas que más admiro. A su vez, con especial afecto a Alejandro Monedero por cambiarme la vida, enseñarme todo lo que sé sobre el mundo de la empresa, confiar ciegamente en mí cuando era un niño y por reforzarme éticamente en lo que es correcto. Unido a esto, otra especial dedicatoria a mi compañero de fatigas laborales, la persona que está presente 24 horas al día en mi chat de WhatsApp y una de las personas sin las que mi vida no sería la misma, Carlos Alix. El futuro es de quien lo crea y entre los 3 no tenemos límites.

Desde lo más profundo de mí, agradecido a mis tíos Javier y Pedro, por ser las mejores personas que han aparecido en mi vida, por sacarme de la oscuridad, darme un futuro y hacerme el hombre que soy a día de hoy. Sin las oportunidades que me brindasteis seguramente esta tesis doctoral no existiría. Todo lo que tengo a día de hoy tiene un componente vuestro. Jamás podré estar lo suficientemente agradecido por “adoptarme”, por tratarme como a vuestro hijo y por educarme en las buenas, en las malas y en las peores.

Así mismo, a mis padres, hermano, madrina y resto de mi familia por toda su calidez, esfuerzo y dedicación durante tantos años que han alimentado mi crecimiento educativo, personal y profesional. Gracias por ayudarme a sobreponerme, hoy puedo decir que ya no soy un niño.

Por supuesto, para todas las personas que durante este camino han participado directamente en esta tesis doctoral o han influido en mi vida personal. Porque, aunque ya no formen parte de la misma, no puedo olvidar sus aportaciones y cariño desinteresado. El futuro se sostiene sobre un baúl de recuerdos que está presente, aunque se cierre.

Finalmente, a los ojos que me guían como un faro en las tinieblas. Por aparecer en un momento lúgubre, por rescatarme de mis propias sombras, por hacerme mejor persona. Gracias por poner parches a un corazón totalmente roto y desolado. Por devolverme el significado de la palabra esperanza.

Gracias a todos. Los corazones salvajes no pueden ser domados.

Summary

Introduction

Dietary supplements are nutritional complements which ingested in conjunction with a healthy diet may improve health and/or sports performance. By their own definition, these substances are legal and not banned by international agents such as the World Antidoping Agency. Accordingly, these products are free-sale, which makes them highly popular between different athletic populations.

As it is presented in chapter 1 capsaicinoids are a group of compounds naturally found in spicy fruits like chili peppers. Due to their clinical and ergogenic potential applications, new scientific research has been conducted around these compounds in the last two decades. Over the last decade, another group of capsaicinoids analogues called capsinoids has also been studied to reduce the discomfort generated when capsaicin is orally ingested. Capsinoids are mainly found in CH-19 Sweet peppers, a type of non-pungent peppers which have been genetically modified to eliminate the natural spiciness of capsaicinoids. Both substances, capsaicinoids and capsinoids, interact with the transient receptor potential vanilloid 1 (TRPV1), which exerts the most important physiological functions of these substances. However, capsinoids are well-documented as a poor bioavailable group of compounds. Moreover, after their ingestion, capsinoids are rapidly metabolized and conjugated, resulting in non-detectable circulating levels in the bloodstream. By contrast, after their ingestion, different capsaicin formulations have a fast effect on different tissues, such as the small intestine, liver, and stomach, which may be extrapolated to peripheral tissues such as the skeletal muscle. On the other hand, the use of oral capsaicin has been reported to be conflictive due to its pungent characteristics that can increase the risk of discomfort during exercise. Therefore, the formulation of new capsaicin analogues that can reduce the optimal physiological dose seems to be a priority approach on this field. For this reason, during the last years a new group of synthetic analogues of traditional capsaicin known as “capsaicyns” has been developed. Among these substances it is found one of special interest called phenylcapsaicin (PC), which has emerged as an alternative to traditional oral purified capsaicin supplementation. PC is a microencapsulation of 98% of PC and 1-1.5% lipidic excipients and cellulose as primary metabolic vehicles.

In addition, as it was aforementioned, TRPV1 agonist drugs have exhibited improvements on several pathophysiological conditions, such as chronic musculoskeletal and neuropathic pain, gastrointestinal disruptions (e.g., gastroduodenal mucosal injury) and

metabolic disorders (e.g., overweight). On the other hand, sport performance seems to be enhanced through lower ratings of perceived exertion, reductions on discomfort and an increase on mechanical performance (e.g., total volume load). In this regard, the physiological effects of capsaicin are mediated by reductions in inflammatory hyperalgesia, downregulating voltage-activated calcium channels, and influencing thermoception, being all of them common links between healthcare and performance fields. TRPV1 are presented in small diameter nerve fibers which are involved in the processing of nervous signals. Accordingly, in the peripheral nervous system TRPV1 are mainly found in III and IV afferent nerve fibers, a type of peripheral afferent fibers linked to the development of central fatigue by affecting both supraspinal and spinal levels of the nervous system during different tasks, as well as to the detection of a large variety of nociceptive stimuli.

Although the mechanisms presented in this section may be relevant to the sports performance field, current direct research on humans' performance is scarce. In this regard, Before the research intervention of this doctoral thesis was conducted, only 5 studies had evaluated the impact of capsaicinoids or capsinoids supplementation on resistance training outcomes in humans. Overall, the findings of these studies suggest a positive impact of capsaicinoids and capsinoids on strength endurance until exhaustion and the perceptual responses to resistance training. In this sense, these substances provide a "counter-fatigue" effect driven by their analgesic improvement in pain and discomfort through their TRPV1 interaction and the enhancement of muscle contraction due to the increase of calcium release from the sarcoplasmic reticulum. For instance, in a previous study, the group that ingested purified capsaicin (12 mg) performed more repetitions in all the sets of a 4x70% of one repetition maximum (1RM) back squats (SQ) protocol until muscle concentric failure. This effect elicited a significantly increase in the total volume performed in the supplementation condition (measured as total weight lifted).

The literature addressing the effects of capsaicinoids or capsinoids on high-intensity interval training is negligible. To date, only two experiments have evaluated this type of exercise under these substances' supplementation. The first study showed a lack of an ergogenic effect on a repeated sprints protocol, which contrasts with the findings of the second study. In this second study, it was reported an enhancement in the number of efforts performed and the time to reach 90% of oxygen uptake consumption peak (VO2 peak) without changes in VO2 values after 12 mg of purified capsaicin supplementation in a high-intensity interval exercise. The neuromuscular improvements of capsaicin increase in 13 extra efforts and 188 s extra time the task in the supplementation condition compared to placebo. Furthermore, overall-body

ratings of perceived exertion (RPE-OB) was maximum in both groups, which suggests that capsaicin improves the total number of efforts until exhaustion or reduces the neuromuscular effort when volume is matched. However, in spite of the positive effect of capsaicin in peripheral neuromuscular performance, how it impacts on sport bioenergetics and central adaptations to exercise remains unclear.

Finally, the metabolic and bioenergetic impact of capsacicin and its analogues is poorly studied in the current literature. In this regard, only two studies have reported the metabolic effects of a capsaicinoids or capsinoids on substrate oxidation in healthy individuals. In the first study, participants exhibited equal respiratory exchange ratios (RER) after ingesting 12 mg of capsiate supplementation during cycling exercise performed at 70% of the maximal aerobic speed. In the second study, a meal with 10 g of hot peppers (i.e., without standardizing the active agent) 2.5 hours prior to 1 hour of aerobic exercise at 60% $VO2_{peak}$ showed an increase in RER compared to placebo. In addition, only one study has addressed substrate oxidation standardizing maximal fat oxidation (MFO), which is critical for a real objective evaluation of these variables. However, this study was conducted in overweight participants, which may not be extrapolated to other populations due to the reduced metabolic flexibility of this population.

Aims

The main aim of this doctoral thesis research project was to assess the potential use of different doses of PC to enhance physical performance and to reduce mechanical, metabolic, biochemical, perceptual and electrophysiological fatigue in different exercise interventions. Before the start of this research project, two preliminary studies were conducted to evaluate the supplementation patterns of national trained athletes and to systematically aggregate the impact of capsaicinoids and capsinoids supplementation in prior studies. With the development of the three studies of this doctoral dissertation, the descriptive, physiological, perceptual and mechanical gaps presented in the introduction section have been assessed.

The design, methodological aspects, main results, and conclusions of each of the three studies that are part of this doctoral thesis and also justify the original contribution of the author, are presented hereunder.

First study: Effects of phenylcapsaicin supplementation on mechanical performance and neuromuscular activity

This study was conceived as a randomized, triple-blinded, crossover, placebo-controlled trial with the purpose of addressing the neural and mechanical responses to resistance exercise

after PC supplementation. The whole development of the present study is presented in chapter 2. Twenty-five healthy men (age = 21.7 ± 3.7 years, body mass = 77.4 ± 9.1 kg, height = 176.7 ± 7.2 cm, 1RM in SQ = 125.6 ± 21.0 kg, 1RM normalized to body mass = 1.64 ± 0.22) with at least two years of experience in resistance training (range = 2-5 years) were enrolled to this study. Participants performed each session at the same individual time of the day under stable environmental conditions (22-24 ºC and 55% humidity).

Two weeks before the beginning of the study, participants were anthropometric (body mass and height), 1RM and load-velocity relationship in SQ tested. Then, participants completed three experimental conditions, each one composed of a main session and a 24 hours second session (post-24h). The three experimental conditions were identical with the only difference of the supplement ingested. In addition, participants randomly ingested either a placebo (PLA) or a low (LD) or high (HD) dose of PC before the first weekly session. During the first weekly session, participants completed a SQ protocol that consisted of three sets of eight repetitions at 70% 1RM. Before (Pre), immediately after (Post), and 24 hours after (Post-24h) the SQ protocol, a battery of tests was conducted to analyze the fatigue induced by each condition: Countermovement jump (CMJ), two SQ repetitions with 60% 1RM, and maximal isometric SQ at 90º, in that order. Since it is important to standardize temporalization to measure the acute responses, the tests were conducted in the following time points at Post: CMJ (one-minute post-exercise), SQ with 60% 1RM (two minutes post-exercise), and isometric SQ (three minutes post-exercise). Electromyographical assessment of each session was recorded while participants were performing the SQ tests. Supplements and placebo were prepared and packaged by a non-involved researcher in independent installations. To ensure the triple blinding, each package was encoded with a number from one to three. Packages were not unblinded until a third-party statistician performed all the analyses. Supplements were composed of a HD of PC (2.5 mg), LD of PC (0.625 mg) or PLA and were ingested 45 min prior to the first session.

A two-way repeated measures analysis of variance (ANOVA) (condition x time) with Bonferroni post-hoc was used to explore the effect of the interventions (LD, HD, PLA) across time on the magnitude of each dependent variable and to address whether an order effect is presented. A one-way repeated measures ANOVA was used to compare the total volume load. Statistical significance was established at $p \leq 0.05$.

Two-way repeated measures ANOVAs did not reveal any condition x time interaction (p range < 0.26 to 0.69). However, a significant time effect for root mean square (RMS) at 60% 1RM, median frequency (MDF) at isometric SQ, MDF at 60% 1RM, and MDF during the SQ

protocol (p range < 0.001 to 0.006) was observed. Post-hoc Bonferroni for time only revealed significant differences at 60% load RMS for 1/2 sets (p = 0.01; d =1.11) and 2/ 3 (p = 0.006; d = 1.19) sets comparisons.

Regarding the SQ test with 60% 1RM, the two-way repeated measures ANOVAs did not reveal significant condition × time interactions (p range = 0.74 to 0.95). However, significant time effect (p < 0.001) and condition effect (p = 0.03) for mean propulsive velocity (MPV) was observed. Moreover, the two-way repeated measures ANOVAs did not reveal significant condition × time interaction (p range = 0.09 to 0.89), or significant differences for time (p range = 0.06 to 0.78), or condition (p range = 0.19 to 0.97) for any of the isometric outcomes.

Two-way repeated measures ANOVA revealed significant condition x time interaction for CMJ height (p < 0.01) and a condition main effect (p < 0.001). Post-hoc Bonferroni tests showed that HD attained higher CMJ height than PLA (p < 0.001; d = 0.72) and LD (p < 0.001; d = 0.37).

One-way repeated measures ANOVAs reported no significant differences between conditions for total volume load (F = 1.09, p = 0.35). The two-way repeated measures ANOVAs did not reveal significant condition × time interaction for any variable (p range = 0.12 to 0.98). However, it revealed significant time effects (p range = 0.001 to 0.008) for all the outcomes analysed. Moreover, a significant condition effect was observed for 60% load (p = 0.03), MPV (p = 0.02)] and Velocity loss [VLoss (p = 0.04)]. Bonferroni post-hoc revealed no significant differences for conditions ((p range = 0.06 to 0.07). Post-hoc Bonferroni for time analyses revealed significant differences between sets 1 and 2 for all outcomes (p range < 0.001 to 0.007; d range = 0.47 to 0.73). However, between sets 1 and 3, Bonferroni post-hoc time analyses only reported significant differences for mean MPV (p < 0.001; d = 0.80). For comparisons between sets 2 and 3, all movement velocity outcomes reported significant time differences (p range < 0.001 to 0.04; d range = 0.21- 0.78). One-way ANOVA for intra-set analysis revealed significant differences in repetitions 13, 15, 16, 17, 23, and 24 in favor of HD and LD (p range = 0.03 to 0.04).

The results of the present study suggest that acute PC ingestion may be considered as ergogenic aid (2.5 mg) for dynamic resistance exercise sessions when more than 1 exercise is performed. Consistently, mechanical fatigue after a submaximal exercise may be delayed and attenuated by PC. In addition, further research must deeply examine if neural mechanisms underlying capsaicinoids ergogenic impact exist.

Second study: Effects of phenylcapsaicin on muscle damage, protein breakdown, recovery and perceptual responses

A randomized, triple-blinded, placebo-controlled crossover trial was used to explore the effects of PC on resistance training performance, muscle damage [aspartate aminotransferase (AST)], protein breakdown (urea) and metabolic (lactate), as well as perceptual responses. These findings are presented in chapter 3.

Participants attended the laboratory twice per week for a total study duration of three weeks. Each week of the study consisted of a main experimental session and a follow-up session. For each condition, six capillary blood extractions, a warm-up, a SQ testing protocol, and a 24-hour recovery and muscle damage follow-up session were performed. All procedures were completed at the same time of the day and under stable environmental conditions for each participant.

Twenty-five healthy men (age = 21.0 ± 2.2 years, body mass = 76.5 ± 9.5 kg, height = 176.4 ± 7.5 cm, SQ 1RM normalized to body mass = 1.66 ± 0.22) enrolled voluntarily to this study. Of the total sample, 2 participants dropped out of the study, 1 due to causes not related to the study and the other after the placebo session. All participants were resistance-trained men with at least 2 years of experience (experience= 3.61 ± 1.43 years). Exclusion criteria comprised cardiovascular, neurological, physical and/or metabolic disorders that may disturb the primary outcomes. To ensure blinding, supplements and placebo were encapsulated and packaged with numbered labels by an independent researcher (i.e., not involved in the study). Packages and capsules were indistinguishable in appearance, smell and taste and their content was only revealed after an independent researcher performed the statistical analyses. Supplements were composed of a HD of PC (2.5 mg), LD of PC (0.625 mg) or PLA and were ingested 45 min prior to the first session.

One week before the beginning of the study, SQ strength and anthropometric measurements (i.e., body mass and height) were tested for all participants. Participants were asked not to consume alcohol, caffeine, or other ergogenic aids. Besides, they could not to perform intense exercise or modify their macronutrients distribution, calorie intake and food selection 24 and 48 hours before each session.

A two-way repeated measures ANOVA (condition x time) was used to explore the effect of the interventions (LD, HD, PLA) across the time on the magnitude of each dependent biochemical and perceptual variable. Bonferroni post-hoc comparison was performed when ANOVA significance was reached. A one-way repeated measures ANOVA was used to

compare velocity analyses. If non-parametric data was examined, Friedman and post-hoc Wilcoxon were used instead. Statistical significance was set at $p \leq 0.05$.

The results of the present study exhibit that lactate and urea absolute changes (pre-post comparisons) were significant for time ($p < 0.001$), although there were not found differences for condition (F = 1.49, $p = 0.23$) or condition × time interaction (F = 0.94, $p = 0.34$). For AST, it was revealed a significant difference in favour of HD in the post-values ($p = 0.03$). Post-hoc test revealed significantly higher post-levels of AST for PLA compared to HD ($p = 0.02$).

Movement velocity outcomes were significant for mean velocity and maximal velocity loss (p range ≤ 0.001 to 0.05) for HD. Bonferroni post-hoc comparisons revealed significant differences between HD and LD for maximal VLoss (p = 0.008) and it was almost achieved between HD and PLA for the mean velocity variable (p = 0.06). The magnitude of the differences between the different conditions ranged from negligible to large.

Concerning ratings of perceived exertion, RPE-OB reported significant differences for time (F = 49.00, $p < 0.001$) but not for condition (F = 2.77, $p = 0.07$) or condition × time interaction (F = 1.339, $p = 0.26$). However, Bonferroni post-hoc analyses revealed no significant differences. For active muscle ratings of perceived exertion (RPE-AM), both condition (F = 9.19, $p < 0.001$) and time (F = 36.154, $p < 0.001$) reached significant differences but not condition × time interaction (F = 0.553, $p = 0.70$). Bonferroni post-hoc analyses showed significant differences for all time comparisons (p range ≤ 0.001 to 0.002) and for comparisons between PLA and HD ($p = 0.004$) and between HD and LD ($p = 0.02$). On the other hand, it was not found significant differences between conditions for perceived recovery status (PRS) (F = 0.698, $p = 0.46$).

Overall, the results of the present study suggest that a HD (2.5 mg) of PC supplementation ingested 45 minutes before exercise may increase SQ performance and reduce muscle damage, as well as peripheral quadriceps perceived exertion in strength-trained participants in comparison to a LD (0.625 mg) and PLA. Therefore, the ergogenic effect of PC may appear after a "dose" threshold is reached.

Third study: Effects of phenylcapsaicin on substrates oxidation, energy expenditure, metabolic, perceptual and thermal responses during exercise

A randomized, triple-blinded, placebo-controlled crossover trial was used to analyze the effects of PC on energy expenditure and substrate oxidation, skin body temperature, heart rate

and perceptual responses to submaximal steady-state and maximal effort cycling tests. This study is further presented in chapter 4.

In this research project, participants attended the laboratory four times, separated by 72-96 h to ensure a complete recovery from central and peripheral fatigue. Before the preliminary session, participants were anthropometric (i.e., height, body mass, % muscle, % body fat) and sociodemographic characterized. Then, a submaximal incremental exercise test followed by a maximal effort incremental test, were conducted in a preliminary session. The submaximal exercise test was used to determine the MFO and the cycling power values (W) at MFO (FATmax intensity). The maximal effort test assessed maximal oxygen consumption ($VO2_{max}$) and the maximal cycling power achieved during the test. The three experimental sessions were identical, only differing in the supplement (PLA, LD of PC, and HD of PC), which was administered 45 min before the first cycling task. In each experimental session, participants performed a steady-state test (60 min at FATmax) followed by a maximal incremental effort test (25 W increments every min until volitional exhaustion). Each participant was constantly tested at the same time of the day and under similar environmental conditions.

To ensure the detection of differences 17 physically active males were enrolled in the study. Participants enrolled in the study through a poster that was shared on social media. None of the participants reported any physical limitation or health condition that could compromise cycling performance. Participants were instructed not to perform any intense physical exercise during the two days preceding each visit to the laboratory and from consuming stimulant beverages or any dietary supplement within 24 h preceding each testing session. To ensure intra and inter-individual reliability in metabolic variables like fat oxidation (FATox), dietary intake was standardized at least 6 hours previous to exercise testing. For this purpose, participants performed each test with at least 6 hours of fasting prior to the start of each session and the last meal before the fasting period was standardized with 45 g of maltodextrin powder and 30 g of protein powder for all participants.

Concerning the supplements used, the contents of the capsules were as follows: a LD of 0.625 mg of PC, a HD of 2.5 mg HD of PC, and a PLA composed of maltodextrin and excipients. Supplements and placebo were encapsulated and packaged with alphanumeric labels to ensure blinding. Accordingly, an independent technician (i.e., not involved in the study) prepared the capsules in the original producer´s industry.

A two-way repeated measures analysis of variance ANOVA (condition x time) was used to analyse the effect of the supplementation (LD, HD, PLA) across the time on each dependent metabolic, performance, and perceptual variable. A Bonferroni post-hoc comparison was

performed when ANOVA significance was reached. A one-way repeated measures ANOVA was used to compare intra-test effects for each stage and for the maximal metabolic values of the steady-state test. For non-parametric data, Friedman`s test and post-hoc Wilcoxon corrections were used instead. Statistical significance was set at $p \leq 0.05$.

For circulating lactate levels, overall-body (RPE-OB), active muscle (RPE-AM) ratings of perceived exertion and heart rate significant differences were not reported among conditions (p range = 0.08 to 0.56). However, significant differences were found for time in the heart rate, RPE-OB, and RPE-AM variables ($p \leq 0.001$). Bonferroni post-hoc analyses revealed significant differences for all time comparisons (pre, '30' and '60') in heart rate, RPE-OB, and RPE-AM (p range < 0.001 to 0.004). Significant condition x time interaction was only found for RPE-OB due to higher values in LD compared to PLA (d = 27) and HD (d = 0.27).

Significant differences in skin body temperature ($p = 0.27$) or mean heart rate ($p = 0.24$) were not detected for the condition analyses. Moreover, maximal carbohydrate oxidation, energy expenditure and RER did not differ between conditions (p ranged from 0.10 to 0.77). However, significant differences were found for maximum heart rate ($p = 0.03$) and MFO ($p = 0.05$), in favor of HD. Nevertheless, Bonferroni post-hoc did not reveal differences between conditions in any of the outcomes (p range = 0.09 to 0.99; d range = 0.20 to 0.31).

Significant differences for condition were not reported for any of the American Society of Heating, Refrigerating, and Air Conditioning Engineers (ASHRAE) outcomes measured (p range = 0.17 to 0.78; ηp^2 range = 0.01 to 0.12). However, significant differences were reported in time for all the variables ($p \leq 0.001$; ηp^2 range = 0.52 to 0.73). A significant condition x time interaction was found for the clavicle ($p = 0.04$; $\eta p^2 = 0.16$) area due to the lower value of HD in comparison to LD an PLA. The other areas did not exhibit condition x time interactions (p range = 0.12 to 0.60; ηp^2 range = 0.04 to 0.12).

No significant differences were found for the area under the curve (AUC) of energy expenditure (EE), FATox (fat oxidation) or CHOox (carbohydrate oxidation) (p range = 0.09 to 0.54; ηp^2 range = 0.04 to 0.18). Non-significant differences were either found for condition in FATox ($p = 0.06$; $\eta p^2 = 0.14$), CHOox ($p = 0.19$; $\eta p^2 = 0.10$), EE ($p = 0.54$; $\eta p^2 = 0.008$), nor RER ($p = 0.21$; $\eta p^2 = 0.10$). Nevertheless, significant differences for time in all the aforementioned variables ($p \leq 0.001$; ηp^2 range = 0.54 to 0.79) but not for condition x time interaction (p range = 0.17 to 0.96; ηp^2 range = 0.003 to 0.06) were revealed. Intra-test analysis only exhibited a significant effect on FATox at min 5 ($p = 0.005$; $\eta p^2 = 0.28$), 10 ($p \leq 0.001$; $\eta p^2 = 0.29$), and 55 ($p = 0.04$; $\eta p^2 = 0.24$) for HD and LD and for CHOox at min 5 ($p = 0.05$; $\eta p^2 = 0.25$) and RER ($p = 0.003$; $\eta p^2 = 0.25$) at min 5 in favor of PLA but not for other variables

in any other stage. Post-hoc Bonferroni reported significant differences in favor of HD and LD between PLA/ HD at 5 min (p = 0.002; d = 0.92), PLA/LD at 5 min (p = 0.002; d = 0.74), PLA/HD (p = 0.002; d = 0.66) and PLA/LD at 10 min (p = 0.002; d = 0.56) for FATox.

None of the variables (i.e., heart rate, lactate, RPE-OB at 30 and 90%, and RPE-AM) recorded during the maximal effort test differed between the experimental conditions (p ranged from 0.011 to 0.915) except RPE-OB at 60% due to the lower values of HD compared to LD and PLA (p = 0.05).

The results of this last study suggest that LD and HD of PC modulate the metabolic response (FATox, CHOox and RER) to exercise and HD of PC reduces maximum heart rate values during aerobic exercise. However, PC only improves the perceptual responses (i.e., RPE-OB and clavicle thermal perception) to exercise when it is consumed in HD.

Conclusions

Collectively, the findings of the present doctoral thesis exhibit through the three research projects conducted that PC supplementation is an effective nutritional ergogenic aid to enhance sports performance in resistance and aerobic training. These results provide a new background to develop future studies addressing the chronic and in new populations effects of this compound.

According to the hypotheses presented in this doctoral thesis and the specific objectives raised, the main results and conclusions are shown hereunder. First, PC is able to increase physical performance due to peripheral mechanisms and to the reduction of acute mechanical and perceptual fatigue during resistance exercise. Due to the improvement in mechanical performance, after PC is ingested the acute post-session fatigue and the following day muscle damage are reduced. Second, although PC is effective enhancing resistance training performance, the neural responses during exercise are not increase, which elicits the need of future studies addressing the peripheral ergogenic mechanisms underlying the effect of this substance. Third, PC increases the metabolic responses to aerobic exercise shifting the contribution of energy substrates during exercise and leading to a higher contribution of FATox, as well as a reduction in maximum heart rate.

Therefore, the compendium of studies that forms this doctoral thesis highlights the plausible use of PC as a nutritional ergogenic aid, which provides valuable information for strength and conditioning, healthcare and specific sports performance professionals.

Resumen

Introducción

Los suplementos dietéticos son complementos nutricionales que consumidos junto a una dieta saludable pueden mejorar la salud y/o el rendimiento deportivo. Bajo su propia definición, estos son sustancias legales, las cuales no están prohibidas por ningún agente internacional como la Agencia Mundial Antidopaje. En consecuencia, estos productos son de venta libre, lo que los hace muy populares entre distintas poblaciones deportistas.

Como queda expuesto en el capítulo 1, los capsaicinoides son un grupo de compuestos que se encuentran de forma natural en frutas picantes como el chile. Debido a sus usos clínicos y ergogénicos, en las dos últimas décadas se han llevado a cabo nuevas investigaciones científicas en torno a estos compuestos. A su vez, en la última década, también se ha estudiado otro grupo de análogos de los capsaicinoides, llamados capsinoides, con el fin de reducir las molestias ocasionadas por el consumo oral de capsaicina. Los capsinoides se encuentran fundamentalmente en los pimientos dulces CH-19, un tipo de pimientos no picantes que han sido modificados genéticamente para eliminar la pungencia natural de los capsaicinoides. Ambas sustancias, capsaicinoides y capsinoides, interactúan con el receptor de potencial transitorio vaniloide 1 (TRPV1), que ejerce las principales funciones fisiológicas de estas sustancias. Sin embargo, la literatura actual ha demostrado que los capsinoides son un grupo de compuestos con escasa biodisponibilidad, ya que, tras su consumo, estos se metabolizan y conjugan rápidamente, lo que da lugar a niveles circulantes en el torrente sanguíneo no detectables. En cambio, tras su consumo, las distintas formulaciones de capsaicina muestran un rápido efecto en varios tejidos, como el intestino delgado, el hígado y el estómago, lo que puede extrapolarse a tejidos periféricos como el músculo esquelético. Por otro lado, se ha documentado que el uso de capsaicina oral puede ser conflictivo debido a sus características picantes, las cuales aumentan el riesgo de molestias durante el ejercicio. Por lo tanto, la formulación de nuevos análogos de capsaicina purificada que puedan reducir la dosis fisiológica óptima parece ser una prioridad en este campo. Por este motivo, en los últimos años se ha desarrollado un nuevo grupo de análogos sintéticos de la capsaicina tradicional conocidos como "capsaicyns". Entre estas sustancias se encuentra la denominada fenilcapsaicina (PC), la cual ha surgido como alternativa a la suplementación oral tradicional con capsaicina purificada. La PC es una microencapsulación de 98% de PC y 1-1,5% de excipientes lipídicos y celulosa como vehículos de transporte primarios.

Más allá de lo expuesto, los fármacos agonistas de TRPV1 han mostrado mejoras en varias condiciones fisiopatológicas, como el dolor musculoesquelético y neuropático crónico, las alteraciones gastrointestinales (por ejemplo, la alteración de la mucosa gastroduodenal) y los trastornos metabólicos (como el sobrepeso). En referencia al rendimiento deportivo, parece que estos compuestos ejercen su efecto generando una disminución de la percepción del esfuerzo, reduciendo la percepción del dolor y aumentando la eficiencia mecánica (por ejemplo, el tonelaje movilizado). En este sentido, los efectos fisiológicos de la capsaicina están mediados por la reducción de la hiperalgesia inflamatoria, la regulación a la baja de los canales de calcio activados por voltaje y la influencia sobre la "termorrecepción", siendo todos ellos vínculos comunes entre los ámbitos de la salud y el rendimiento. Los TRPV1 se encuentran en una serie de fibras nerviosas de pequeño diámetro que intervienen en el procesamiento de señales nerviosas. En consecuencia, en el sistema nervioso periférico los TRPV1 se encuentran principalmente en las fibras nerviosas aferentes III y IV, un tipo de fibras aferentes periféricas vinculadas al desarrollo de la fatiga central al afectar tanto a los niveles supraespinales como espinales del sistema nervioso durante diferentes tareas, así como a la detección de una gran variedad de estímulos nociceptivos.

Aunque los mecanismos presentados en esta sección pueden ser relevantes para el campo del rendimiento deportivo, la investigación actual directa sobre el rendimiento en humanos es escasa. En este sentido, antes de realizar la intervención experimental de esta tesis doctoral, sólo 5 estudios habían evaluado el impacto de la suplementación con capsaicinoides o capsinoides en el entrenamiento de fuerza en humanos. Los resultados de estos estudios sugieren un impacto positivo de los capsaicinoides y capsinoides sobre la resistencia a la pérdida de fuerza y las respuestas perceptivas al entrenamiento de fuerza. Por ello, estas sustancias proporcionan un efecto "anti-fatiga" explicado por su mejora analgésica del dolor a través de su interacción con los TRPV1 y a la mejora de la contracción muscular debido al aumento de la liberación de calcio del retículo sarcoplasmático. Por ejemplo, en un estudio previo, el grupo que ingirió capsaicina purificada (12 mg) realizó más repeticiones en todas las series de un protocolo de sentadilla trasera (SQ) de cuatro series al 70% de la máxima repetición (1RM) hasta alcanzar el fallo muscular concéntrico. Esto a su vez provocó un aumento significativo del volumen total realizado en el grupo que consumió capsaicina.

La bibliografía que evalúa los efectos de los capsaicinoides o capsinoides en el entrenamiento interválico de alta intensidad es escasa. Hasta la fecha, sólo dos estudios han evaluado este tipo de ejercicio bajo la suplementación de estas sustancias. El primer estudio mostró una ausencia de efecto ergogénico en un protocolo de esprines repetidos, lo que

contrasta con los resultados del segundo estudio. En este segundo estudio se observó una mejora en el número de esfuerzos realizados hasta la extenuación y en el tiempo hasta alcanzar el 90% del pico de consumo pico de oxígeno (VO2peak), mientras que los valores de VO2peak no fueron incrementados tras la suplementación con 12 mg de capsaicina purificada en un protocolo de ejercicio de intervalos de alta intensidad. Concretamente, las mejoras neuromusculares de la capsaicina incrementaron en 13 esfuerzos extra y 188 s el tiempo de duración de la tarea en la condición de suplementación en comparación con el placebo. Además, el esfuerzo percibido general (RPE-OB) fue máximo en ambos grupos, lo que sugiere que la capsaicina mejora el número total de esfuerzos hasta el agotamiento o reduce el esfuerzo neuromuscular si se iguala el volumen. Sin embargo, a pesar del efecto positivo de la capsaicina en el rendimiento neuromuscular periférico, sigue sin estar claro cómo influye en la bioenergética deportiva y en las adaptaciones centrales al ejercicio.

Por último, el impacto metabólico y bioenergético de la capsacicina y sus análogos está poco estudiado en la bibliografía actual. A este respecto, sólo dos estudios han informado de los efectos metabólicos de un capsaicinoide o capsinoide sobre la oxidación de sustratos energéticos en individuos sanos. En el primer estudio, los participantes mostraron tasas de intercambio respiratorio (RER) iguales tras ingerir 12 mg de un suplemento de capsiato durante un protocolo en cicloergómetro realizado al 70% de la velocidad aeróbica máxima. En el segundo estudio, una comida con 10 g de pimientos picantes (es decir, sin estandarizar el principio activo) 2,5 horas antes de 1 hora de ejercicio aeróbico al 60% del VO2peak mostró un aumento del RER en comparación con el placebo. Más allá de esto, sólo un estudio ha evaluado la oxidación de sustratos energéticos estandarizando la máxima oxidación de ácidos grasos (MFO), lo cual es fundamental para garantizar la evaluación objetiva de estas variables. Sin embargo, este estudio se realizó en participantes con sobrepeso, lo que puede no ser extrapolable a otras poblaciones debido a la menor flexibilidad metabólica de esta población.

Objetivos

El objetivo principal de esta tesis doctoral fue evaluar el uso potencial de diferentes dosis de PC para mejorar el rendimiento físico y reducir la fatiga mecánica, metabólica, bioquímica, perceptiva y electrofisiológica en diferentes intervenciones de ejercicio. Antes de iniciar este proyecto de investigación, se realizaron dos estudios preliminares para evaluar los patrones de suplementación de atletas entrenados a nivel nacional y para compendiar sistemáticamente la literatura previa sobre el impacto de la suplementación con capsaicinoides y capsinoides. Con el desarrollo de los tres estudios de esta tesis doctoral se han evaluado las

lagunas descriptivas, fisiológicas, perceptivas y mecánicas presentadas en el apartado de introducción.

A continuación, se presenta el diseño, aspectos metodológicos, resultados y conclusiones de cada uno de los tres estudios que forman parte de esta tesis doctoral y que además justifican la aportación original del autor como contribuciones científicas originales.

Primer estudio: Efectos de la suplementación con fenilcapsaicina en el rendimiento mecánico y la actividad neuromuscular

Este estudio fue concebido bajo un diseño aleatorizado, triple ciego, cruzado y controlado con placebo con el propósito de abordar las respuestas neurales y mecánicas al ejercicio de fuerza tras la suplementación con PC. La totalidad del presente estudio puede ser hallada en el capítulo 2 de esta tesis doctoral. Veinticinco varones sanos (edad = 21,7 ± 3,7 años, masa corporal = 77,4 ± 9,1 kg, altura = 176,7 ± 7,2 cm, 1RM en sentadilla trasera SQ = 125,6 ± 21,0 kg, 1RM normalizado a la masa corporal = 1,64 ± 0,22) con al menos dos años de experiencia en el entrenamiento de fuerza (rango = 2-5 años) participaron en este estudio. Los participantes realizaron cada sesión a la misma hora individual del día en condiciones ambientales estables (22-24 ºC y 55% de humedad).

Dos semanas antes del inicio del estudio, los participantes fueron sometidos a evaluaciones antropométricas (masa corporal y altura), de la 1RM y de la relación carga-velocidad en SQ. En las sesiones experimentales los participantes fueron sometidos a tres condiciones de suplementación, cada una compuesta por una sesión principal y una segunda sesión a las 24 horas (post-24h). Las tres condiciones experimentales eran idénticas con la única diferencia del suplemento ingerido. Los participantes ingirieron aleatoriamente un placebo (PLA) o una dosis baja (LD) o alta (HD) de PC antes de la primera sesión semanal. Durante la primera sesión semanal, los participantes completaron un protocolo de SQ que consistió en tres series de ocho repeticiones al 70% 1RM. Antes (Pre), inmediatamente después (Post) y 24 horas después (Post-24h) del protocolo de SQ, se realizó una batería de pruebas para analizar la respuesta a la fatiga inducida en cada condición en el siguiente orden: Salto contramovimiento (CMJ), dos repeticiones de SQ con 60% 1RM, y SQ isométrica máxima a 90º. Dado que es importante estandarizar la temporalización para medir las respuestas agudas, las pruebas se realizaron en los siguientes puntos temporales en el momento Post: CMJ (un minuto post-ejercicio), SQ con 60% 1RM (dos minutos post-ejercicio), y SQ isométrica (tres minutos post-ejercicio). La evaluación electromiográfica de cada sesión se registró mientras los participantes realizaban las pruebas de SQ. Los suplementos y el placebo fueron preparados por un

investigador no implicado en el desarrollo del estudio en instalaciones independientes. Para garantizar el triple cegamiento, cada paquete se codificó con un número del uno al tres. Los paquetes no se desenmascararon hasta que un estadístico externo realizó todos los análisis. Los suplementos se componían por una HD de PC (2.5 mg), LD de PC (0.625 mg) o PLA, que fueron ingeridos 45 min previos a la primera sesión.

Se utilizó un análisis de varianza (ANOVA) de medidas repetidas de dos vías (condición x tiempo) con ajuste post-hoc de Bonferroni para explorar el efecto de las intervenciones (LD, HD, PLA) a lo largo del tiempo sobre la magnitud de cada variable dependiente y para abordar si se presenta un efecto de orden. Se utilizó un ANOVA de medidas repetidas de una vía para comparar el tonelaje total. La significación estadística se estableció en $p \leq 0,05$.

Los ANOVA de medidas repetidas de dos vías no revelaron ninguna interacción condición x tiempo (rango de $p < 0,26$ a 0,69). Sin embargo, se observó un efecto temporal significativo para la raíz de la media cuadrática (RMS) al 60% 1RM, frecuencia mediana (MDF) en SQ isométrica, MDF al 60% 1RM y MDF durante el protocolo de SQ (rango de $p < 0,001$ a 0,006). El ajuste post-hoc de Bonferroni reveló diferencias significativas al 60% RM para el tiempo en la RMS para las comparaciones de 1/2 series ($p = 0,01$; $d = 1,11$) y 2/ 3 ($p = 0,006$; $d = 1,19$) series.

En cuanto a la prueba de SQ con un 60% de 1RM, los ANOVA de medidas repetidas de dos vías no revelaron interacciones significativas entre condición × tiempo (rango de $p = 0,74$ a 0,95). Sin embargo, se observó un efecto significativo del tiempo ($p < 0,001$) y de la condición ($p = 0,03$) para la velocidad media propulsiva (MPV). Además, los ANOVAs de medidas repetidas de dos vías no revelaron una interacción significativa condición × tiempo (rango $p = 0,09$ a 0,89), o diferencias significativas para el tiempo (rango $p = 0,06$ a 0,78), o condición (rango $p = 0,19$ a 0,97) para cualquiera de los resultados isométricos.

El ANOVA de medidas repetidas de dos vías reveló una interacción significativa de condición x tiempo para la altura en el CMJ ($p < 0,01$) y un efecto principal de condición ($p < 0,001$). Las pruebas post-hoc de Bonferroni mostraron que HD alcanzó una mayor altura en CMJ que PLA ($p < 0,001$; $d = 0,72$) y LD ($p < 0,001$; $d = 0,37$).

Los ANOVA de medidas repetidas de una vía no revelaron diferencias significativas entre condiciones para el tonelaje movilizado ($F = 1,09$, $p = 0,35$). Los ANOVAs de medidas repetidas de dos vías no revelaron una interacción significativa condición × tiempo para ninguna variable (p rango = 0,12 a 0,98). Sin embargo, se encontraron efectos temporales significativos (rango $p = 0,001$ a 0,008) para todos los resultados analizados. Además, se observó un efecto significativo de la condición para la carga del 60% ($p = 0,03$), la MPV media

(p = 0,02) y la pérdida de velocidad [VLoss (*p* = 0.04)]. El ajuste post-hoc de Bonferroni no reveló diferencias significativas para las condiciones (rango p = 0,06 a 0,07). Los análisis post-hoc de Bonferroni para el tiempo revelaron diferencias significativas entre las series 1 y 2 para todos los resultados (rango p < 0,001 a 0,007; rango d = 0,47 a 0,73). Sin embargo, entre las series 1 y 3, los análisis post hoc de Bonferroni para el tiempo sólo revelaron diferencias significativas para la MPV media (p < 0,001; d = 0,80). Para las comparaciones entre las series 2 y 3, todos los resultados de velocidad de movimiento mostraron diferencias temporales significativas (rango p < 0,001 a 0,04; rango d = 0,21- 0,78). El ANOVA de una vía para el análisis intra-set reveló diferencias significativas en las repeticiones 13, 15, 16, 17, 23 y 24 en favor de HD y LD (rango p = 0,03 a 0,04).

Los resultados del presente estudio sugieren que el consumo agudo de PC (2,5 mg) puede ejercer un efecto ergogénico para las sesiones de ejercicios de fuerza dinámica cuando se realiza más de un ejercicio. En relación a esto, la fatiga mecánica tras un ejercicio submáximo puede retrasarse y atenuarse con el uso de PC. Más allá de esto, las investigaciones futuras deben evaluar en profundidad si existen mecanismos neurales subyacentes al impacto ergogénico de los capsaicinoides.

Segundo estudio: Efectos de la fenilcapsaicina sobre el daño muscular, la degradación proteica, la recuperación y las respuestas perceptivas.

En este estudio fue utilizado un diseño cruzado aleatorizado, triple ciego y controlado con placebo para examinar los efectos de la PC en el entrenamiento de fuerza, el daño muscular [aspartato aminotransferasa (AST)], la degradación proteica (urea), así como en las respuestas metabólicas (lactato) y perceptivas. El desarrollo de este estudio puede encontrarse en el capítulo 3.

Los participantes acudieron al laboratorio dos veces por semana durante un total de tres semanas de estudio. Cada semana del estudio consistió en una sesión experimental principal y una sesión de seguimiento. Para cada condición, se realizaron seis extracciones de sangre capilar, un calentamiento, un protocolo de sentadilla libre y una sesión de seguimiento a las 24 horas para evaluar la recuperación y el daño muscular. Todos los procedimientos se realizaron a la misma hora del día y en condiciones ambientales estables para cada participante.

Veinticinco varones sanos (edad = 21,0 ± 2,2 años, masa corporal = 76,5 ± 9,5 kg, altura = 176,4 ± 7,5 cm, SQ 1RM normalizada a la masa corporal = 1,66 ± 0,22) participaron voluntariamente en este estudio. De la muestra total, dos participantes abandonaron el estudio, uno por causas no relacionadas con el estudio y el otro tras la primera sesión con placebo. Todos

los participantes eran hombres experimentados en el entrenamiento de fuerza (experiencia= 3,61 ± 1,43 años). Los criterios de exclusión estaban compuestos por la presencia de trastornos cardiovasculares, neurológicos, físicos y/o metabólicos que pudieran alterar los resultados principales. Para garantizar el cegamiento, los suplementos y el placebo fueron encapsulados y envasados con etiquetas numeradas por un investigador independiente (es decir, no implicado en el estudio). Los envases y las cápsulas eran indistinguibles en apariencia, olor y sabor, y su contenido sólo fue revelado después de que un investigador independiente realizara los análisis estadísticos. Los suplementos se componían por una HD de PC (2.5 mg), LD de PC (0.625 mg) o PLA, que fueron ingeridos 45 min previos a la primera sesión.

Una semana antes del inicio del estudio, se evaluó la fuerza en SQ y se determinaron las medidas antropométricas (la masa corporal y la altura) de todos los participantes.

Se solicitó a los participantes que no consumieran alcohol, cafeína u otras ayudas ergogénicas previo al estudio. Además, no podían realizar ejercicio intenso ni modificar su distribución de macronutrientes, ingesta calórica y selección de alimentos 24 y 48 horas antes de cada sesión respectivamente.

Se utilizó un ANOVA de medidas repetidas de dos vías (condición x tiempo) para explorar el efecto de las intervenciones (LD, HD, PLA) a lo largo del tiempo sobre la magnitud de cada variable dependiente bioquímica y perceptiva. Se realizó una comparación post-hoc de Bonferroni cuando se alcanzó la significación en el test ANOVA. Se utilizó un ANOVA de medidas repetidas de una vía para comparar las variables mecánicas de velocidad. Para aquellas variables que resultaban no paramétrica, fueron utilizadas las pruebas de Friedman y Wilcoxon post-hoc. La significación estadística se fijó en $p \leq 0,05$.

Los resultados del presente estudio muestran que las variaciones absolutas de lactato y urea (comparaciones pre-post) fueron significativas para el tiempo ($p < 0,001$), aunque no se encontraron diferencias para la condición ($F = 1,49$, $p = 0,23$) o la interacción condición × tiempo ($F = 0,94$, $p = 0,34$). Para el marcador AST, se reveló una diferencia significativa en favor de HD en los valores post ($p = 0,03$). La prueba post-hoc reveló niveles significativamente más altos de AST para PLA en comparación con HD ($p = 0,02$).

Los resultados de las variables de velocidad fueron significativos para la velocidad media y la pérdida de velocidad máxima (rango de $p \leq 0,001$ a $0,05$) en favor de HD. Las comparaciones post-hoc de Bonferroni revelaron diferencias significativas entre HD y LD para la pérdida de velocidad máxima ($p = 0,008$) y casi se alcanzó entre HD y PLA para la variable de velocidad media ($p = 0,06$). La magnitud de las diferencias entre las distintas condiciones osciló entre insignificante y grande.

En cuanto al esfuerzo percibido, para el RPE-OB se registraron diferencias significativas para el tiempo ($F = 49,00$, $p < 0,001$), pero no para la condición ($F = 2,77$, $p = 0,07$) o la interacción condición × tiempo ($F = 1,339$, $p = 0,26$). Sin embargo, los análisis post-hoc de Bonferroni no revelaron diferencias significativas. Para el esfuerzo percibido local (RPE-AM), tanto la condición ($F = 9,19$, $p < 0,001$) como el tiempo ($F = 36,154$, $p < 0,001$) alcanzaron diferencias significativas, pero no la interacción condición × tiempo ($F = 0,553$, $p = 0,70$). Los análisis post hoc de Bonferroni mostraron diferencias significativas para todas las comparaciones temporales (rango de $p \leq 0,001$ a $0,002$) y para las comparaciones entre PLA y HD ($p = 0,004$) y entre HD y LD ($p = 0,02$). Por otro lado, no se encontraron diferencias significativas entre condiciones para el estado de recuperación percibido (PRS) ($F = 0,698$, $p = 0,46$).

Los resultados del presente estudio sugieren que una HD (2,5 mg) de suplementación de PC ingerida 45 minutos antes del ejercicio puede aumentar el rendimiento en SQ y reducir el daño muscular, así como el esfuerzo percibido periférico del cuádriceps en participantes entrenados en fuerza en comparación con una LD (0,625 mg) y PLA. Por lo tanto, el efecto ergogénico de la PC puede aparecer tras alcanzar un umbral "dosis-respuesta".

Tercer estudio: Efectos de la fenilcapsaicina sobre la oxidación de sustratos, el gasto energético y las respuestas metabólicas, perceptuales y de temperatura durante el ejercicio.

En este último estudio se recurrió a un diseño cruzado, aleatorizado, triple ciego y controlado con placebo para analizar los efectos de la PC sobre el gasto energético y la oxidación de sustratos, la temperatura corporal cutánea, la frecuencia cardiaca y las respuestas perceptivas en una prueba continua submáxima en cicloergómetro, así como en un test incremental hasta la extenuación. Este estudio se presenta con más detalle en el capítulo 4.

En este proyecto de investigación, los participantes acudieron al laboratorio cuatro veces, separadas por 72-96 h para garantizar una recuperación completa de la fatiga central y periférica entre sesiones. Antes de la sesión preliminar, los participantes se sometieron a una caracterización antropométrica (altura, masa corporal, % de músculo, % de grasa corporal) y sociodemográfica. A continuación, en una sesión preliminar, se realizó una prueba de ejercicio incremental submáxima seguida de una prueba incremental de esfuerzo máximo. La prueba de ejercicio submáximo se utilizó para determinar la MFO y los valores de potencia de pedaleo (W) asociados a la MFO (intensidad FATmax). La prueba de esfuerzo máximo evaluó el consumo máximo de oxígeno (VO2max) y la potencia de pedaleo máxima alcanzada durante

la prueba. Las tres sesiones experimentales fueron idénticas, diferenciándose únicamente en el suplemento ingerido (PLA, LD de PC y HD de PC), el cual se administró 45 min antes de la primera prueba en cicloergómetro. En cada sesión experimental, los participantes realizaron la prueba continua (60 min a FATmax) seguida de la prueba de esfuerzo máximo incremental (incrementos de 25 W cada min hasta el agotamiento). Las pruebas se realizaron respetando las mismas condiciones temporales y ambientales para cada participante.

Para garantizar la detección de diferencias, fueron incluidos en el estudio 17 varones físicamente activos. Los participantes se inscribieron en el estudio a través de un póster que se compartió en las redes sociales. Ninguno de los participantes declaró ninguna limitación física o de estado de salud que pudiera comprometer su rendimiento en las pruebas. Los participantes fueron instruidos para no realizar ningún tipo de ejercicio físico intenso durante los dos días anteriores a cada visita al laboratorio y de no consumir bebidas estimulantes o cualquier suplemento dietético dentro de las 24 h anteriores a cada sesión experimental. Para garantizar la fiabilidad intra e interindividual en las variables metabólicas como la oxidación de grasas (FATox) se estandarizó la ingesta dietética al menos 6 horas antes de las pruebas experimentales. Para ello, los participantes realizaron cada prueba con al menos 6 horas de ayuno antes del inicio de cada sesión y estandarizando la última comida antes del periodo de ayuno con 45 g de maltodextrina en polvo y 30 g de proteína en polvo.

En cuanto a los suplementos utilizados, el contenido de las cápsulas era el siguiente: una LD de 0,625 mg de PC, una HD de 2,5 mg de PC y un PLA compuesto de maltodextrina y excipientes. Los suplementos y el placebo se encapsularon y envasaron con etiquetas alfanuméricas para garantizar el cegamiento. Esto fue posible ya que un técnico independiente (es decir, no implicado en el estudio) preparó las cápsulas en las instalaciones de origen.

Se utilizó un ANOVA de medidas repetidas de dos vías (condición x tiempo) para analizar el efecto de la suplementación (LD, HD, PLA) a lo largo del tiempo sobre cada variable metabólica, de rendimiento y perceptiva dependiente. Se realizó una comparación post-hoc de Bonferroni cuando se alcanzó la significación del ANOVA. Se utilizó un ANOVA de medidas repetidas de una vía para comparar los efectos intra-prueba para cada etapa y para los valores metabólicos máximos de la prueba continua. Para las variables no paramétricas, se utilizó en su lugar la prueba de Friedman y las correcciones post-hoc de Wilcoxon. La significación estadística se fijó en $p \leq 0,05$.

Los niveles de lactato circulante, esfuerzo percibido general (RPE-OB) y de músculo activo (RPE-AM), así como de frecuencia cardiaca no reportaron diferencias significativas entre condiciones (rango $p = 0,08$ a $0,56$). Sin embargo, se encontraron diferencias

significativas para el tiempo en las variables de frecuencia cardiaca, RPE-OB y RPE-AM ($p < 0,001$). Los análisis post-hoc de Bonferroni revelaron diferencias significativas para todas las comparaciones temporales (pre, '30' y '60') en la frecuencia cardiaca, RPE-OB y RPE-AM (rango $p < 0,001$ a 0,004). Sólo se encontró interacción significativa condición x tiempo para RPE-OB debido a los valores más altos en LD en comparación con PLA ($d = 27$) y HD ($d = 0,27$).

No se detectaron diferencias significativas en la temperatura corporal cutánea ($p = 0,27$) ni en la frecuencia cardiaca media ($p = 0,24$) para los análisis de condición. Además, la oxidación máxima de carbohidratos, el gasto energético y la RER no difirieron entre condiciones (p osciló entre 0,10 y 0,77). Sin embargo, se encontraron diferencias significativas para la frecuencia cardiaca máxima ($p = 0,03$) y la MFO ($p = 0,05$), donde PLA alcanzó los valores máximos y la HD los más bajos. Sin embargo, el post-hoc de Bonferroni no reveló diferencias entre condiciones en ninguno de los resultados (p rango = 0,09 a 0,99; d rango = 0,20 a 0,31).

No se observaron diferencias significativas de condición para ninguno de los resultados medidos de la escala de American Society of Heating, Refrigerating, and Air Conditioning Engineers (ASHRAE) (p rango = 0,17 a 0,78; ηp^2 rango = 0,01 a 0,12). Sin embargo, se registraron diferencias significativas en el tiempo para todas las variables ($p < 0,001$; rango $\eta p^2 = 0,52$ a 0,73). Se encontró una interacción significativa condición x tiempo para la zona de la clavícula ($p = 0,04$; $\eta p^2 = 0,16$) debido al menor valor de HD en comparación con LD y PLA. Las demás zonas no mostraron interacciones condición x tiempo (rango $p = 0,12$ a 0,60; rango $\eta p^2 = 0,04$ a 0,12).

No se encontraron diferencias significativas para el área bajo la curva (AUC) del gasto energético (EE), FATox (oxidación de grasas) o CHOox (oxidación de carbohidratos) (p rango = 0,09 a 0,54; ηp^2 rango = 0,04 a 0,18). Tampoco se encontraron diferencias significativas por condición en la FATox ($p = 0,06$; $\eta p^2 = 0,14$), CHOox ($p = 0,19$; $\eta p^2 = 0,10$), EE ($p = 0,54$; $\eta p^2 = 0,008$), ni RER ($p = 0,21$; $\eta p^2 = 0,10$). No obstante, se revelaron diferencias significativas para el tiempo en todas las variables mencionadas ($p < 0,001$; ηp^2 rango = 0,54 a 0,79), pero no para la interacción condición x tiempo (p rango = 0,17 a 0,96; ηp^2 rango = 0,003 a 0,06). El análisis intra-prueba sólo mostró un efecto significativo sobre FATox en min 5 ($p = 0,005$; $\eta p^2 = 0,28$), 10 ($p < 0,001$; $\eta p^2 = 0,29$) y 55 ($p = 0,04$; $\eta p^2 = 0.\ 24$) para HD y LD; y para CHOox en min 5 ($p = 0,05$; $\eta p^2 = 0,25$) y RER ($p = 0,003$; $\eta p^2 = 0,25$) en min 5 a favor de PLA, pero no para ninguna otra variable en ninguna otra etapa. El análisis post-hoc de Bonferroni post-hoc informó de diferencias significativas en favor de HD y LD entre PLA/ HD a los 5 min (p =

0,002; d = 0,92), PLA/LD a los 5 min (p = 0,002; d = 0,74), PLA/HD (p = 0,002; d = 0,66) y PLA/LD a los 10 min (p = 0,002; d = 0,56) para FATox.

Ninguna de las variables (es decir, frecuencia cardiaca, lactato, RPE-OB al 30 y 90%, y RPE-AM) registradas durante la prueba de esfuerzo máximo difirió entre las condiciones experimentales (p osciló entre 0,011 y 0,915) excepto el RPE-OB al 60% debido a los valores más bajos de HD en comparación con LD y PLA (p = 0,05).

Los resultados de este último estudio sugieren que LD y HD de PC modulan la respuesta metabólica (FATox, CHOox y RER) al ejercicio y HD de PC reduce los valores de frecuencia cardiaca máxima durante el ejercicio aeróbico. Sin embargo, la PC sólo mejora las respuestas perceptivas (es decir, RPE-OB y percepción térmica de la clavícula) al ejercicio cuando se consume en HD.

Conclusiones

En conjunto, los hallazgos de la presente tesis doctoral demuestran a través de los tres proyectos de investigación realizados, que la suplementación con PC es una ayuda ergogénica nutricional eficaz para mejorar el rendimiento deportivo en el entrenamiento de fuerza y aeróbico. Estos resultados proporcionan un nuevo marco teórico para desarrollar futuros estudios que aborden los efectos crónicos y en nuevas poblaciones de este compuesto.

De acuerdo con las hipótesis planteadas en esta tesis doctoral y los objetivos específicos planteados, a continuación, se muestran los principales resultados y conclusiones. En primer lugar, la PC es capaz de aumentar el rendimiento físico debido a mecanismos periféricos y a la reducción de la fatiga mecánica y perceptiva aguda durante el ejercicio de fuerza. Debido a la mejora del rendimiento mecánico, tras la ingesta de PC se reduce la fatiga aguda post-sesión y el daño muscular el día posterior. En segundo lugar, aunque la PC es eficaz para mejorar el rendimiento en el entrenamiento de fuerza, las respuestas nerviosas durante el ejercicio no se ven aumentadas, lo que plantea la necesidad de futuros estudios que aborden los mecanismos ergogénicos periféricos subyacentes al efecto de esta sustancia. En tercer lugar, la PC aumenta las respuestas metabólicas al ejercicio aeróbico, modificando la contribución de los sustratos energéticos durante el ejercicio y dando lugar a una mayor contribución de la oxidación de grasas, así como a una reducción de la frecuencia cardíaca máxima.

Por lo tanto, el compendio de estudios que conforma esta tesis doctoral pone de manifiesto el plausible uso de la PC como ayuda ergogénica nutricional, lo que aporta una valiosa información para los profesionales del acondicionamiento físico, la salud y el rendimiento deportivo específico.

Contents

Symbols and abbreviations

1RM: One repetition maximum

ANOVA: Analysis of variance.

ASHRAE: American Society of Heating, Refrigerating, and Air Conditioning Engineers

AST: Aspartate aminotransferase

AUC: Area Under the curve

CHOox: Carbohydrate oxidation

CMJ: Countermovement jump

CV: Coefficient of variation

d: Cohen's *d* as a measure of the effect size in two-group comparisons.

EE: Energy expenditure

EFSA: European Food Safety Authority

EMG: Electromyography

F: Analysis of variance statistic

FATmax: cycling power values at maximal fat oxidation

FATox: Fat oxidation

FOpeak: Fat oxidation peak

HD: High dose

ICC: Intraclass correlation coefficient

LD: Low dose

MDF: Median frequency

MFO: Maximal fat oxidation

MIF: Maximal isometric force

MPS: Muscle protein synthesis

MPV: Mean Propulsive Velocity

ηp^2: Eta partial squared as a measure of the effect size

p: *p*-value of significance

PC: Phenylcapsaicin

PLA: Placebo

PRS: Perceived recovery status

RER: Respiratory exchange ratio

RFD: Rate of force development

RMS: Root mean square

RPE-AM: Active muscle ratings of perceived exertion

RPE-OB: Overall body ratings of perceived exertion

SQ: Back squats

TRPV1: Transient receptor potential vaniloid 1

VCO2: Carbon dioxide production

VL: Vastus lateralis

VLoss: Velocity loss

VM: Vastus medialis

VO2 peak: Oxygen uptake consumption peak

$VO2_{max}$: Maximal oxygen consumption

Table list

Figure list

Regulation and structure

According to the regulations approved and guaranteed by the Academic Committee of the PhD Program in Phyisical Activity and Sport and the Doctoral School of the University of Valencia, this doctoral dissertation is presented as a compilation of three scientific publications:

1. **Jiménez-Martínez, P.**, Sánchez-Valdepeñas, J., Cornejo-Daza, P. J., Cano-Castillo, C., Asín-Izquierdo, I., Alix-Fages, C., Pareja-Blanco, F., & Colado, J. C. (2023). Effects of different phenylcapsaicin doses on neuromuscular activity and mechanical performance in trained male subjects: A randomized, triple-blinded, crossover, placebo-controlled trial. *Frontiers in Physiology*, 14. https://doi.org/10.3389/fphys.2023.1215644

2. **Jiménez-Martínez, P.**, Cornejo-Daza, P. J., Sánchez-Valdepeñas, J., Asín-Izquierdo, I., Cano-Castillo, C., Alix-Fages, C., Pareja-Blanco, F., & Colado, J. C. (2023). Effects of different phenylcapsaicin doses on resistance training performance, muscle damage, protein breakdown, metabolic response, ratings of perceived exertion, and recovery: a randomized, triple-blinded, placebo-controlled, crossover trial. *Journal of the International Society of Sports Nutrition*, *20*(1), 2204083. https://doi.org/10.1080/15502783.2023.2204083

3. **Jiménez-Martínez, P.**, Alix-Fages, C., Janicijevic, D., Miras-Moreno, S., Chacón-Ventura, S., Martín-Olmedo, J. J., De La Cruz-Márquez, J. C., Osuna-Prieto, F. J., Jurado-Fasoli, L., Amaro-Gahete, F. J., García-Ramos, A., & Colado, J. C. (2023). Effects of phenylcapsaicin on aerobic capacity and physiological parameters in active young males: a randomized, triple-blinded, placebo-controlled, crossover trial. *Frontiers in physiology*, *14*, 1190345. https://doi.org/10.3389/fphys.2023.1190345

The details about the indexation of each journal and other activities of the doctoral candidate that justify this compendium are presented in Chapter 8. The justification of each of the included articles is presented in the section 1.7.

Chapter 1. Introduction.

1. Introduction

Dietary supplements are nutritional complements which ingested in conjunction with a healthy diet may improve health and/or sports performance (Maughan et al., 2018). By their own definition, these substances are legal and not banned by international agents such as the World Antidoping Agency (Maughan et al., 2018). Accordingly, these products are free-sale, which makes them highly popular between different athletic populations (Daher et al., 2022; Montuori et al., 2021). Within this context, amateur and professional athletes are well-known targets for the marketing of companies that sell these products (Montuori et al., 2021). These findings are also reinforced with the exponential growth of this industry in the last years, when a wide variety of new nutraceuticals have emerged (Kamiński et al., 2020; Piccolella et al., 2019). However, some of these new compounds are sold without strong scientific research supporting their effects (AlAli et al., 2021). On other occasions, these new compounds are based in new formulations of substances previously described in scientific literature without a direct testing of the new developments (AlAli et al., 2021). This topic will be discussed in the next sub-sections of this introduction, as well as in future chapters of this doctoral dissertation based on the approach of the supplement capsaicin and its new technological formulations.

1.1 A structural overview of capsaicinoids and capsinoids

Capsaicinoids are a group of compounds naturally found in spicy fruits like chili peppers (Luo et al., 2011). Due to their clinical and ergogenic potential applications, new scientific research has been conducted around these compounds in the last two decades (Arora et al., 2021; Hayman & Kam, 2008).The most abundant and well-documented capsaicinoid in chili peppers is capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide), a vanilloid-structured substance found in the placental tissue of *Capsicum* (Basith et al., 2016). The vanillyl moiety of capsaicin seems to be the responsible of its pungency as well as of some of its pharmacological effects in health, pain relief, weight loss and sports performance (Luo et al., 2011; Sharma et al., 2013).

Over the last decade, another group of capsaicinoids analogues called capsinoids has also been studied to reduce the discomfort generated when capsaicin is orally ingested (Lang et al., 2009; Luo et al., 2011). Capsinoids are primarily found in CH-19 Sweet peppers, a type of non-pungent peppers which have been genetically modified to eliminate the natural spiciness of capsaicinoids (Lang et al., 2009). Furthermore, although some structural similarities between both active principles can be mentioned (an aliphatic hydroxyl group in vanillyl alcohol with a

fatty acid), their central linkages are different, changing an amide moiety for an ester moiety, which elicits their main functional differences (Luo et al., 2011).

Both substances, capsaicinoids and capsinoids, interact with the transient receptor potential vanilloid 1 (TRPV1) (Hayman & Kam, 2008; Jiménez-Martínez, Ramírez-Campillo, et al., 2022), which exerts the most important physiological functions of these substances. TRPV1 agonists display an important role in the management of perceived heat and analgesia due to the downregulation of the voltage activated calcium channels and the reduction of inflammatory hyperalgesia states (de Moura e Silva et al., 2021). Although the effects of the stimulation of TRPV1 will be discussed in the next sub-section of this introduction (1.2), some concerns should be addressed previously. In this regard, capsinoids are well-documented as a poor bioavailable group of compounds (EFSA, 2012). Moreover, after their ingestion, capsinoids are rapidly metabolized and conjugated (EFSA, 2012), resulting in non-detectable circulating levels in the bloodstream (EFSA, 2012). By contrast, after their ingestion, different capsaicin formulations have reported a fast effect on different tissues, such as the small intestine, liver, and stomach (Turck, et al., 2019), which may be extrapolated to peripheral tissues such as the skeletal muscle. On the other hand, their specific interaction with TRPV1 is conflictive and should be pointed out (Sasahara et al., 2010). Although capsiates were able to provide a significant circulating concentration, their final binding with TRPV1 is approximately 1/10 compared to capsaicinoids such as capsaicin (Sasahara et al., 2010). This biochemical mechanism may be a secondary reason of the current differences reported in scientific literature between both types of compounds (Jiménez-Martínez, Ramírez-Campillo, et al., 2022). Therefore, it could be stated that based on a physiological perspective, capsaicinoids and mainly capsaicin, are the best options to evaluate the impact of TRPV1 modulators on physical performance.

1.2 Transient receptor potential vanilloid 1: more than a biochemical approach

In the previous sub-section, capsaicin was defined as a vanilloid-structured substance, which makes it possible to interact with the TRPV1 (Hayman & Kam, 2008). TRPV1 are receptors chemically composed by six transmembrane domains and a short pore-forming hydrophobic stretch that provides a similar configuration to other ion channels (Tominaga & Tominaga, 2005). This receptor belongs to the TRP family, which it is generally involved in the regulation of perception (Hayman & Kam, 2008). Furthermore, the lipophilic nature of capsaicin and its analogues provide some advantages for their interaction with this receptor (Tominaga & Tominaga, 2005). In this sense, capsaicin seems to be able to bind with the

cytosolic domain of TRPV1, something that may be explained by its ability to pass through the cell membrane until reaching the intracellular surface of TRPV1 (Baamonde et al., 2005). In addition, TRPV1 agonist drugs have exhibited improvements on several pathophysiological conditions, such as chronic musculoskeletal and neuropathic pain, gastrointestinal disruptions (e.g., gastroduodenal mucosal injury) and metabolic disorders (e.g., overweight) (Basith et al., 2016). On the other hand, sport performance seems to be enhanced through lower ratings of perceived exertion, reductions on discomfort and an increase on mechanical performance (e.g., total volume load) (Jiménez-Martínez, Ramírez-Campillo, et al., 2022; Taylor et al., 2016). In this regard, the physiological effects of capsaicin are mediated by reductions in inflammatory hyperalgesia, downregulating voltage-activated calcium channels, and influencing thermoception, being all of them common links between healthcare and performance fields (Baamonde et al., 2005; Fattori et al., 2016; Hayman & Kam, 2008).

Moreover, how TRPV1 specifically interacts with nervous system may also be detailed to understand the underlying mechanisms of capsaicin. Nervous system provides afferent and efferent feedback from the peripheral to the central nervous system and back to modulate human motor responses (Alix-Fages, Del Vecchio, et al., 2022). TRPV1 are presented in small diameter nerve fibers which are involved in the processing of nervous signals (Kaufman et al., 1982). Accordingly, in the peripheral nervous system TRPV1 are mainly found in III and IV afferent nerve fibers, a type of peripheral afferent fibers linked to the development of central fatigue by affecting both supraspinal and spinal levels of the nervous system during different tasks, as well as to the detection of a large variety of nociceptive stimuli (Alix-Fages, Del Vecchio, et al., 2022; Okano et al., 2015).

These afferent fibers are highly sensitive to metabolic changes, which also makes them possible to link the peripheral and central mechanisms of fatigue (Taylor et al., 2016). Accordingly, bearing in mind the close relationship between these fibers and the onset of discomfort, some responses related to exercise fatigue such as calcium overload, as well as the accretion and accumulation of metabolic waste subproducts during exercise, the modulation of peripheral nerve TRPV1 activity may be a biochemical target to improve sports performance (de Moura e Silva, Cholewa, Billaut, et al., 2021; Taylor et al., 2016). For instance, recent evidence points out that the infiltration of lumbar intrathecal fentanyl, a substance that provides afferent blockage, during an intermittent knee-extension exercise performed until exhaustion is effective attenuating afferents III and IV feedback (Broxterman et al., 2018). This blockage produced a large reduction on the perceived discomfort and exertion, as well as an improvement on physical performance (Broxterman et al., 2018). For this reason, this finding clearly suggests

that the metabolic responses to mechanical forces are detected by afferent nerves fibers contributing to the development of peripheral and central fatigue (Blain et al., 2016; Sidhu et al., 2017).

On the other hand, chronic training adaptations (i.e., exercise experience) modulate the tolerance of the nervous system to fatigue and discomfort, eliciting a higher ability to perform a task until exhaustion (Alix-Fages, Del Vecchio, et al., 2022). Nevertheless, although it could be hypothesized that capsaicin may provide different responses depending on the training experience of participants, current research has shown that capsaicin and its analogs modulate the neuromuscular responses to exercise in a similar extent in trained and untrained participants (Jiménez-Martínez, Ramírez-Campillo, et al., 2022). Therefore, afferents III and IV responses to parameters such as peripheral fatigue and metabolic stress or mechanical tension are generally expressed in humans fatigue without differences according to the fitness level or training experience of the participants when capsaicin is ingested (Carroll et al., 2017; Jiménez-Martínez, Ramírez-Campillo, et al., 2022; Taylor et al., 2016).

Finally, one explanation underlying the link between afferents and force production may be related to the firing behavior of these fibers during strenuous physical exercise (Boat & Taylor, 2017). During exercise, the aforementioned fibers increase their firing to spinal and supraspinal structures of the nervous system, which directly or indirectly inhibits motor neurons activity (Taylor et al., 2016). As a consequence, the neural drive finally received by the muscle fibers is impaired, resulting in lower rates of force production (Del Vecchio et al., 2018). Then, if the firing from afferents III and IV is reduced, lower levels of pain and discomfort during exercise will be manifested, which also preserves motor units' performance (Boat & Taylor, 2017). Note that once the action potential is propagated along the muscle fiber membrane and spread through the T-tubules, it provokes the release of the calcium ions from the sarcoplasmic reticulum after interacting with the dihydropyridine (that act as voltage sensors) and ryanodine receptors (González-Badillo & Gorostiaga, 1995). With the transient increase of calcium concentration in the muscle cell cytoplasm and its interaction with troponin, the crossbridge cycling is caused by the interaction between myosin and actin allowing the muscle contraction to produce force (González-Badillo & Gorostiaga, 1995). All these processes mentioned occur because of the neural stimulation, being the action potentials propagated by the axon of the motor neuron and initiated in the muscle fibers through the transmission produced in the neuromuscular junction by the release of acetylcholine neurotransmitters (González-Badillo & Gorostiaga, 1995). Hence, being the action potential the regulator of the excitation–contraction coupling (i.e., the series of events that happen from the generation of the action potential and

its propagation through the membrane of the muscle fibers to the beginning of the muscle force after calcium release), it could be stated that the nervous system controls muscle force (Alix-Fages, Del Vecchio, et al., 2022). In this sense, if motor neurons are working in a greater manner because of a lower afferent firing, muscle fibers are going to receive a higher degree of neural drive and this will improve the calcium release (Alix-Fages, Del Vecchio, et al., 2022).

Overall, the neuromuscular implications of TRPV1 and the link with neuromuscular fatigue must be highlighted when the onset of neuromuscular fatigue is discussed. Therefore, it could be hypothesized that from a physiological point of view, capsaicinoids and mainly capsaicin may be highly valuable substances to improve sports performance due to their agonistic TRPV1 activity (Jiménez-Martínez, Ramírez-Campillo, et al., 2022).

1.3 Phenylcapsaicin as a new technological analog

A common concern about the drug delivery profile of a substance is the bioavailability that it presents (Fernandes & Jozala, 2022). Bioavailability can be defined as a measure of the fraction of a drug that after entering in the body is able to bind successfully to its target domain or site of action (Fernandes & Jozala, 2022). Moreover, oral bioavailability is determined by several factors, such as drug permeability, dissolution rate, pre and first-pass metabolism and the hydrophilic or lipophilic nature of the drug (Gomez-Orellana, 2005). To improve the bioavailability of drugs, the pharmaceutical industry has documented some useful strategies that can be used. This includes the use of: (a) inactive prodrugs that human organism transforms in active drugs, (b) chemical medicine that optimizes oral absorption, (c) formulation strategies (i.e., solubility, hydrophilic ability, sustained release and “gastroretentive” systems) and macromolecules and biopharmaceuticals techniques (Gomez-Orellana, 2005).

In this regard, the use of oral capsaicin has been reported to be conflictive due to its pungent characteristics that can increase the risk of discomfort during exercise (Jiménez-Martínez, Ramírez-Campillo, et al., 2022). Therefore, the formulation of new capsaicin analogues that can reduce the optimal physiological dose seem to be a priority approach on this field. For this reason, during the last years a new group of synthetic analogues of traditional capsaicin known as “capsaicyns” has been developed (Turck et al., 2019).

Capsaicyns are characterized by providing a composition wherein R is alkyl, trifluoromethyl, cycloalkyl, phenyl, or halogen, and when the substituent R comprises a carbon chain, it is straight-chained or branched and optionally further substituted with alkyl, alkenyl, alkynyl, allyl, aryl, alkoxy, aryloxy, alkanoyl, aroyl, amino alkylthio, arylthio, cyano, cycloalkyl, cycloalkenyl, halo, hydroxy, oxo, nitro, or trifluoromethyl (Turck et al., 2019).

Among these substances it is found one of special interest called phenylcapsaicin (PC), which has emerged as an alternative to traditional oral purified capsaicin supplementation (Turck et al., 2019). PC is a microencapsulation of 98% of PC and 1-1.5% lipidic excipients and cellulose as primary metabolic vehicles (Turck et al., 2019). PC levels are fast presented in different tissues after it ingestion and the hepatic glucuronidation pass (Turck et al., 2019). For this reason, high concentrations of PC are observed after 30 minutes of it ingestion in the liver, small intestine, stomach and possibly in peripheral tissues such as the skeletal muscle (Turck et al., 2019). Within this context, the use of lipidic excipients as a vehicle of microencapsulation might reduce the risk of digestive system mucosa irritation while improving the bioavailability of capsaicin (Framroze, 2022). Therefore, given that purified capsaicin ergogenic dose is set around 12 mg consumed 45 min prior to exercise (de Freitas, Cholewa, Freire, et al., 2018), PC might exert a positive ergogenic effect reducing the ergogenic dose below 2.5 mg. The rationale of this hypothesis is in line with the current European Food Safety Authority (EFSA) experts panel opinion about the security of PC as a dietary supplement (Turck et al., 2019). In this sense, the current upper limit of PC as a dietary supplement is fixed in the aforementioned dose (i.e., 2.5 mg) (Turck et al., 2019).

1.4. Capsaicinoids and capsinoids on resistance training performance

Before the research intervention of this doctoral thesis was conducted, only 5 studies had evaluated the impact of capsaicinoids or capsinoids supplementation on resistance training outcomes in humans. The methodology employed in each of these studies is detailed in **Table 1**.

Table 1. Characteristic of the studies involving capsaicin supplementation or its analogues and resistance training interventions.

Study	Sample	Training status	Exercise protocol	Supplementation protocol	Main Outcomes
Walter et al. (2009)	20 (20M) 21.5±1.4 years	Recreationally active	Graded 25 W incremental test every 2 min until exhaustion 1-RM in bench press and leg press	Acute SUP: 200 mg caffeine, 33.34 mg capsaicin, 5 mg bioperine, 20 mg niacin CON: 175 calcium carbonate, 160 mg microcrystalline cellulose, 5 mg stearic acid, 5 mg magnesium stearate	Bench press (kg) Leg press (kg)
de Freitas et al. (2018)	10 (10M) 22.7±4.0 years	Young men. At least 1 year experience	4 sets of back squats until muscular failure with 70% 1RM/ 90 s rest.	Acute SUP: 12 mg of purified capsaicin CON: 50 mg of starch	Total weight lifted (kg) Total repetitions until failure RPE
de Freitas et al. (2019)	11 (11 M) 23.3 ±2.2 years	At least 1 year experience in resistance training	5-km intermittent run in 1:1 effort and pause ratio; 10 minutes passive rest and 4x70% 1RM back squats until failure.	Acute SUP: 2 dose of 12 mg of purified capsaicin CON: 2 dose of 12 mg of starch	RPE Total repetitions until failure Total weight lifted (kg)
Cross et al. (2020)	9 (5F/4M) 23.6±1.5 years	Recreationally active participants	120 maximal isokinetic knee extensions at 120º per second with passive flexion at 240º per second	Acute SUP: 1.2 mg of capsaicin containing gummy. CON: eucaloric placebo	Peak torque (N·m-1) Summed torque (N·m-1) Fatigue index (%)
de Moura et al. (2021)	20 (M) 18-30 years	Young untrained males	3 sets of 45º leg press, 3 sets of bench press until failure with 70% of 1RM and 90s rest between sets.	Chronic (6 weeks)/ mixed SUP: 12 mg of capsiate capsules CON: Starch capsules	Strength at 45 leg press (kg) Strength at bench press (kg) Weight lifted at 45 leg press (kg) Repetitions until failure at 45 leg press Weight lifted at bench press (kg) Repetitions until failure at bench press Peak power for 3 sets (W)

CON, Control; F, Female; M, Male; RPE: Rating of perceived exertion; SUP, Supplement; 1RM, 1Repetition maximum. Adapted from: Jiménez-Martínez, P., Ramirez-Campillo, R., Flandez, J., Alix-Fages, C., Baz-Valle, E., & Colado, J.C. (2023). Effects of oral capsaicinoids and capsinoids supplementation on resistance and high intensity interval training: A systematic review of randomized controlled trials. Journal of Human Sport and Exercise, 18(2), 375-389.

Overall, the findings of these studies suggest a positive impact of capsaicinoids and capsinoids on strength endurance until exhaustion and the perceptual responses to resistance training (de Freitas, Cholewa, et al., 2019; de Freitas, Cholewa, Freire, et al., 2018). In this sense, these substances provide a "counter-fatigue" effect driven by their analgesic improvement in pain and discomfort through their TRPV1 interaction (Knotkova et al., 2008; Lebovitz et al., 2012) and the enhancement of muscle contraction due to the increase of calcium release from the sarcoplasmic reticulum (Ito et al., 2013; Zhai et al., 2020). For instance, in a previous study (de Freitas, Cholewa, Freire, et al., 2018), the group that ingested purified capsaicin (12 mg) performed more repetitions in all the sets of a 4x70% of one repetition maximum (1RM) in a back squats (SQ) protocol until muscular concentric failure. This effect elicited a significantly increase in the total volume performed in the supplementation condition (measured as total weight lifted).

Moreover, the effects of capsaicin on strength endurance have also been reported when resistance training is performed imbedded in a concurrent protocol with high-intensity interval training (de Freitas, Cholewa, et al., 2019). In this experiment, resistance training consisted of the same protocol as in the previous study cited (de Freitas, Cholewa, Freire, et al., 2018) with the only difference of being completed after the interval exercise task. Therefore, 4 sets with 70% 1RM until concentric exhaustion were completed after 5-km of running at 1:1 work to rest ratio also performed until exhaustion. To date, this is the unique study in which a mono-ingredient (i.e., only composed of one ingredient) of capsaicin (12 mg) is assessed on concurrent training. Authors reported similar findings that those provided by their previous study (de Freitas, Cholewa, Freire, et al., 2018), exhibiting a benefit on strength endurance due to the reductions of perceived exertion during the sets and the overall session, as well as impacting positively on the total weight lifted.

Only one study have evaluated the chronic effects of a capsaicin analogue on resistance training performance (de Moura e Silva, Cholewa, Jäger, et al., 2021). In this study, significant effects were not reported for lower body strength, peak power, inflammatory response or total repetitions until failure (acutely or chronically) after 12 mg of capsiate (i.e., a type of capsinoid) supplementation. Nevertheless, fat-free mass changes were augmented significantly in the supplementation group. For this reason, two elements may have modulated the null response of the supplement on resistance training performance. First, it is possible that the use of a capsinoid could not stimulate TRPV1 to a required extent (EFSA, 2012). Second, if capsiates were able to stimulate TRPV1, this was done in a chronic way. It is known that long term TRPV1 agonists

use produce endocytosis and lysosomal degradation of TRPV1 (Sanz-Salvador et al., 2012). Thus, there is a potential mechanism which suggests that TRPV1 agonist supplements may only provide benefits with acute consumption or after wash-out periods.

In the other two studies, some critical limitations may be addressed. First, in Walter et al. (2009) the supplement ingested was a multi-ingredient formulation (200 mg caffeine, 33.34 mg of capsaicin, 5 mg piperine, 20 mg niacin) instead of a purify capsaicin or capsiate form. Besides, in Cross et al. (2020) participants consumed a low dose (1.2 mg) of capsaicin. Hence, the non-significant results reported in both studies may be biased by these two points.

Overall, capsaicin or its analogues supplementation seem to be safe and do not increase injury risk in exhaustion resistance training tasks (de Freitas, Cholewa, Freire, et al., 2018). For this reason, it could be stated that capsaicin can improve the tolerance to neuromuscular fatigue without a total disinhibition of nervous system alarm mechanisms.

1.5 Capsaicinoids and capsinoids on high-intensity interval training performance

The literature addressing the effects of capsaicinoids or capsinoids on high-intensity interval training is scarce. To date, only two experiments have evaluated this type of exercise under these substances' supplementation. These studies are detailed in **Table 2**.

Table 2. Characteristic of the studies involving capsaicin supplementation or its analogues and high-intensity interval training interventions.

Study	Sample	Training status	Exercise protocol	Supplementation protocol	Main Outcomes
Opheim et al. (2012)	19 (9M) 22.6±2.6 years	Experienced athletes	15x30-m sprints on 35 s intervals	One week SUP: capsaicin, 6 capsules of 4.3 mg) CON: 6 gelatin capsules of 500 mg of toasted wheat flour	Medium sprint time (s) Total sprint time (s)
de Freitas et al. (2019)	13 (13M) 24.4±4.0 years	Active men. At least 6 months experience	15 s at 120% of sVO2 peak/ 15 s of passive recovery until exhaustion	Acute SUP: 12 mg of purified capsaicin CON: 12 mg of starch	Time to reach 90% VO2 peak (s): Number of efforts performed RPE

CON, Control; F, Female; M, Male; RPE: Rating of perceived exertion; SUP, Supplement. Adapted from: Jiménez-Martínez, P., Ramirez-Campillo, R., Flandez, J., Alix-Fages, C., Baz-Valle, E., & Colado, J.C. (2023). Effects of oral capsaicinoids and capsinoids supplementation on resistance and high intensity interval training: A systematic review of randomized controlled trials. Journal of Human Sport and Exercise, 18(2), 375-389.

In the first study, Opheim et al. (2012) did not exhibit improvements in medium sprint time or total sprint time with 25.8 mg of capsaicin versus placebo. The selected protocol included a 15x30-m interval repeated sprint test and it was conducted in experienced athletes. The null effects reported in this study may contrast with those of the previous sub-section due to the time of force application and the rest differences of both types of tasks. While resistance training studies include efforts with a usual 90 s rest between sets performed until failure, sprints protocols used 35 seconds, which could modify the contribution of each energy system leading to different manifestations of fatigue (Wells et al., 2009). Other reason may be related to the higher gastrointestinal discomfort rates reported in this study (Opheim & Rankin, 2012). In this sense, a 13.5% of participants withdrew before the end of the intervention due to intestinal cramps, diarrhea, nausea and/or flatulence in the supplementation group. This finding is valuable because although in other research in which capsaicin was consumed in a high dose (Walter et al., 2009) discomfort was not reported, the use of a dose of 25.8 mg may impair sports performance.

Nevertheless, de Freitas et al. (2019) exhibited an enhancement in the number of efforts performed and time to reach 90% of the oxygen uptake consumption peak (VO2 peak) without changes in VO2 values after 12 mg of purified capsaicin supplementation in high-intensity interval exercise. The neuromuscular improvements of capsaicin increase in 13 extra efforts and 188 s extra time the task in the supplementation condition compared to placebo. Furthermore, overall-body ratings of perceived exertion (RPE-OB) was maximum in both groups, which suggests that capsaicin improves the total number of efforts until exhaustion or reduces the neuromuscular effort when volume is matched. However, in spite of the positive effect of capsaicin in peripheral neuromuscular performance, how it impacts on sport bioenergetics and central adaptations to exercise remain unclear.

1.6 Capsaicinoids and capsinoids: metabolic and bioenergetic effects

In previous sub-sections, it was described how capsaicinoids and capsinoids can modulate peripheral adaptations to exercise, however, the metabolic and bioenergetic effects of these substances are poorly studied in the current literature. Hence, an overview of the scarce scientific literature about this topic is provided in this sub-section.

Lipids comprise the main energy substrate that human body stores as an energy pool (Chait & den Hartigh, 2020). The use of lipids as energy fuel occurs due to a process named as beta oxidation (Duncan et al., 2007). Fatty acids are stored esterified in the form of triglycerides.

When adipose tissue detects biochemical signals like catecholamines release, these triglycerides are broken down during the lipolysis process, resulting in three free fatty acids molecules and a molecule of glycerol. Then, fatty acids are transported inside the mitochondria to be oxidized as energy fuel (Duncan et al., 2007). In some aerobic sports, the maximal capacity to oxidize fatty acids (MFO) efficiently may be an important factor in performance (Purdom et al., 2018). 2018). For this reason, some critical factors must be pointed out when MFO is discussed.

First, training level, primarily in trained athletes, is a strong moderator of MFO during long time exercise (i.e., more than 90 minutes) at moderate or low intensities (45-75% VO2 max) (Purdom et al., 2018). In these athletes, intramuscular fatty acids stores are increased and the metabolic respiratory thresholds shifted (Purdom et al., 2018). The underlying mechanisms that explain these adaptations are related to the chronic stimulation of different molecular pathways that increase (a) the content and density of mitochondria, (b) enzymatic efficiency (e.g., b-hydroxyacyl-CoA dehydrogenase), (c) the function of fatty acids transporter proteins, as well as (d) capillary density (Maunder et al., 2018; Purdom et al., 2018; Yeo et al., 2011). Moreover, sexual dimorphism also contributes to MFO due to the higher levels of AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor gamma co-activator-1α (PGC-1α) reported in women (Maunder et al., 2018). This can be explained because of the upper levels of estrogens presented in females in comparison to males (Chenevière et al., 2011; Maunder et al., 2018; Purdom et al., 2018). Finally, dietary factors such as the amount of dietary carbohydrates ingested can acutely modify the use of energy substrates during exercise (Yeo et al., 2011). However, low carbohydrates diets can impair sports performance due to reductions on the pyruvate dehydrogenase (PDH) enzyme activity (Volek et al., 2015; Yeo et al., 2011). Within this context, some nutritional ergogenic aids have demonstrated being able to increase fatty acid oxidation during physical activity. Among these substances, caffeine, teacrine, green tea extract and its polyphenols, as well as p-synephrine clearly enhance MFO (González-Cano et al., 2022; Gutiérrez-Hellín et al., 2020; Gutiérrez-Hellín & Del Coso, 2016). Nevertheless, although some potential mechanisms of TRPV1 agonists have been linked to an increase of free fatty acids for beta-oxidation, to date, there are several gaps in the use of capsaicinoids and capsinoids for this purpose (de Moura e Silva, Cholewa, Billaut, et al., 2021). Accordingly, research addressing capsaicinoids and capsinoids is mostly conducted in preclinical models, and human studies are poorly standardized in healthy athletes (Jiménez-Martínez, Ramirez-Campillo, et al., 2022).

In this regard, only two studies have reported the metabolic effects of a capsaicinoid or capsinoid on substrate oxidation in healthy individuals (Lim et al., 1997; Rossi et al., 2022). In

the first study, participants exhibited equal respiratory exchange ratios (RER) after ingesting 12 mg of capsiate supplementation during exercise performed at 70% of the maximal aerobic speed (Rossi et al., 2022). In the second study, a meal with 10 g of hot peppers (i.e., without standardizing the active agent) 2.5 hours prior to 1 hour of aerobic exercise at 60% $VO2_{peak}$ showed an increase in RER compared to placebo (Lim et al., 1997). Furthermore, other studies where these substances are evaluated do not provide reports of substrate oxidation (Santos et al., 2022). In addition, only one study has addressed substrate oxidation standardizing MFO, which is critical for a real objective evaluation of these variables (Amaro-Gahete et al., 2018, 2019; Osuna-Prieto et al., 2022). However, this study was conducted in overweight participants (Osuna-Prieto et al., 2022), which may not be extrapolated to other populations due to the reduced metabolic flexibility of this population (San-Millán & Brooks, 2018). In spite of these limitations, in the aforementioned study (Osuna-Prieto et al., 2022), 12 mg of dihydrocapsiate supplementation did not exhibited significant differences in fat oxidation (FATox), carbohydrate oxidation (CHOox), non-esterified fatty acids, energy expenditure, and skin temperature in sedentary overweight men.

Moreover, other metabolic and central variables such as cardiovascular responses to exercise or lactate circulating levels have not been evaluated after TRPV1 agonists ingestion in low or moderate aerobic exercise. Nonetheless, one recent study has shown that the supplementation with purified capsaicin does not alter heart rate during an incremental exercise test in cycloergometer until exhaustion at high intensity (i.e., above 85% of maximal work rate) (Giuriato et al., 2022). In addition, although preclinical research has shown that lactate is a potent endogenous inhibitor of TRPV1 activity (de la Roche et al., 2016), there is no current data that this mechanism is presented in humans during aerobic exercise.

Overall, the current research of the metabolic impact of capsaicin and its analogues on bioenergetic variables is poor and scarce, which may hinder their real applicability of these compounds in sports performance.

1.7 Aims of this doctoral research project

The main aim of this doctoral thesis research project was to assess the potential use of different doses of PC to enhance physical performance and to reduce mechanical, metabolic, biochemical, perceptual and electrophysiological fatigue in different exercise interventions. Before the start of this research project, two preliminary studies were performed to evaluate the supplementation patterns of trained athletes (González-Cano et al., 2022) and to systematically aggregate the impact of capsaicinoids and capsinoids supplementation in prior studies

(Jiménez-Martínez, Ramírez-Campillo, et al., 2022). With the development of the three studies of this doctoral dissertation, the descriptive, physiological and mechanical gaps presented in the introduction section have been assessed. Therefore, as it is shown in the sub-section 1.7.1, all the articles of this compendium doctoral thesis are justified to evaluate the aims of this project.

The specific objectives, which were assessed each one in one of the studies, are detailed hereunder in the order presented in this doctoral thesis:

1. Examine the effects of two doses of PC supplementation on mechanical (velocity, force and power) and neuromuscular [root mean square (RMS) and median frequency (MDF)] performance and fatigue outcomes on the squat exercise (velocity loss), as well as in a battery of strength and conditioning physical tests [countermovement jump (CMJ) and isometric squat] in young males.

2. Evaluate the impact of two doses of PC on the squat exercise performance, general and specific perceived exertion (RPE-OB and RPE-AM) muscle damage [aspartate aminotransferase (AST)], protein breakdown (urea), metabolic response (lactate), and 24 hours perceived recovery (PRS) in young males.

3. Assess the effects of two PC doses on aerobic capacity (VO2max), substrate oxidation (FATox, CHOox, RER), energy metabolism (energy expenditure) and exercise physiological variables (lactate, RPE, thermal perception and heart rate) in young males.

1.7.1 Hypotheses

All the current hypotheses that are going to be presented are based on the previous evidence that was presented throughout the introduction section of this doctoral thesis. Accordingly, it was hypothesized that:

1. PC would be able to increase physical performance due to peripheral mechanisms and to the reduction of acute fatigue during the exercise.

2. As a consequence of the greater intensity achieved, a higher level of mechanical, metabolic, biochemical, perceptual and electrophysiological fatigue would be expected in the post-exercise window after ingesting PC.

3. PC would increase the metabolic, cardiovascular and perceptual responses to aerobic exercise shifting the contribution of energy substrates during exercise.

These hypotheses are linearly developed in chapters 2,3 and 4 in a chronological order. Each chapter corresponds to one article of the present compendium. Particularly, the first study (Jiménez-Martínez, Sánchez-Valdepeñas, et al., 2023) was used to examine the neuromuscular

and mechanical responses to PC supplementation on resistance training, the second (Jiménez Martínez et al., 2023) to assess the perceptual, protein breakdown, mechanical and muscle damage responses to PC supplementation on resistance training, and the third (Jiménez-Martínez, Alix-Fages, et al., 2023) to evaluate the physiological and metabolic responses to PC supplementation on aerobic exercise.

Chapter 2. Effects of phenylcapsaicin on mechanical performance and neuromuscular activity.

Effects of different phenylcapsaicin doses on neuromuscular activity and mechanical performance in trained male subjects: A randomized, triple-blinded, crossover, placebo-controlled trial

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2. Effects of phenylcapsaicin on mechanical performance and neuromuscular activity

In the next three chapters (chapters 2, 3 and 4), the ergogenic use of PC in different exercise tasks is discussed. Accordingly, in this first study the mechanical and neuromuscular impact of PC on resistance exercise performance and fatigue is presented. Besides, this information is complemented in the next chapters.

Human voluntary movement and force production are determined by the nervous system behavior (Alix-Fages, Grgic, et al., 2022). During strenuous exercise, changes in neuronal circuitry (e.g., supraspinal structures excitability) lead to a decline in fatigue tolerance and sports performance (Alix-Fages, Del Vecchio, et al., 2022). In this regard, as it was explained in the introduction section, a heterogeneous group of substances known as capsaicinoids, which are found in chili peppers, have emerged as plausible ergogenic nervous system modulators (Jiménez-Martínez, Ramírez-Campillo, et al., 2022). As a vanilloid-structured substance, capsaicin interacts with the TRPV1 (Hayman & Kam, 2008). TRPV1 are receptors related to afferent feedback from III and IV nerve fibers, a type of peripheral afferent fibers that are linked to the detection of pain and the development of central fatigue by affecting both supraspinal and spinal levels of the nervous system (Alix-Fages, Del Vecchio, et al., 2022; Okano et al., 2015). In this regard, TRPV1 agonists display their main physiological functions through reductions in inflammatory hyperalgesia, downregulating of voltage-activated calcium channels, and influencing thermoception (Baamonde et al., 2005; Fattori et al., 2016; Hayman & Kam, 2008). Accordingly, some discomfort related responses to exercise, such as metabolites accumulation and calcium overload, are linked to III and IV afferent nerve fibers activity during exercise (de Moura e Silva, Cholewa, Billaut, et al., 2021; Taylor et al., 2016). Capsaicin supplementation is able to reduce the afferent signals of pain that are driven from the peripheral to the central nervous system, delaying the onset of fatigue in the neuromuscular junction (Jiménez-Martínez, Ramírez-Campillo, et al., 2022; Taylor et al., 2016). Consequently, the upregulation of TRPV1 lead to a decline in ratings of perceived exertion and the perception of pain, as well as discomfort during exercise (Jiménez-Martínez, Ramírez-Campillo, et al., 2022).

As it was previously presented PC is a capsaicin's synthetic analog composed of 98% of PC and excipients (Turck et al., 2019). PC pharmacokinetics has shown a fast metabolism (i.e., 30 minutes) after administration (Turck et al., 2019). For these reasons, although the PC ergogenic dose may be lower than traditional purified capsaicin, this remains to be verified. On

the other hand, capsaicin has demonstrated a positive influence on repetitions until failure, total volume load, and rate of perceived exertion during resistance exercise interventions (de Freitas, Cholewa, Freire, et al., 2018). Previous research has elucidated the effects of capsaicin on dynamic exercise (Jiménez-Martínez, Ramírez-Campillo, et al., 2022). In this regard, de Freitas et al., (2018) observed that 12 mg of capsaicin enhanced repetitions until exhaustion, total mass lifted, and the rate of perceived exertion of four sets at 70% of 1RM in the SQ of a double-blinded, randomized and placebo-controlled intervention. However, these previous studies have not addressed different concerns that are still present in the literature. First, previous research is based on strength endurance protocols (Jiménez-Martínez, Ramírez-Campillo, et al., 2022), which may not be extrapolated to other types of exercise tasks such as isometric contractions. In addition, the electromyographical mechanisms underlying the effects of capsaicin on resistance training performance and most of the mechanical outcomes used in the strength and conditioning field, which includes the assessment of mechanical fatigue, have not been evaluated yet (de Moura e Silva, Cholewa, Billaut, et al., 2021). Moreover, in these previous studies, submaximal (i.e., not performed until failure) intensities were not assessed (Jiménez-Martínez, Ramírez-Campillo, et al., 2022), which reduce the real applicability of capsaicin on physical conditioning because of the induction of excessive fatigue can disturb training adaptations (Pareja-Blanco, Alcazar, et al., 2020). Furthermore, most of the current research has not used objective measures of performance (e.g., infrared detection of jump height or linear velocity), which may hinder the estimate of the real impact of this substance on mechanical performance and fatigue (e.g., linear velocity loss) (Sánchez-Medina & González-Badillo, 2011). This new objective approach is presented in chapters 2, 3 and 4.

Besides, as capsaicin may produce an analgesic effect, its impact on direct muscle force production and electromyographical outcomes is relevant. To date, there are not previous data evaluating the influence of oral capsaicin on force production and neural responses during exercise. In addition, the electromyographical effects of capsaicin have only been evaluated for topical administration (Evans et al., 2021). In this study, topical capsaicin elicited significant changes in the motor unit recruitment pattern, which violated Henneman´s size principle in free-of-pain adults during voluntary trapezius and infraspinatus contractions (Evans et al., 2021). However, these neural responses have not been assessed during dynamic exercise after the ingestion of capsaicin, which makes difficult to extrapolate this finding to sport performance. In addition, this issue must be highlighted because of the neural strategies of the nervous system during exercise are reflected in force production during sports and in the induction of fatigue (Alix-Fages, Del Vecchio, et al., 2022). Concerning force production, none

of the previous studies have directly evaluated the effects of capsaicin on this variable. Although this can be easily addressed with the use of a force platform (Piqueras-Sanchiz et al., 2021), the information obtained from this approach can be useful to determine if this substance alters the amount, slope or time of force production. On the other hand, the "desensitizer" effect of capsaicin on a sport task may lead to a higher degree of fatigue in the post-exercise window. Nevertheless, the effects of capsaicin on mechanical recovery outcomes have not been assessed yet (Jiménez-Martínez, Ramírez-Campillo, et al., 2022). This issue must be pointed out because an acute increase of fatigue could lead to a reduction on force production and sport performance during the subsequent training sessions or competitions (Pareja-Blanco, Rodríguez-Rosell, et al., 2020). Overall, information about how capsaicin may modulate resistance training performance and fatigue, as well as the neural and mechanical mechanisms underlying these effects are still lacking in current research.

Therefore, this second study aims to examine the effects of two different doses of PC on fatigue and short-term mechanical responses, by measuring isometric and dynamic performance, as well as neural activity, in CMJ and SQ exercises. This study included for the first time the confluence of neural responses, dynamic and isometric exercise performance and mechanical fatigue after a capsaicinoid ingestion. It was hypothesized that PC may acutely increase velocity in the SQ exercise, neural excitability, and CMJ height. However, due to the improvements in performance, a higher degree of fatigue and a detrimental effect on recovery in the post-test measurements were also expected.

2.1 Materials and methods

2.1.1 Experimental design

This study was conceived as a randomized, triple-blinded, crossover, placebo-controlled trial. Two weeks before the beginning of the study, participants were anthropometric (body mass and height), 1RM and load-velocity relationship in SQ tested (see dynamic full squat test sub-section). Then, participants completed three experimental conditions, each one composed of a main session and a 24 hours second session (post-24h). The three experimental conditions were identical with the only difference of the supplement ingested. In addition, participants randomly ingested either a placebo (PLA) or a low (LD) or high (HD) dose of PC before the first weekly session. During the first weekly session, participants completed a SQ protocol that consisted of three sets of eight repetitions at 70% 1RM. Before (Pre), immediately after (Post), and 24 hours after (Post-24h) the SQ protocol, a battery of tests was conducted to

analyze the fatigue induced by each condition: CMJ, two SQ repetitions with 60% 1RM, and maximal isometric SQ at 90º, in that order. Since it is important to standardize temporalization to measure the acute responses, the tests were conducted in the following time points at Post: CMJ (one-minute post-exercise), SQ with 60% 1RM (two minutes post-exercise), and isometric SQ (three minutes post-exercise). Electromyographical assessment of each session was recorded while participants were performing the SQ tests. Participants performed each session at the same individual time of the day under stable environmental conditions (22-24 ºC and 55% humidity). The overall design of the study is depicted in **Figure 1**.

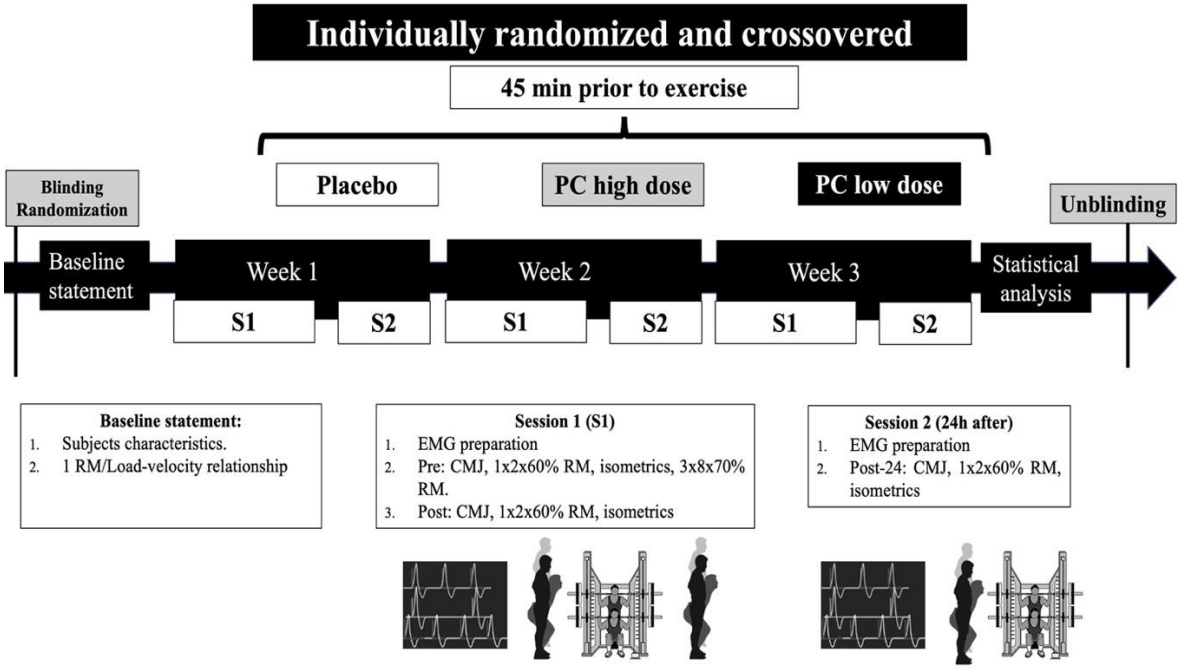

Figure 1. Timeline of the experimental protocol. PC, Phenylcapsaicin; CMJ, Countermovement Jump; EMG, Electromyography; 1RM, one-repetition maximum; S1/S2, Sesssion 1/2.

2.1.2 Participants

Sample size calculation was performed using the G*POWER software (Heinrich-Heine-Universität Düsseldorf, Germany) with an alpha value of 0.05. Statistical power was fixed at 0.80 and effect size at 0.60. Based on the total volume in the SQ exercise of previous research (de Freitas, Cholewa, Freire, et al., 2018), at least 21 participants were required for this study. Finally, twenty-five healthy men (age = 21.7 ± 3.7 years, body mass = 77.4 ± 9.1 kg, height = 176.7 ± 7.2 cm, 1RM in SQ = 125.6 ± 21.0 kg, 1RM normalized to body mass = 1.64 ± 0.22) with at least two years of experience in resistance training (range = 2-5 years) were enrolled to this study. If participants suffered from any cardiovascular, muscular,

neurological, and/or metabolic disorder, they were directly excluded. Once participants were informed about the aim of the study, procedures and possible risks, participants freely signed the informed consent sheet. The present research was approved by the Local Research Ethics Committee of Junta de Andalucía (Code: 0513-N-22) in accordance with the tenets of the Declaration of Helsinki. Each condition was established under the safety ranges proposed by European Food Safety Authority (EFSA) expert panel (Turck et al., 2019).

Participants were asked not to ingest stimulants (e.g., caffeine) or other ergogenic aids prior to each session, not to perform strenuous physical activity, and not to modify their dietary intake two days before the tests. One of the researchers reminded participants to maintain their normal dietary intake and not to train each one of the two days before the weekly testing sessions with a text message. During the three weeks of the study, two participants withdrew from the study. One of them due to injury and the other because of missing a session.

2.1.3 Procedures

Supplementation procedures

Supplements and placebo were prepared and packaged by a non-involved researcher in independent installations (Life Pro Nutrition industries, Madrid, Spain). To ensure the triple blinding, each package was encoded with a number from one to three. Packages were not unblinded until a third-party statistician performed all the analyses. Packages and capsules were identical in appearance, color, and taste, and their content was only revealed after the statistician independent researcher finished all the analyses. Capsules composition included: I) PLA: a maltodextrin and excipients placebo with a red dye; II) HD: 2.5 mg of PC (Axivite, Malmö, Sweden); and III) LD: 0.625 mg of PC. Randomization and crossover were performed two weeks before the beginning of the study. To reduce possible bias, a third-party researcher assigned participants to each condition with the Research Randomizer website (www.randomizer.org). Each participant consumed one condition per week during the three total weeks of the study. PC doses or placebo were ingested 45 minutes previous to the first exercise session. Researchers encourage participants to freely select a capsule from the daily assigned condition package. Capsules were taken with water under the supervision of at least one researcher. The randomized, counterbalanced crossover sequences of each week of the study were: eight HD, eight LD, nine PLA in week one; seven HD, ten LD, eight PLA in week two; ten HD, seven LD, eight PLA in week three. This information was pooled after unblinding.

Adverse effects were not reported for any of the supplementation conditions. Additionally, to ensure the absence of a maturation effect, order effect statistical analyses were also performed.

Electromyography

Before electromyography (EMG) recording, a researcher checked if participants were correctly shaved. Then, a black permanent marker was used to ensure consistency in electrodes position across conditions (Pareja-Blanco, Alcazar, et al., 2020). Surface EMG electrodes were placed over the vastus medialis (VM) and vastus lateralis (VL) of the right leg according to SENIAM criteria (Hermens et al., 2000) and previous research of the field (Ortega-Becerra et al., 2021; Piqueras-Sanchiz et al., 2021). EMG signals were evaluated for 60% and 70% 1RM sets and isometric tests in both sessions. EMG signals were recorded continuously with a bipolar, parallel-bar surface electromyographic wireless Trigno™ sensor (Delsys Inc, MA, USA) (r range = 0.92–0.99) (Poitras et al., 2019). Baseline noise was established on < five µV peak-to-peak and sampling rate was 1926 Hz. EMG system was set on an inter-electrode distance of ten mm, common mode rejection ratio >80 dB, and bandwidth filter between 20 and 450 Hz ± 10% (Delsys Inc, MA, USA). Data was stored using EMG works Acquisition software (Delsys Inc, MA, USA). For each measure, MDF [ICC (95% CI): 0.95 (0.90–0.98) and CV: 5.3%] and RMS [ICC (95% CI): 0.95 (0.90–0.98) and CV: 7.4%] were individually calculated for VM and VL as excitatory muscle activity assessments and averaged for further analyses according to previous research (Kattla & Lowery, 2010; Piqueras-Sanchiz et al., 2021). All the outcomes measured were recorded for each repetition (over sliding windows of 500ms with an overlap of 499ms) and averaged for further analysis for the dynamic and isometric tests. According to previous research, data were normalized under the daily maximal value of the first isometric signal (Piqueras-Sanchiz et al., 2021). Thus, EMG values were expressed as a percentage of the maximal daily value obtained (Hermens et al., 2000).

Resistance exercise protocol

Warm-up

A standardized warm-up was performed 30 minutes after capsule ingestion in the first session and immediately after participants arrived at the laboratory in the second session. All participants were inspected regarding EMG marks before the warm-up. The general warm-up consisted of five minutes of continuous running at nine $km \cdot h^{-1}$. Then, a specific warm-up was conducted before each test. The specific warm-up consisted of three sets of ten repetitions of bodyweight SQs followed by three progressive CMJs, and two maximal CMJs. After that three

SQ sets of two repetitions with 40%, 50%, and 60% 1RM were performed before the main SQ test.

Countermovement jump test

CMJ height was determined using an infrared timing system (OptojumpNext, Microgate, Bolzano, Italy) (r = 0.99) (Glatthorn et al., 2011). Participants were instructed in performing CMJs with their arms akimbo during eccentric and concentric phases. Accordingly, CMJ technique was established as about 90° of knee flexion, followed by a maximal vertical jump. For each attempt, the landing was required to be in an upright position without knees bending until the movement was completed. For each measurement, participants were required to perform two attempts separated by ten seconds and the mean value was calculated for further analyses [ICC (95% CI): 0.99 (0.97–0.99) and CV: 1.9%] (Piqueras-Sanchiz et al., 2021). If jump height difference was greater than two cm between trials, a third measurement was required and the two nearest values were averaged.

Dynamic full squat test

An initial test with increasing loads was performed before the start of the study for the individual calculation of the 1RM and the load-velocity relationship in the full SQ exercise (González-Badillo et al., 2017). A smith machine with no counterweight mechanism was used (Multipower Fitness Line, Peroga, Murcia, Spain). Mean propulsive velocity (MPV) was directly measured for each repetition with a linear velocity transducer (T-Force System, Ergotech, Murcia, Spain) (r = 0.99) (Sánchez-Medina & González-Badillo, 2011) attached perpendicularly to the barbell (González-Badillo & Sánchez-Medina, 2010). Although all the participants were experienced in all the tests performed as they had participated in at least two previous studies where these mechanical variables were assessed in the previous three months, the initial session was also used as familiarization. Accordingly, all the participants performed after the progressive loading test the isometrics and CMJs tests.

Regarding the progressive loading test, the initial load used was 30 kg and it was progressively increased in ten kg until reaching a mean propulsive velocity (MPV) of 0.50 $m \cdot s^{1}$ or lower. Then, the load was gradually increased (2.5–5.0 kg) until the repetition could not be completed. Three repetitions were completed for light loads (≥ 1.00 $m \cdot s^{-1}$), two for medium loads (1.00–0.80 $m \cdot s^{-1}$), and one for the heaviest loads (≤ 0.80 $m \cdot s^{-1}$). Rest periods were set in three minutes for light and medium loads and five minutes for heavy loads. Load-velocity

relationship was calculated with the best repetition of each attempt (i.e., highest MPV) (Piqueras-Sanchiz et al., 2021).

For the dynamic full SQ evaluation, two SQ repetitions with 60% 1RM were performed. The SQ was performed on a Smith Machine (Fitness Line, Peroga, Murcia, Spain) with participants starting from the upright position with the knees and hips fully extended, parallel feet and stance approximately shoulder-width apart, and the barbell resting across the back at the level of the acromion. Each participant descended in a continuous motion until the top of the thighs were below the horizontal plane, the posterior thighs and shanks making contact with each other (~35-40° knee flexion), then immediately reversed motion and raised back to the upright position. Unlike the eccentric phase that was performed at a controlled mean velocity (~0.50-0.65 $m \cdot s^{-1}$), participants were encouraged to always perform the concentric phase of the SQ at maximal intended velocity (Pareja-Blanco et al., 2014). The mean propulsive values of velocity (MPV) were acquired from a linear velocity transducer (T-Force System, Ergotech, Murcia, Spain) attached perpendicularly to the barbell. The highest values of each variable were recorded for further analysis. Resting between sets was fixed in two minutes.

Isometric squat test

Maximal isometric SQ test was performed on 90° of knee flexion position (180° = full extension) for elucidating the effects of PC on maximal isometric force (MIF) [ICC (95% CI): 0.99 (0.97–0.99) and CV: 3.4%] and maximal rate of force development (RFDmax) [ICC (95% CI): 0.94 (0.86–0.97) and CV: 13.8%] (Martinopoulou et al., 2022; Piqueras-Sanchiz et al., 2021). For this purpose, a Smith machine with customizable height supports was equipped with an 80 x 80-cm dynamometric platform (FP-500, Ergotech, Murcia, Spain). Participants were instructed to push with their legs against the floor of the platform as hard as possible after the cue "ready, set, go!". Participants were required to execute two 5-s attempts separated by one minute of rest per test. External forces of each attempt were collected at a sampling rate of 1000 Hz and processed with specific software (T-Force System, Ergotech, Murcia, Spain) (r = 0.99) (Sánchez-Medina & González-Badillo, 2011). For RFDmax assessment, the maximum slope in the force-time curve in 20-ms time intervals was selected. Furthermore, as RFD data was represented for different discriminable time gaps, RFD was calculated for the 0-50, 0-100, 0-150, 0-200, and 0-400 ms intervals. RFD and MIF outcomes were both averaged for further analysis. MIF was presented as the percentage of change from the pre-values. The specific

warm-up consisted of two submaximal attempts at 70% and 90% of maximal perceived exertion.

Full-squat protocol

The execution technique and setting have been described in the "Dynamic full squat test" sub-section. The SQ protocol consisted of three sets of eight repetitions with 70% 1RM with a two-minute rest period between sets. According to warm-up sets and individual load-velocity relationships, a 70% 1RM load was established daily for each participant. The MPV values for every repetition were recorded. Velocity loss (VLoss) induced within the set was calculated as the relative difference between the fastest repetition velocity and the last repetition velocity of each set (Sánchez-Medina & González-Badillo, 2011). The total volume load accumulated within the session was calculated as absolute load lifted (kg) x total repetitions.

2.1.4 Statistical analysis

The normal distribution of the variables and homoscedasticity were tested with Shapiro-Wilk and Levene's test, respectively ($p > 0.05$). A two-way repeated measures analysis of variance (ANOVA) (condition x time) with Bonferroni post-hoc was used to explore the effect of the interventions (LD, HD, PLA) across time on the magnitude of each dependent variable and to address whether an order effect is presented. A one-way repeated measures ANOVA was used to compare the total volume load. The Greenhouse-Geisser correction was applied when Mauchly's sphericity test was significant ($p \leq 0.05$). Statistical analyses were performed using the software package SPSS (IBM SPSS version 25.0, Chicago, IL, USA). Statistical significance was established at $p \leq 0.05$. To assess the magnitude of the differences, partial eta squared values (ηp^2) were obtained derived from ANOVAs and were interpreted as low (<0.04), moderate (0.04–0.13) and large (>0.13). Bonferroni post-hoc comparisons were used to evaluate pairwise differences. The effect size of the post-hoc comparisons was calculated by means of Cohen's d, which was interpreted as a low (<0.50), moderate (0.50–0.79), or large effect (>0.80) (Cohen, 1988).

2.2 Results

2.2.1 Electromyography

Descriptive values and statistical comparisons for EMG outcomes are presented in **Table 3**. Two-way repeated measures ANOVAs did not reveal any condition x time interaction (p range < 0.26 to 0.69). However, a significant time effect for RMS at 60% 1RM, MDF at isometric SQ, MDF at 60% 1RM, and MDF during the SQ protocol (p range < 0.001 to 0.006) was observed. Post-hoc Bonferroni for time only revealed significant differences at 60% load RMS for 1/2 sets (p = 0.01; d =1.11) and 2/ 3 (p = 0.006; d = 1.19) sets comparisons.

Table 3. Two-way repeated measures analysis of variance (ANOVA) comparing the electromyographical responses to the three different supplementation conditions.

Variable		Time	Condition			ANOVA		
			PLA	LD	HD	Condition	Time	Condition × time
Isometric (%)	RMS	Post	100.99 ± 23.72	101.31 ± 20.39	97.97 ± 35.00	F = 1.13; p = 0.33 ηp^2 = 0.05	F = 2.64; p = 0.11 ηp^2 =0.12	F = 0.37; p = 0.69 ηp^2 = 0.02
		Post-24	105.59 ± 19.10	127.49 ± 34.42	107.19 ± 24.37			
60% 1RM load RMS (%)		Pre	119.77 ± 24.11	125.20 ± 25.01	129.27 ± 39.11	F = 1.095; p = 0.35 ηp^2 = 0.06	F = 10.67; p <0.001* ηp^2 = 0.40	F = 1.14; p = 0.34 ηp^2 = 0.07
		Post	102.82 ± 29.83	123.39 ± 40.81	102.84 ± 35.18			
		Post 24	120.41 ± 31.13	132.88 ± 33.33	131.66 ± 26.60			
Isometric (%)	MDF	Post	98.09 ± 10.34	95.87 ± 6.93	97.04 ± 8.23	F = 1.16; p = 0.32 ηp^2 = 0.05	F = 9.51; p = 0.006* ηp^2 = 0.32	F = 0.49; p = 0.62 ηp^2 = 0.02
		Post-24	102.96 ± 15.81	95.95 ± 7.30	99.94 ± 8.14			
60% 1RM load MDF (%)		Pre	88.22 ± 8.42	91.72 ± 9.65	89.81 ± 9.45	F = 1.20; p = 0.88 ηp^2 = 0.01	F = 3.52; p = 0.006* ηp^2 = 0.18	F = 1.35; p = 0.26 ηp^2 = 0.08
		Post	87.50 ± 11.17	83.64 ± 6.36	88.98 ± 10.57			
		Post-24	95.51 ± 16.42	89.16 ± 10.84	90.93 ± 11.88			
SQ protocol RMS (%)		Set 1	88.89 ± 8.27	89.47 ± 10.05	90.69 ± 10.80	F = 0.85; p = 0.36 ηp^2 = 0.04	F = 3.42; p = 0.07 ηp^2 = 0.13	F = 0.43; p = 0.52 ηp^2 = 0.02
		Set 2	87.13 ± 8.30	87.09 ± 9.85	89.23 ± 11.11			
		Set 3	86.07 ± 8.80	85.91 ± 7.64	89.73 ± 10.77			
SQ protocol MDF (%)		Set 1	89.31 ± 8.74	90.55 ± 9.63	91.40 ± 9.93	F = 0.64; p = 0.54 ηp^2 = 0.03	F = 13.68; p <0.001* ηp^2 = 0.44	F = 1.23; p = 0.29 ηp^2 = 0.07
		Set 2	87.06 ± 8.85	87.97 ± 9.83	90.19 ± 11.39			
		Set 3	86.01 ± 8.32	86.14 ± 8.48	90.73 ± 11.06			

Mean ± standard deviation. PLA, Placebo; HD, High dose; LD, Low dose; RMS, Root mean square; MDF; Median frequency; VL, Vastus lateralis; VM, Vastus medialis. Post: post-exercise measure; Post24: 24-hours post-exercise measure. Isometric: values obtained from the isometric squat test; 60% 1RM load: values obtained from the 2 full-squat (SQ) repetitions against the 60% 1RM load; SQ protocol; values obtained from the SQ protocol, i.e., from 3 SQ sets of 8 repetitions with 70% 1RM load. * Significant difference ($p \leq 0.05$).

2.2.2 Countermovement jump test

Two-way repeated measures ANOVA revealed significant condition x time interaction for CMJ height ($p < 0.01$) and a condition main effect ($p < 0.001$) (**table 5**). Post-hoc Bonferroni tests showed that HD attained higher CMJ height than PLA ($p < 0.001$; d = 0.72) and LD ($p < 0.001$; d = 0.37).

2.2.3 Dynamic and isometric squat tests

Regarding the SQ test with 60% 1RM, the two-way repeated measures ANOVAs did not reveal significant condition × time interactions (p range = 0.74 to 0.95). However, significant time effect ($p < 0.001$) and condition effect ($p = 0.03$) for MPV was observed (**Table 4**). Moreover, the two-way repeated measures ANOVAs did not reveal significant condition × time interaction (p range = 0.09 to 0.89), or significant differences for time (p range = 0.06 to 0.78), or condition (p range = 0.19 to 0.97) for any of the isometric outcomes (**Table 4**).

Table 4. Two-way repeated measures analysis of variance (ANOVA) comparing the isometric mechanical responses to the three supplementation conditions.

Variable	Time	Condition			ANOVA		
		PLA	LD	HD	Condition	Time	Condition × time
MIF (%)	Post	0.88 ± 0.13	0.90 ± 0.12	0.92 ± 0.13	F = 0.57; p = 0.57	F = 0.005; p = 0.95	F = 0.20, p = 0.81
	Post-24	0.93 ± 0.17	0.92 ± 0.19	0.95 ± 0.17	ηp^2 = 0.045	ηp^2 = 0.001	ηp^2 = 0.02
RFDmax (N·s^{-1})	Pre	4,562.8 ± 1607.7	5,041.3 ± 1676.7	4,363.2 ± 1234.2	F = 0.03; p = 0.97	F = 0.30; p = 0.78	F = 1.46; p = 0.22
	Post	4,395.9 ± 1,689.3	3935.8 ± 1,153.0	4,678.5± 1,658.9			
	Post-24	4,894.2 ± 1601.3	4,075.7 ± 1337.7	4,752.2 ± 1585.1	ηp^2 = 0.07	ηp^2 = 0.12	ηp^2 = 0.06
RFD$_{0\text{-}50}$ (N·s^{-1})	Pre	2,262.4 ± 1,189.2	2,765.4± 1,725.4	2,544.1 ± 1,150.3	F = 1.28; p = 0.29	F = 0.78; p = 0.46	F = 1.55; p = 0.19
	Post	2,467.7 ± 1,396.8	2,335.8 ± 1,408.5	2,375.3 ± 1,172.7			
	Post-24	2.512,4 ± 1,332.5	2,006.3 ± 773.6	2,190.1 ± 1,249.6	ηp^2 = 0.08	ηp^2 = 0.05	ηp^2 = 0.10
RFD$_{0\text{-}100}$ (N·s^{-1})	Set 1	2,377.2 ± 1325.0	2,921,4 ± 1,353.3	2,585.6± 1,998.9	F = 1.73; p = 0.19	F = 1.14; p = 0.33	F = 2.43; p = 0.10
	Set 2	2,039.6 ± 1,283.3	2,320.4 ± 1,229.5	1,968.5 ± 1,373.4			
	Set 3	2,666.2 ± 1,432.5	2,129.3 ± 942.0	2,377.3 ± 1,196.7	ηp^2 = 0.11	ηp^2 = 0.07	ηp^2 = 0.14
RFD$_{0\text{-}150}$ (N·s^{-1})	Set 1	2,380.8 ± 1,655.6	2,958.4 ± 1,280.9	2.635,7 ± 1,075.2	F = 0.54; p = 0.58	F = 1.29; p = 0.28	F = 2.09; p = 0.09
	Set 2	2,670.8 ± 1,002.9	2.388.1 ± 1,184.6	2.129,5 ± 1,279.2			
	Set 3	2,600.7 ± 1,330.5	2,260.5 ± 1,032.9	2.450,5 ± 1,275.4	ηp^2 = 0.04	ηp^2 = 0.09	ηp^2 = 0.13
RFD$_{0\text{-}200}$ (N·s^{-1})	Set 1	2,400.9 ± 1,322.2	2,697.3 ± 1,219.1	2,456.9 ± 1,052.1	F = 0.28; p = 0.76	F = 2.56; p = 0.10	F = 1.69; p = 0.16
	Set 2	2,384.1 ± 918.5	2,178.3 ± 1,101.1	1,973.3 ± 1,214.8			
	Set 3	2,295.3 ± 1,430.7	2,067.3 ± 978.1	2.361,8 ± 1,214.6	ηp^2 = 0.02	ηp^2 = 0.15	ηp^2 = 0.10
RFD$_{0\text{-}400}$ (N·s^{-1})	Set 1	1,787.1 ± 974.8	1,764.9 ± 898.6	1,784.8 ± 809.4	F = 0.47; p = 0.62	F = 3.94; p = 0.06	F = 0.27; p = 0.89
	Set 2	1,652.5 ± 772.0	1,489.6 ± 733.9	1,521.3 ± 853,9			
	Set 3	1,631.8 ± 1,045.1	1,610.3 ± 773.9	1,664.4 ± 912.0	ηp^2 = 0.03	ηp^2 = 0.23	ηp^2 = 0.02

Mean ± standard deviation. PLA, Placebo; HD, High Dose; LD, Low dose; MIF; Maximal isometric force; RFDmax, maximal rate of force development; RFD0-50: rate of force development from the onset of force production to 50 ms; RFD0-100: rate of force development from the onset of force production to 100 ms; RFD0-150: rate of force development from the onset of force production to 150 ms; RFD0-200: rate of force development from the onset of force production to 200 ms; RFD0-400: rate of force development from the onset of force production to 400 ms.

2.2.4 Full-squat protocol

One-way repeated measures ANOVAs reported no significant differences between conditions for total volume load (F = 1.09, p = 0.35). The two-way repeated measures ANOVAs did not reveal significant condition × time interaction for any variable (p range = 0.12 to 0.98). However, it revealed significant time effects (p range = 0.001 to 0.008) for all the outcomes analysed. Moreover, a significant condition effect was observed for 60% load (p = 0.03), MPVmean (p = 0.02) and VLoss (p = 0.04) (**Table 5**). Bonferroni post-hoc revealed no significant differences for conditions ((p range = 0.06 to 0.07). Post-hoc Bonferroni for time analyses revealed significant differences between sets 1 and 2 for all outcomes (p range < 0.001 to 0.007; d range = 0.47 to 0.73). However, between sets 1 and 3, Bonferroni post-hoc time analyses reported significant differences for mean MPV (p < 0.001; d = 0.80). For comparisons between sets 2 and 3, all movement velocity outcomes reported significant time differences (p range < 0.001 to 0.04; d range = 0.21- 0.78). One-way ANOVA for intra-set analysis revealed significant differences in repetitions 13, 15, 16, 17, 23, and 24 (p range = 0.03 to 0.04). Post-hoc Bonferroni reported significant differences between HD and PLA in repetition 23 (p = 0.01; d = 0.65) and for HD and LD in repetitions 15 (p = 0.008; d = 0.50) and 16 (p = 0.004; d = 0.46) (**figure 2**).

Table 5. Two-way repeated measures analysis of variance (ANOVA) comparing the mechanical responses to the three supplementation conditions and one-way repeated measures ANOVA comparing the descriptive total volume of the squat protocol between the three supplementation conditions.

Variable	Time	Condition			ANOVA		
		PLA	LD	HD	Condition	Time	Condition × time
Total volume load (kg)		2,089.1± 357.6	2,014.6 ± 380.9	2,101.7 ± 352.9	F = 1.09; *p* =0.35		
60% load MPV ($m \cdot s^{-1}$)	Pre	0.91 ± 0.07	0.93 ± 0.06	0.93 ± 0.06	F = 3.94; *p* = 0.03*	F = 50.89; *p*<0.001*	F = 0.48; *p* = 0.74
	Post	0.77 ± 0.09	0.80 ± 0.08	0.81 ± 0.12			
	Post-24	0.89 ± 0.07	0.93 ± 0.09	0.91 ± 0.08	$\eta p^2 = 0.19$	$\eta p^2 = 0.75$	$\eta p^2 = 0.03$
MPV best ($m \cdot s^{-1}$)	Set 1	0.77± 0.04	0.78 ± 0.05	0.79 ± 0.06	F = 1.40; *p* = 0.14	F = 65.53; *p* <0.001*	F = 0.01; *p* = 0.90
	Set 2	0.72 ± 0.04	0.72 ± 0.07	0.74 ± 0.07			
	Set 3	0.70 ± 0.05	0.71 ± 0.07	0.73 ± 0.05	$\eta p^2 = 0.09$	$\eta p^2 = 0.73$	$\eta p^2 = 0.013$
MPV mean ($m \cdot s^{-1}$)	Set 1	0.65 ± 0.04	0.66 ± 0.07	0.68 ± 0.07	F = 4.14; *p* = 0.02*	F = 98.13; *p* <0.001*	F = 0.50; *p* = 0.77
	Set 2	0.60 ± 0.05	0.60 ± 0.07	0.64 ± 0.08			
	Set 3	0.57 ± 0.06	0.58 ± 0.08	0.61 ± 0.08	$\eta p^2 = 0.17$	$\eta p^2 = 0.83$	$\eta p^2 = 0.02$
VLoss (%)	Set 1	29.4 ± 8.5	29.7 ± 10.9	26.6 ± 9.4	F = 3.73; *p* = 0.04*	F = 5.43; *p* = 0.008*	F = 0.11; *p* = 0.98
	Set 2	29.9 ± 11.8	30.9 ± 10.2	26.6 ± 11.7			
	Set 3	34.2 ± 11.9	34.4 ± 12.2	29.8 ± 10.9	$\eta p^2 = 0.14$	$\eta p^2 = 0.21$	$\eta p^2 = 0.006$
CMJ height (cm)	Pre	39.94 ± 9.63	40.15 ± 9.61	40.18 ± 9.88	F = 97.10; *p* <0.001*	F = 2.82; *p* = 0.07	F = 3.48; *p* = 0.01*
	Post	32.92 ± 8.24	33.13 ± 8.29	34.73 ± 9.08			
	Post-24	39.07 ± 9.12	40.02 ± 9.68	39.71 ± 9.71	$\eta p^2 = 0.80$	$\eta p^2 = 0.10$	$\eta p^2 = 0.13$

Mean ± standard deviation. PLA, Placebo; HD, High Dose; LD, Low dose; Mean propulsive velocity; VLoss, Percentage of velocity loss during a set; Best, the highest value of each set; Mean, the mean value of all repetitions conducted in each set; CMJ, Countermovement jump. * Significant difference ($p \leq 0.05$).

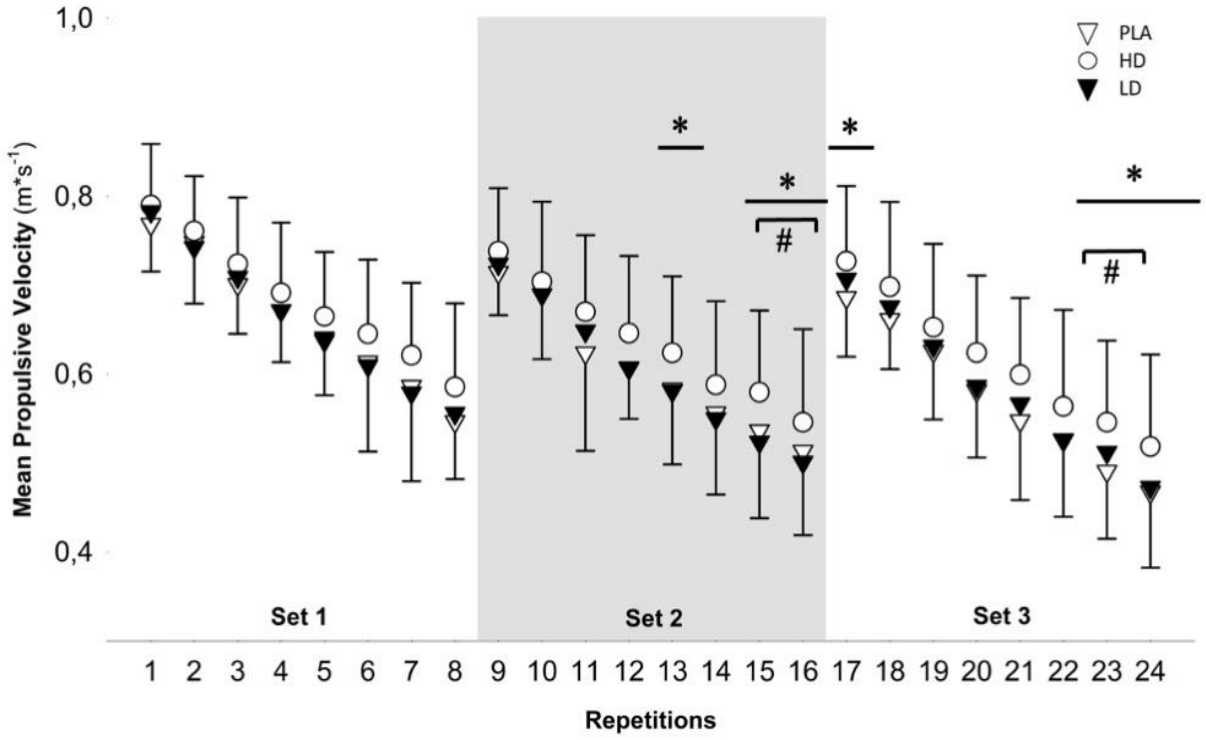

Figure 2. One-way repeated measures analysis of variance (ANOVA) of the intra-set comparisons of individual repetitions and repetitions across time for the different supplementation conditions (PLA, HD, LD). PLA, Placebo; HD, High dose; LD, Low dose. (* $p_{ANOVA} \leq 0.05$; # $p_{Bonferroni} \leq 0.05$).

2.3 Discussion

The objective of this second study was to explore for the first time the neuromuscular and mechanical responses to a capsaicinoid supplement in dynamic and isometric exercises. The main findings of this research indicate that a LD or HD doses of PC do not modulate the electrical signals of quadriceps muscles compared to PLA. However, HD condition attained higher velocity values during the main SQ test than LD and PLA. Furthermore, HD condition also exhibited higher CMJ values across time compared to PLA and LD. Supporting our initial hypothesis, PC may produce an ergogenic effect on SQ performance and may improve mechanical recovery outcomes (e.g., CMJ height and post-test lifting velocity). On the other hand, the initial hypothesis of a nervous system modulator effect was rejected according to the collected electrical signals. Therefore, HD of PC supplementation may attenuate mechanical fatigue after a submaximal high-intensity session. These effects on fatigue outcomes may not be mediated by the traditional neural mechanisms proposed in previous literature (Jiménez-Martínez, Ramírez-Campillo, et al., 2022).

In the present study, changes in the electrical signals were not detected for RMS or MDF between PLA and LD or HD. However, the protocol was effective inducing fatigue due to the reduction of the intensity of the electrical signals across the SQ sets. In this regard, increments in RMS may be explained by a higher degree of motor unit recruitment, higher firing rates, or the recruitment of higher-threshold motor units when greater electrical outputs are needed (Bigland-Ritchie et al., 1986; Hunter et al., 2004). By contrast, lower MDF values are highly correlated with a decline in force production from the fresh state due to impairments in neural conduction velocity and discharge rates of motor units (Allison & Fujiwara, 2002). Thus, during and after high-intensity exercise, EMG can reflect the changes in neural strategies of the muscle related to the increase in the metabolic activity, hydrogen ions accumulation and other physiological events inducing fatigue (Mendez-Villanueva et al., 2008). Contrary to the present findings, previous research has documented that topical capsaicin administration may alter Henneman´s size principle (De Luca & Contessa, 2012) eliciting a greater number of motor units recruited in upper-limbs isometric contractions (Evans et al., 2021). Nevertheless, the effects of an oral capsaicin supplement on electrical muscle signals have not been evaluated previously. In addition, although mechanical performance was higher in the HD of PC condition (i.e., lower velocity loss in all the sets), the null changes on electrical signals may be interpreted bearing in mind that other physiological mechanisms may be presented in the ergogenic effect of PC supplementation (de Moura e Silva, Cholewa, Billaut, et al., 2021). Furthermore, if a direct neural mechanism would have appeared improving the neural output, HD may have attained higher RMS values during and in the post-squat window (Alix-Fages, Del Vecchio, et al., 2022), which suggests that PC does not provide an ergogenic effect on the acute recovery of the neural output. For this reason, PC may exert its main effects directly in the muscle junction upregulating the efflux of calcium channels and the acetylcholine turnover without a direct effect on the neural raw output (de Moura e Silva, Cholewa, Billaut, et al., 2021).

Regarding mechanical outcomes, the present results have not displayed an ergogenic mechanical effect of PC on post-hoc comparisons of velocity outcomes, although CMJ height and intra-set lifting velocities were reported significant in the post-hoc comparisons. However, as velocity were higher in both PC conditions, a plausible delaying effect on neuromuscular fatigue may have been presented (i.e., lower velocity loss in all the sets and higher CMJ height). Within this context, previous research has focused on the effects of capsaicin supplementation on strength endurance tasks performed until exhaustion (Jiménez-Martínez, Ramírez-Campillo, et al., 2022). For instance, a four sets at 70% 1RM until failure SQ protocol

documented an ergogenic response to 12 mg of purified capsaicin in comparison to a placebo (de Freitas, Cholewa, Freire, et al., 2018). In this study, authors reported higher number of repetitions until exhaustion, and consequently, a rise in total mass lifted. However, the effects of capsaicin on mechanical fatigue were only approached by the inter-set analysis of the number of repetitions in the SQ exercise. Moreover, the effects of capsaicin on a non-exhaustive task after an SQ exercise have not been assessed previously (de Moura e Silva, Cholewa, Billaut, et al., 2021; Jiménez-Martínez, Ramírez-Campillo, et al., 2022). Accordingly, these previous protocols may provide a low applicability on the strength and conditioning or sports performance fields due to the high levels of fatigue induced (Pareja-Blanco, Rodríguez-Rosell, et al., 2020). Nevertheless, according to the current results and previous research, capsaicin may improve efforts performed near to muscular failure due to the regulation of TRPV1 in the context of high degrees of metabolic fatigue during exercise (de Freitas, Cholewa, et al., 2019; de Freitas, Cholewa, Freire, et al., 2018), due to the responses of III and IV nerve afferent fibers (Taylor et al., 2016). For this reason, it could be hypothesized that the differences reported between the current study and previous research may be explained by the fact that in the present study volume was matched. This is corroborated by the growing gap reported between lifting MPVs in the intra-set analysis, where most of the significant differences were found in favor of the HD condition at the end of the last repetitions and mainly in the last set.

Concerning the influence of HD on mechanical recovery, the two dynamic indicators of fatigue, (i.e., lifting MPV with 60% 1RM and CMJ height), exhibited higher values in the post exercise window in favor of HD. According to previous literature, SQ dynamic strength (Nuzzo et al., 2008) is highly correlated with CMJ height (Bauer et al., 2019). Consequently, these effects on CMJ performance may have been mediated by the less mechanical fatigue accumulated according to the % of VLoss reported in the HD group. These findings are meaningful due to the reported improvements in the mechanical outcomes can be associated to a 2.5% enhancement of force production (Sánchez-Medina et al., 2017). Nonetheless, in both dynamic mechanical recovery variables, the post-24 values returned near the baseline, which suggests that this mechanical effect may be only considered during the first hours of the acute time course recovery window (Pareja-Blanco et al., 2019). Regarding isometric testing, as it was aforementioned, the null effects of PC on MIF and RFD outcomes may be explained by the lower metabolic demands across the time required in isometric tasks (Wells et al., 2009). Accordingly, previous research did not report performance improvements from a low dose of capsaicin in knee-extension isokinetic exercise when the range of motion was restricted (Cross

et al., 2020). Thus, PC's TRPV1 activity may enhance resistance training during dynamic exercises but not in short-duration isometric tasks. Moreover, this rationale could also be the cause of the contrary results observed between dynamic fatigue and isometric fatigue in the present study, given that afferent III and IV nerve fibers mainly detect metabolic discomfort during exercise (Alix-Fages, Del Vecchio, et al., 2022; Collins et al., 2018). Within this context, exercise "perceived pain" could be explained as a manifestation of these nerve fibers firing, which is a biochemical target of PC supplementation, and leads to a lesser extent efficiency in the neuromuscular junction (e.g., regulation of calcium overload) reducing the velocity of crossbridge cycling, a mechanism directly involved in dynamic contractions (Collins et al., 2018; de Moura e Silva, Cholewa, Billaut, et al., 2021; Jiménez-Martínez, Ramírez-Campillo, et al., 2022). Additionally, the lack of a dose-response effect in most of the variables studied (i.e., isometric and electrical) may be explained by the existence of a threshold in TRPV1 peripheral activation, which is in line with the different doses of capsaicin employed in previous literature (Jiménez-Martínez, Ramírez-Campillo, et al., 2022). In this sense, the current HD dose of PC may correspond to the most used evidenced ergogenic dose of capsaicin due to its near five-fold higher bioavailability (Framroze, 2022; Jiménez-Martínez, Alix-Fages, et al., 2023; Jiménez-Martínez, Ramírez-Campillo, et al., 2022; Turck et al., 2019).

Collectively, an acute dose of HD (i.e., 2.5 mg) of PC may reduce mechanical fatigue (e.g., higher CMJ height) after submaximal resistance exercise, as a consequence of a positive effect on the mechanical performance (i.e., higher MPV values and lower % of VLoss) compared to a LD and PLA. Therefore, PC may be a tool for high-volume resistance exercise workouts. Finally, this study presented important strengths such as a triple-blind, crossover, placebo-controlled design and as previous research suggested (de Freitas, Cholewa, Freire, et al., 2018), the enrollment of trained participants. Nevertheless, some limitations may be pointed out. First, nutritional follow-up of participant's diet and supplementation was not directly registered. Secondly, this study was conducted in trained men under laboratory conditions, for this reason, the present finding may be cautiously interpreted in other populations and environments. Moreover, bipolar surface EMG might not detect the contractile properties of the muscle in isolation. Accordingly, future research may add more accurate measures such as high-density EMG for the assessment of the neural effects of PC. Finally, ratings of perceived exertion or perceived pain were not evaluated in this study which may be useful in future studies to understand the sensitivity mechanisms underlying the supplementation with capsaicin. On the other hand, it would be valuable to determine the impact of PC in protocols

where exercise volume was not matched. In addition, sets may be performed until exhaustion and a force platform and linear transducer may be used.

2.4 Conclusion

The results of the present study suggest that acute PC ingestion may be considered as ergogenic aid (2.5 mg) for dynamic resistance exercise sessions when more than one exercise is performed. Consistently, mechanical fatigue after a submaximal exercise may be delayed and attenuated by PC. In addition, further research must deeply examine if neural mechanisms underlying capsaicinoids ergogenic impact exist.

Chapter 3. Effects of phenylcapsaicin on muscle damage, protein breakdown, recovery and perceptual responses.

Effects of different phenylcapsaicin doses on resistance training performance, muscle damage, protein breakdown, metabolic response, ratings of perceived exertion and recovery: a randomized, triple-blinded, placebo-controlled, crossover trial

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3. Effects of phenylcapsaicin on muscle damage, protein breakdown, recovery and perceptual responses

As the neuromuscular responses to PC supplementation were evaluated in the previous study (Jiménez-Martínez, Sánchez-Valdepeñas, et al., 2023), the present study was focused on the impact of PC supplementation on resistance training biochemical and perceptual responses. Furthermore, in the previous study it was found a positive effect of PC on dynamic mechanical performance (i.e., higher velocity with a matched load) and mechanical fatigue (i.e., lower CMJ height loss across time). However, the physiological mechanisms underlying these effects were not reported before under reliable conditions in scientific literature.

Within this context, as supplements are popular ergogenic aids among athletes aiming to improve their sport performance (González-Cano et al., 2022; Maughan et al., 2018), capsaicinoids have emerged as a plausible ergogenic aid for strength conditioning and high-intensity sports (de Freitas, Cholewa, Freire, et al., 2018; Jiménez-Martínez, Ramírez-Campillo, et al., 2022). As it was described in the introduction section, capsaicinoids are a group of compounds naturally found in spicy chili peppers which are characterized by their vanilloid structure (Lang et al., 2009). Capsaicin (8-methyl-N-vanillyl-trans-6-nonenamide), which is found in the placental tissue of *Capsicum* fruit, has emerged as the primary and most abundant capsaicinoid with therapeutic and physical performance relevance (de Freitas, Cholewa, Freire, et al., 2018; Sharma et al., 2013). In this sense, capsaicin has exhibited improvements on several pathophysiological conditions, such as chronic musculoskeletal and neuropathic pain, gastrointestinal disruptions (e.g., gastroduodenal mucosal injury) and metabolic disorders (e.g., overweight) (Basith et al., 2016; Framroze, 2022). On the other hand, capsaicin seems to enhance sport performance by reducing ratings of perceived exertion and perceived discomfort while improving mechanical performance (e.g., total volume load) (Jiménez-Martínez, Ramírez-Campillo, et al., 2022; Taylor et al., 2016).

The aforementioned physiological effects of capsaicin have been associated to the TRPV1 (de Moura e Silva, Cholewa, Billaut, et al., 2021). TRPV1 content is specially high in afferents III and IV nerve fibers, which are linked to peripheral and central fatigue during high-intensity tasks (Alix-Fages, Del Vecchio, et al., 2022; Alix-Fages, Grgic, et al., 2022; Collins et al., 2018). The feedback from afferents III and IV could directly or indirectly reduce motoneuron firing and motor unit recruitment (Alix-Fages, Del Vecchio, et al., 2022; Alix-Fages, Grgic, et al., 2022). Thus, as a TRPV1 agonist, capsaicin could improve muscle contraction by increasing the perceived heat-analgesia and the release of calcium from the

sarcoplasmic reticulum, which are linked to motor units function (Ito et al., 2013; Jiménez-Martínez, Ramírez-Campillo, et al., 2022; Zhai et al., 2020).

To date, capsaicinoids supplementation has mainly been tested on sports performance with encapsulated capsaicin formulations (Jiménez-Martínez, Ramírez-Campillo, et al., 2022). Purified acute (i.e., 45 minutes before exercise) oral capsaicin supplementation has demonstrated an ergogenic effect in upper- and lower-limbs resistance training tasks (da Silva et al., 2022; Jiménez-Martínez, Ramírez-Campillo, et al., 2022). For instance, de Freitas et al. (de Freitas, Cholewa, Freire, et al., 2018) in a double-blind, randomized placebo-controlled trial found that acute 12 mg of capsaicin increased significantly the number of repetitions until failure, the total weight lifted, and reduced RPE-OB in SQ with a not-volume-matched design. However, although the effects of capsaicinoids have been tested in well-controlled conditions (Jiménez-Martínez, Sánchez-Valdepeñas, et al., 2023), acute muscle damage, protein breakdown, and recovery variables have not been assessed in response to capsaicin supplementation yet. As intense exercise may increase metabolic, biochemical, and neuromuscular fatigue in acute and short-term ways (Izquierdo et al., 2010; Taylor et al., 2016), exploring the biochemical, perceptual, and neuromuscular effects of capsaicin on these topics may also be relevant for researchers and practitioners.

Besides, although the TRPV1 activity of capsaicinoids may be linked to their spiciness (Basith et al., 2016; Sharma et al., 2013), encapsulated capsaicin has not got a direct pungent taste. However, previous research has reported intestinal discomfort after high-dose oral encapsulated-capsaicin supplementation (25.8 mg) (Opheim & Rankin, 2012), which suggests that capsaicin may be irritating, in spite of the vehicle of administration used. For this reason, in agreement with the previous study (Jiménez-Martínez, Sánchez-Valdepeñas, et al., 2023), PC was also used in this study as an alternative to traditional oral purified capsaicin supplementation (Turck et al., 2019). Furthermore, as it was explained in the introduction section, PC is rapidly metabolized through hepatic glucuronidation, which presents high levels of PC in tissues such as the small intestine, stomach, and liver after 0.5 hours of ingestion (Turck et al., 2019). Therefore, it is supposed that microencapsulation might entail a lower pungency power, less digestive system mucosa irritation, and potentially a higher bioavailability (Framroze, 2022; Turck et al., 2019). Therefore, given that purified capsaicin ergogenic dose oscillates around 12 mg (de Freitas, Cholewa, Freire, et al., 2018), as it was corroborated in the previous study, the ergogenic PC is lower (Jiménez-Martínez, Sánchez-Valdepeñas, et al., 2023) (i.e., less than 2.5 mg). This reasoning may be aligned with current European Food Safety Authority (EFSA) PC permitted limits for dietary supplements (i.e., 2.5

mg) (Turck et al., 2019).

Overall, the aim of this study was to explore the effects of a LD and HD of PC on lower-limbs performance in the SQ exercise under velocity-based control, metabolic responses to exercise, acute biochemical muscle damage and protein breakdown, RPE, and perceived recovery in resistance-trained men. These assessments were approached under a randomized, triple-blinded, placebo-controlled, crossover design. It was hypothesized that PC may exert a positive impact in a dose-response way on velocity outcomes and RPE. However, a concomitant increment of perceived fatigue, protein breakdown, and muscle damage would have appeared after PC supplementation due to the increase in physical performance. It was expected that impairments in performance as well as muscle and protein damage were higher in the HD condition.

3.1 Materials and methods

3.1.1 Participants

Twenty-five healthy men (age = 21.0 ± 2.2 years, body mass = 76.5 ± 9.5 kg, height = 176.4 ± 7.5 cm, SQ 1RM normalized to body mass = 1.66 ± 0.22) enrolled voluntarily to this study. Of the total sample, two participants dropped out of the study, 1 due to causes not related to the study and the other after the placebo session. All participants were resistance-trained men with at least 2 years of experience (experience= 3.61 ± 1.43 years). Exclusion criteria comprised cardiovascular, neurological, physical and/or metabolic disorders that may disturb the primary outcomes.

One week before the beginning of the study, SQ strength and anthropometric measurements (i.e., body mass and height) were tested for all participants. Participants were asked not to consume alcohol, caffeine, or other ergogenic aids. Besides, they could not to perform intense exercise or modify their macronutrients distribution, calorie intake and food selection 24 and 48 hours before each session. The experimental protocol was explained before the informed consent and sample collection agreement was signed prior to the first experimental session. The study protocol adhered to and respected the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of Pablo of Olavide University (Code: 0513-N-22).

3.1.2 Experimental design

A randomized, triple-blinded, placebo-controlled crossover trial was used to explore the effects of PC on resistance training performance, muscle damage, and metabolic responses. Participants attended the laboratory twice per week for a total study duration of 3 weeks. Each week of the study consisted of a main experimental session and a follow-up session. For each condition, six capillary blood extractions, a warm-up, a SQ testing protocol, and a 24-hour recovery and muscle damage follow-up session were performed.

The order of the interventions was randomized for all participants by an external researcher in a balanced way before the beginning of the study in order to reduce training bias risk. The Research Randomizer website (www.randomizer.org) was used. All procedures were completed at the same time of the day and under stable environmental conditions (22-24 ºC and 55% humidity) for each participant. The overall design of the study is depicted in **Figure 3**.

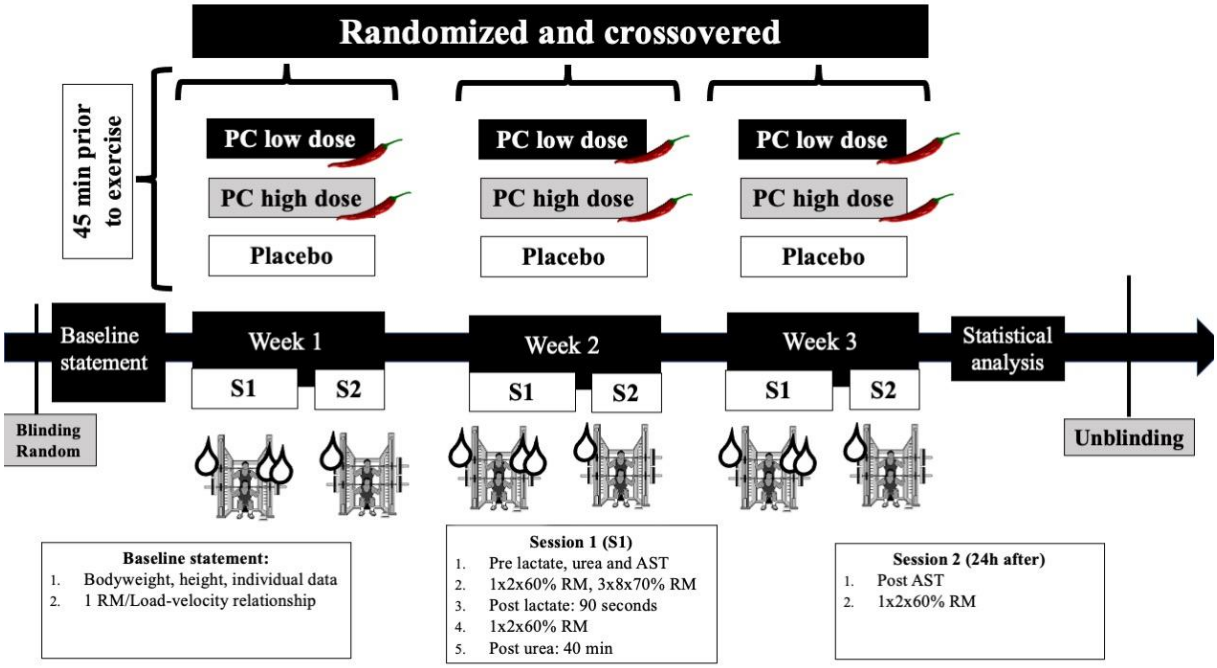

Figure 3. Overview of the experimental protocol and methodological aspects of the timeline. PC, phenylcapsaicin; S1/2, Session 1/2; 1RM, one-repetition maximum; AST aspartate aminotransferase**.**

3.1.3 Supplementation procedures

To ensure blinding, supplements and placebo (Life Pro Nutrition industries, Madrid, Spain) were encapsulated and packaged with numbered labels by an independent researcher (i.e., not involved in the study). Packages and capsules were indistinguishable in appearance,

smell and taste and their content was only revealed after an independent researcher performed the statistical analyses.

Oral ingestion of each condition was performed 45 minutes before the first physical testing session of each week according to previous research (Jiménez-Martínez, Ramírez-Campillo, et al., 2022; Turck et al., 2019). Participants were encouraged to freely select one capsule of the assigned numbered condition under researchers' supervision. Participants were only allowed to consume the selected capsule with water. Capsules contained a 0.625 mg LD of PC, a 2.5 mg HD of PC (Axivite, Malmö, Sweden), and a PLA composed of maltodextrin and excipients. According to EFSA, both PC doses are considered in the safety range proposed by its expert panel judgment (Turck et al., 2019).

3.1.4 Blood testing

Blood samples were extracted and analyzed at 4 different times each week. Before the beginning of each first session, baseline capillary lactate, blood urea, and aspartate aminotransferase (AST) samples were collected from the index fingertip of each participant. The timeline for each biomarker was chosen due to suitability and reproducibility according to previous data (Izquierdo et al., 2010; Pal et al., 2018). Capillary blood extractions were conducted with a sterilized lancet after cleaning and drying the fingertip of participants before each attempt. Lactate Pro 2 LT-1730 (Arkray, Kyoto, Japan) was used for lactate measurements as it has been shown previously as reliable throughout the physiological range of 1.0-18.00 $mmol \cdot l^{-1}$ (Pyne et al., 2000). Post-test lactate was approached 90 seconds after the last SQ set as a metabolic indicator of the exercise intensity (Mora-Custodio et al., 2018). Urea and AST were tested with 28.5-31.5 µl blood samples using automatic reflectance photometry (Reflotron, Roche, Boheringer Mannhein, Germany) (Cattozzo et al., 1988; Pearson et al., 1988) as whole-body protein breakdown and muscle damage measurements, respectively. For the determination of urea and AST, heparinized capillary tubes, pipettes, and the manufacturer reagent strips were used immediately after each extraction (Izquierdo et al., 2010). According to previous research, post-tests extractions and analyses were performed 40 minutes (Izquierdo et al., 2010) after the last SQ set for urea as a purine cycle indicator (Warburton, 2002) and before starting the 24 hours follow-up session for AST (Pal et al., 2018) (**figure 3**).

3.1.5 Resistance training protocol

Progressive loading test

Before the beginning of the study, each participant undertook an initial test with increasing loads for the individual determination of the 1RM and the load-velocity relationship in the SQ exercise (González-Badillo et al., 2017). For this purpose, a smith machine with no counterweight mechanism was used (Multipower Fitness Line, Peroga, Murcia, Spain). MPV was directly measured for each repetition with a linear velocity transducer (T-Force System, Ergotech, Murcia, Spain) attached perpendicularly to the barbell (González-Badillo & Sánchez-Medina, 2010).

Concerning the progressive loading test, the initial load was set at 30 kg and it was progressively increased in 10 kg until the mean propulsive velocity (MPV) was 0.50 $m \cdot s^{-1}$. Then, the load was increased with smaller increments (2.5–5.0 kg) until the repetition could not be completed. Three repetitions were completed for light loads (≥ 1.00 $m \cdot s^{-1}$), 2 for medium loads (1.00–0.80 $m \cdot s^{-1}$), and one for the heaviest loads (≤ 0.80 $m \cdot s^{-1}$). Rest periods were 3 minutes for light and medium loads and 5 minutes for heavy loads. Only the best repetition (i.e., highest MPV) with each load was considered for load-velocity relationship calculation (Piqueras-Sanchiz et al., 2021).

The SQ was performed with participants starting from the upright position with the knees and hips fully extended, parallel feet and stance approximately shoulder-width apart, and the barbell resting across the back at the level of the acromion. Each participant descended in a continuous motion until the top of the thighs was below the horizontal plane (~35-40° knee flexion), then immediately reversed motion and raised back to the upright position. Unlike the eccentric phase that was performed at a controlled mean velocity (~0.50-0.65 $m \cdot s^{-1}$), participants were encouraged to always perform the concentric phase of the SQ at maximal intended velocity (Pareja-Blanco, Alcazar, et al., 2020).

Warm-up

The standardized warm-up of the two weekly SQ sessions consisted of (I) five minutes of running at nine $km \cdot h^{-1}$, (II) three sets of ten repetitions of bodyweight squat, (III) three progressive CMJ, and (IV) three sets of two SQ repetitions with the 40%, 50%, and 60% of 1RM resting two minutes between sets. These warm-up intensities were chosen based on previous research on the field (Piqueras-Sanchiz et al., 2021) in order to progressively prepare participants but not to fatigue them.

Full Squat protocol

The execution technique has been described in the progressive loading test section. The SQ protocol consisted of three sets of eight repetitions with 70% 1RM with a two-minutes rest period between sets. Therefore, training volume was matched for all sessions. Thus, velocity loss could be compared for the same training performed after different interventions.

According to warm-up sets velocities and the individual load-velocity relationship, 70% 1RM load was daily established for each participant. This load was chosen because this 1RM percentage may involve a submaximal non-extenuating (i.e., without reaching muscle failure) high effort for the selected repetitions (Sánchez-Medina & González-Badillo, 2011). Finally, as MPV and consequently, the percentage of VLoss are indicators of neuromuscular fatigue (Sánchez-Medina & González-Badillo, 2011), two repetitions with 60% 1RM load were performed 3 minutes and 24 hours after the last set of the 3x8 protocol. Velocity values were treated as the fastest, mean and slowest obtained for the three sets. The percentage of mean and maximal VLoss were calculated in agreement to previous research (González-Badillo et al., 2015).

3.1.6 Rating of perceived exertion and perceived recovery status assessment

Subjective fatigue and recovery assessment was conducted using PRS, RPE-OB, and active muscle RPE-AM scales. PRS, RPE-AM, and RPE-OB are subjective recovery and fatigue status pictographs where cut-off points ranged from 0 to 10. Although participants were familiarized with both pictographs before the start of the study, both pictographs were presented during the previous session to the study start. In PRS, the perceived recovery is set between "very poorly recovered/extremely tired" (value 0) and "very well recovered/highly energetic" (value 10) (Colado et al., 2018; Laurent et al., 2010). PRS was explained again and evaluated before the start of the post 24 hours follow-up session for each condition.

Fatigue was evaluated using RPE-AM and RPE-OB immediately after each 3x8 set (Swank & Robertson, 1989). RPE scales were explained after the 60% 1RM load and before the 3x8 protocol for each condition. Maximum perceived exertion was set on the value 10, which corresponds to reaching exhaustion (i.e., last set rating of exertion of the progressive loading test), and basal intensity is represented with the value 0. RPE-AM was set up as the locally perceived exertion of the quadriceps and RPE-OB as the traditional general perceived exertion of the whole body (Swank & Robertson, 1989). Both validated scales were printed and participants were able to visualize each one when they required them.

3.1.7 Sample size calculation

Sample size calculation was performed using the G* POWER software (Heinrich-Heine-Universität Düsseldorf, Germany) with an alpha of 0.05, a statistical power of 0.80, and an effect size of 0.60 based on previous resistance training performance (i.e., total mass lifted) in previous literature (de Freitas, Cholewa, Freire, et al., 2018). Accordingly, at least 21 participants were required for this study. Plausible drop-out rate was set on 15% and 25 participants were recruited (de Morton, 2009; Sánchez-Moreno et al., 2020).

3.1.8 Statistical analysis

Data are presented as means and standard deviations (Mean ± SD). The normal distribution of the variables (Shapiro-Wilk test) and the homogeneity of the variances (Levene's test) were tested for each variable ($p > 0.05$). A two-way repeated measures ANOVA (condition x time) was used to explore the effect of the interventions (LD, HD, PLA) along the time on the magnitude of each dependent biochemical and perceptual variable. Bonferroni post-hoc comparison was performed when ANOVA significance was reached. A one-way repeated measures ANOVA was used to compare velocity analyses. The Greenhouse-Geisser correction was applied when Mauchly's sphericity test was significant ($p \leq 0.05$). The Cohen's *d* effect size (ES) with 95% confidence intervals was calculated to evaluate the magnitude of the differences using the following scale: negligible (< 0.20), small (0.20–0.49), moderate (0.50–0.79), and large (≥ 0.80) (Cohen, 1988). If non-parametric data was examined, Friedman and post-hoc Wilcoxon were used instead. Statistical analyses were performed using the software package SPSS (IBM SPSS version 25.0, Chicago, IL, USA). Statistical significance was set at $p \leq 0.05$.

3.2 Results

3.2.1 Blood testing

Two-way repeated measures ANOVAs reported significant differences of lactate and urea concentrations for time (pre-post comparisons) ($F = 269.62$, $p < 0.001$) but not for condition ($F = 1.49$, $p = 0.23$) or condition × time interaction ($F = 0.94$, $p = 0.34$) (**Table 6**). For AST, Friedman test revealed no significant differences between conditions in pre-values ($p = 0.77$), showing similar baseline levels. However, significant differences between conditions were found for AST post-values ($p = 0.03$, **Table 6**). Post-hoc Wilcoxon test revealed significantly higher post-levels of AST for PLA compared to HD ($p = 0.02$).

Table 6. Metabolic response to the different conditions of phenylcapsaicin supplementation.

Variable	Time	Condition			ANOVA		
		PLA	LD	HD	Condition	Time	Condition × time
Lactate (mmol/L)	Pre	1.9 ± 0.6	1.7 ± 0.4	1.6 ± 0.4	F = 1.49	F = 269.62	F = 0.94
	Post	12.4 ± 3.5	12.7 ± 4.5	11.5 ± 2.8	$p = 0.23$	$p < 0.001$*	$p = 0.34$
Urea (mg/dl)	Pre	32.0 ± 11.6	29.8 ± 10.7	30.3 ± 10.4	F = 0.11	F = 32.44	F = 0.94
	Post	38.9 ± 17.4	40.8 ± 14.2	37.5 ± 18.2	$p = 0.89$	$p < 0.001$*	$p = 0.39$
AST (U/L)	Pre	18.6 ± 9.9	17.4 ± 11.3	16.4 ± 11.3	Pre; $p^{\#} = 0.77$		
	Post	29.6 ± 8.6	27.1 ± 14.7	22.4 ± 10.9	Post; $p^{\#} = 0.03$*		

Mean ± standard deviation. AST, Aspartate aminotransferase; PLA, Placebo; HD, High dose; LD, Low dose. Pre; measurements; Taken each week before the first session. Post; Taken 90 seconds after squat testing for lactate, 40 min for urea and 24 hours for AST. * Significant difference ($p \leq 0.05$). # non-parametric outcome.

3.2.2 Resistance training protocol

One-way repeated measures ANOVAs of movement velocity outcomes revealed significant effects for mean velocity and maximal VLoss (p range ≤ 0.001 to 0.05, **Table 7**). Bonferroni post-hoc comparisons revealed significant differences between HD and LD for maximal VLoss (p = 0.008) and it was almost achieved between HD and PLA for the mean velocity variable (p = 0.06). The magnitude of the differences between the different conditions ranged from negligible to large (**Table 8**).

Table 7. Mechanical characteristics of the training session carried out under different conditions of phenylcapsaicin supplementation.

Variable	Condition			ANOVA
	PLA	LD	HD	
Repetitions (n)	23.8 ± 0.6	24.0 ± 0.0	24.0 ± 0.0	F =#, *p*=#
Fastest-V ($m \cdot s^{-1}$)	0.78 ± 0.04	0.78 ± 0.03	0.80 ± 0.06	F= 2.45; *p* = 0.09
Mean-V_t ($m \cdot s^{-1}$)	0.59 ± 0.05	0.61 ± 0.07	0.64 ± 0.07	F = 4.14, *p* = 0.02*
Slowest-V_t ($m \cdot s^{-1}$)	0.43 ± 0.10	0.45 ± 0.09	0.49 ± 0.09	F = 2.69, *p* = 0.08
MeanLoss-V (%)	31.2 ± 9.9	31.7 ± 9.3	27.7 ± 8.8	F = 2.79, *p* = 0.07
MaxLoss-V (%)	36.9 ± 10.8	38.0 ± 10.6	32.8 ± 8.3	F = 0.33, *p* = 0.05*

Mean ± standard deviation. Repetitions, Repetitions performed in the protocol; fastest-V, the highest velocity measured in the 3 sets; mean-V, mean velocity of all repetitions during the 3 sets; Slowest-V, Slowest velocity measured in the 3 sets; MeanLoss-V, mean percent loss in velocity from the fastest to the slowest repetition over the 3 sets; MaxLoss-V, maximum percent loss in velocity from the fastest to the slowest repetition over the 3 sets; PLA, Placebo; LD, Low dose; HD, High dose; * Significant difference ($p \leq 0.05$); # value not defined.

Table 8. Cohen's *d* effect size (ES) with 95% confidence intervals (CI) comparing mechanical outcomes between conditions.

	LD *vs.* PLA	HD *vs.* PLA	LD *vs.* HD
Fastest-V ($m \cdot s^{-1}$)	2.22 (1.76, 2.67)	0.41 (0.00, 0.82)	1.17 (0.65, 1.17)
Mean-V_t ($m \cdot s^{-1}$)	0.08 (-0.36, 0.51)	0.53 (0.08, 0.99)	-0.37 (-0.65, -0.08)
Slowest-V_t ($m \cdot s^{-1}$)	0.16 (-0.36, 0.069)	0.53 (-0.06, 1.13)	-0.37 (-0.65, -0.08)
MeanLoss-V ($m \cdot s^{-1}$)	0.09 (-0.26, 0.44)	-0.33 (-0.80, 0.14)	0.38 (0.06, 0.69)
MaxLoss-V ($m \cdot s^{-1}$)	-0.15 (-0.21, 0.52)	-0.28 (-0.73, 0.17)	0.45 (0.15, 0.75)

Mean ± standard deviation. Repetitions, Repetitions performed in the protocol; 60% fastest-V, highest velocity measured in the 60% sets; fastest-V, highest velocity measured in the 3 sets; mean-V, mean velocity of all repetitions during the 3 sets; Slowest-V, Slowest velocity measured in the 3 sets; MeanLoss-V, mean percent loss in velocity from the fastest to the slowest repetition over the 3 sets; MaxLoss-V, maximum percent loss in velocity from the fastest to the slowest repetition over the 3 sets; PLA, Placebo; LD, Low dose; HD, High dose. A positive ES indicates a higher value for HD compared to PLA, LD compared to PLA, and LD compared to HD.

3.2.3 Rating of perceived exertion and perceived recovery status assessments

Two-way repeated measures ANOVAs for RPE-OB reported significant differences for time (F = 49.00, $p < 0.001$) but not for condition (F = 2.77, $p = 0.07$) or condition × time interaction (F = 1.339, $p = 0.26$) (**Figure 4A**). However, Bonferroni post-hoc analyses revealed no significant differences. For RPE-AM, both condition (F = 9.19, $p < 0.001$) and time (F = 36.154, $p < 0.001$) reached significant differences but not condition × time interaction (F = 0.553, $p = 0.70$). Bonferroni post-hoc analyses showed significant differences for all time

comparisons (p range ≤ 0.001 to 0.002) and for comparisons between PLA and HD (p = 0.004) and between HD and LD (p = 0.02) (**Figure 4B**). On the other hand, one-way repeated measures ANOVAs found no significant differences between conditions for PRS (F = 0.698, p = 0.46, **Figure 4C**).

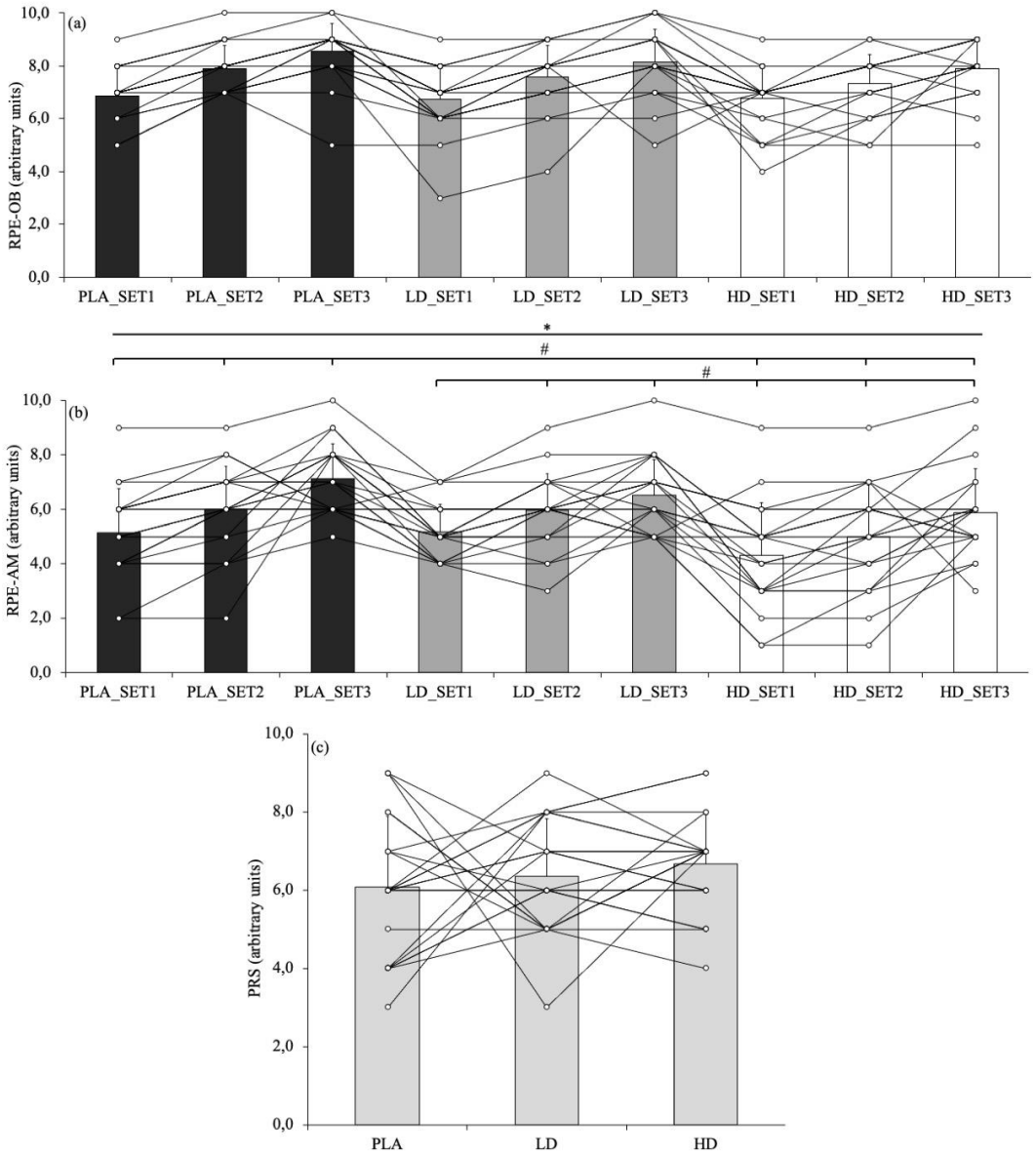

Figure 4. Individual (points) and mean (bars) values of: (a) RPE-OB, Overall body rating of perceived exertion; (b) RPE-AM, Active muscle rating of perceived exertion; (c) PRS, Perceived recovery status for the different supplementation conditions (PLA, HD, LD). PLA, Placebo; HD, High dose; LD, Low dose. (* $p_{ANOVA} \leq 0.05$; # $p_{Bonferroni} \leq 0.05$).

3.3 Discussion

This is the first study where the impact of a capsaicinoid has been evaluated on several topics such as muscle damage, protein breakdown, recovery and peripheral perceived exertion,

with an acute 24 hours design in strength-trained men. The main finding of this study was that, although HD reduced RPE-AM and enhanced mechanical performance, it also exhibited lower muscle damage in comparison to PLA and LD. The main HD effects were documented through reductions in maximal VLoss and eliciting a positive trend in the velocity of the slowest repetitions compared to LD and PLA. Collectively, contrary to the initial hypothesis, the ergogenic effects of PC on performance variables were only verified for HD. In addition, a significant dose-response relationship for LD and HD was not fulfilled for any of the particular outcomes. On the other hand, contrary to our initial hypothesis, HD was effective in reducing muscle damage. Therefore, the results of the present study confirm a plausible ergogenic effect of PC on strength training and performance, but an HD seems to be necessary.

Regarding metabolic blood testing, previous literature has described that the involvement of major muscle mass areas (e.g., lower-limbs exercise) and the execution time are key factors in lactate response (Wirtz et al., 2014). In this sense, a higher velocity loss is linearly correlated with an increase in lactate levels after SQ exercise (Sánchez-Medina & González-Badillo, 2011). Accordingly, the resistance training stimulus applied in the present study was effective inducing a lactate response between pre and post exercise states. These results agree with the only previous study where lactate was assessed after capsaicin supplementation (de Freitas, Cholewa, Freire, et al., 2018). Within this context, in our study post exercise lactate levels trended to be lower in the HD condition compared to PLA and LD. Preclinical data has shown that lactate is a potent endogenous inhibitor of TRPV1 activity (de la Roche et al., 2016). For this reason, PC may have modulated sarcoplasmic calcium efflux channels lowering lactate levels (de la Roche et al., 2016). This finding agrees with a previous study (de Freitas, Cholewa, Freire, et al., 2018), in which although participants significantly performed more repetitions after capsaicin ingestion compared to placebo, non-significant lower lactate levels were reported for capsaicin condition. This reduction on lactate kinetics after oral capsaicin administration has also been reported in other high-intensity exercise modalities, such as running time trial (de Freitas, Cholewa, Gobbo, et al., 2018).

Concerning, protein breakdown, previous literature has observed an accelerated catabolic effect on purine nucleotides in skeletal muscle after strenuous exercise (Sutton et al., 1980; Warburton, 2002). As a waste product of ammonium, urea levels are linked to exercise intensity (i.e., closeness to muscle failure), which can be assessed through VLoss (Sánchez-Medina & González-Badillo, 2011; Warburton, 2002). Previous research has fixed purine breakdown threshold on 35% of VLoss in the SQ exercise (Sánchez-Medina & González-Badillo, 2011). In the present study, all protocols induced moderate increases in urea levels

following exercise. However, none of the protocols achieved this cut-off threshold. Nevertheless, although significant differences were not reported between conditions, the absolute increase trended to be lower in HD, which reported a significantly lower maximal VLoss. In agreement with protein breakdown, strenuous exercise also raises inflammation and oxidative stress, which can be observed in different muscle damage biomarkers (Baird et al., 2012; Bessa et al., 2016). AST is a liver and musculoskeletal enzyme which react 24 hours after demanding exercise (Bessa et al., 2016; Pal et al., 2018; Villavicencio-Kim & Wu, 2020). In this regard, HD exhibited lower levels of AST than PLA after 24 hours. This finding may be linked to the lower mechanical stress suffered during the HD conditions, since HD obtained higher velocities and lower VLoss values. This mechanical hypothesis may be more intuitive instead of a direct "antioxidant" or recovery effect of PC due to its synthetic composition (Turck et al., 2019).

Related to the SQ protocol, the present results agree with previous literature where capsaicin enhanced performance when it was acutely ingested (de Freitas, Cholewa, Freire, et al., 2018; Jiménez-Martínez, Ramírez-Campillo, et al., 2022). In previous research, capsaicin (i.e., 12 mg) raised the total number of repetitions performed until failure in a 4x70% 1RM protocol (de Freitas, Cholewa, Freire, et al., 2018). Within this context, as MPV is a reliable predictor of the number of repetitions (Morán-Navarro et al., 2019), the observed trend of higher MPV values in the last repetitions (i.e. the slowest repetitions) of HD might have implied an increase in the total repetitions until exhaustion if the volume had not been matched. Furthermore, as previous research elicited further benefits of capsaicin in the last set of higher-volume training protocols, plausible greater effects would have been reported by increasing the total number of sets (de Freitas, Cholewa, et al., 2019; de Freitas, Cholewa, Freire, et al., 2018). Regarding the potential ergogenic mechanisms underlying capsaicin effects on SQ performance, according to previous research, they may be linked to an increase of calcium released by the sarcoplasmic reticulum, higher acetylcholine levels, and its analgesic effect (de Moura e Silva, Cholewa, Jäger, et al., 2021; Jiménez-Martínez, Ramírez-Campillo, et al., 2022). As afferents III and IV fibers seem to contribute to the development of central fatigue at spinal and supraspinal levels of the central nervous system (Collins et al., 2018; Kaufman et al., 1982), this "desensitizer" agent may provide a higher tolerance to firing rate reductions during strenuous exercise. Thus, as muscle force is critically affected by the motor unit activity (Del Vecchio et al., 2019), and MPV is a mechanical manifestation of muscle force (Sánchez-Medina & González-Badillo, 2011), the analgesic effect of PC may have played a retardant effect on neuromuscular fatigue improving MPV, especially at the last repetitions. Consequently, the

ergogenic effect of PC on mechanical outcomes might be explained by a plausible reduction in neural fatigue (Heckman & Enoka, 2004; Jiménez-Martínez, Ramírez-Campillo, et al., 2022).

Concerning perceived exertion variables, previous research has reported RPE-OB reductions after 12 mg of acute capsaicin supplementation (de Freitas, Cholewa, et al., 2019; de Freitas, Cholewa, Freire, et al., 2018). However, although a positive trend was observed, our results did not support these RPE-OB reductions when PC, in HD or LD, was ingested in comparison to PLA. In this sense, most of the previous research protocols were performed until muscle failure where the plausible analgesic effect of capsaicinoids may be higher (de Freitas, Cholewa, Freire, et al., 2018; de Moura e Silva, Cholewa, Jäger, et al., 2021). On the other hand, in the present study, local quadriceps RPE-AM was highly affected by HD in comparison to LD and PLA. Considering that RPE can be modulated by changes in the neuronal circuitry of the brain (Alix-Fages et al., 2020; Okano et al., 2015) and afferent feedback from III and IV afferents may raise exercise-related discomfort (Taylor et al., 2016) and RPE (Amann et al., 2010), this effect of PC on RPE-AM could be mediated by TRPV1 interaction. In addition, since HD but not LD produced significant effects on RPE-AM, peripheral analgesic effects of PC (Arora et al., 2021) during exercise could be dose-dependent. This conjecture agrees with the only previous study where a low dose of a regular-bioavailable capsaicin supplement was consumed before a resistance training protocol (Cross et al., 2020). In this study, fatigue index and isometric knee torque was not affected after 1.2 mg of capsaicin (Cross et al., 2020). On the other hand, although PC in HD exerted a positive effect on muscle damage and RPE-AM, PC effects did not produce any improvements in PRS. Hence, the possible analgesic effect of PC may only appear acutely. Intriguingly, as previous research (Jiménez-Martínez, Ramírez-Campillo, et al., 2022) hypothesized that capsaicin may increase injury risk due to its analgesic effect, in this study only two participants dropped out, one for personal reasons and the other during the placebo session.

Collectively, our findings suggest that a 2.5 mg dose of PC provides a plausible ergogenic effect on strength performance, muscle damage, and peripheral perceived exertion in comparison to PLA and a lower dose of 0.625 mg. This novel information is valuable because never before a capsaicinoid has been evaluated concerning muscle damage, protein breakdown, and peripheral fatigue. Finally, this study presents important strengths as a triple-blinded, placebo-controlled crossover design, trained participants enrollment, and the uses of velocity measures as performance indicators. However, some limitations should be addressed. First, electromyographical assessment may have completed the internal reliability of the peripheral effects of PC (see chapter 2). Another possible limitation is that the study was only carried out

in male athletes, for this reason, these results may not be extrapolated to other populations such as female athletes or untrained participants. Furthermore, these results should be cautiously interpreted for other exercises and tasks (see chapter 4) not performed in a laboratory environment. Finally, as in this study only 3 sets of SQ in a single training session were performed, further research may evaluate the effects of PC during longer workouts and chronic trials to verify whether or not these benefits are replicated in these conditions.

3.4 Conclusion

The results of the present study suggest that a HD (2.5 mg) of PC supplementation ingested 45 minutes before exercise may increase SQ performance and reduce muscle damage, as well as peripheral quadriceps perceived exertion in strength-trained participants in comparison to a LD (0.625 mg) and PLA. Therefore, the ergogenic effect of PC may appear after a "dose" threshold is reached.

Chapter 4. Effects of phenylcapsaicin on substrates oxidation, energy expenditure, metabolic, perceptual and thermal responses during exercise.

Effects of phenylcapsaicin on aerobic capacity and physiological parameters in active young males: a randomized, triple-blinded, placebo-controlled, crossover trial

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4. Effects of phenylcapsaicin on substrates oxidation, energy expenditure, metabolic, perceptual and thermal responses during exercise

Scientific literature concerning the impact of capsaicinoids or capsinoids in resistance and high-intensity training is larger and more robust than that addressing the effects of these substances in the physiological responses to aerobic exercise (Jiménez-Martínez, Ramirez-Campillo, et al., 2022). In the previous chapters, it has been discussed if the pungent properties of capsaicinoids, are really associated with their ergogenic effects (Jiménez-Martínez, Ramirez-Campillo, et al., 2022). In this sense, although capsaicin spiciness has been proposed as an athletic enhancer mechanism, capsinoids (i.e., new non-pungent analogues) have been proposed as a plausible alternative (Luo et al., 2011). It is important to stand out this difference between both type of substances as research evaluating the physiological responses to exercise has used both types of compounds.

As it was explained in the introduction section capsinoids composed a group of capsaicinoids non-pungent analogues (e.g., capsiate) found in sweet peppers (Lang et al., 2009). Although capsaicinoids and capsinoids have a similar chemical structure, these substances present differences in their functional properties and their metabolism. Accordingly, capsinoids are rapidly metabolized and conjugated after their ingestion (EFSA, 2012), resulting in non-detectable circulating levels in the bloodstream (EFSA, 2012). By contrast, after their ingestion, different capsaicin formulations, including PC, have a fast effect on different tissues, such as the small intestine, liver, and stomach (Turck, et al., 2019).

Once again, the ergogenic effects of capsaicinoids and capsinoids seems to be mediated by their interaction with the TRPV1 in afferent III and IV nerve fibers (Jiménez-Martínez, Ramirez-Campillo, et al., 2022). Besides, ingesting these substances enhance muscle contraction as a consequence of the improvement in motoneurons recruitment, calcium release, and perceived analgesia (de Moura e Silva, Cholewa, Billaut, et al., 2021). Additionally, other potential ergogenic mechanisms related to TRPV1 stimulation concern metabolic effects such as an increase in fatty acid oxidation (more FFAs available for beta-oxidation), an increase in glycogen sparing, and a positive effect in the acetylcholine turnover (de Moura e Silva, Cholewa, Billaut, et al., 2021). However, current human evidence regarding the positive metabolic impact of these substances is scarce, albeit the plausible mechanisms aforementioned are well documented in preclinical models (Basith et al., 2016; Jiménez-Martínez, Ramirez-Campillo, et al., 2022).

In the present study, only active males were recruited as previous research has exhibited that the effects of capsaicin on substrate oxidation are unaffected by the sex of the participants (Lejeune et al., 2003). To date, only two studies have reported the metabolic effects of a capsaicinoid on substrate oxidation (Lim et al., 1997; Rossi et al., 2022). In the first study, participants exhibited equal RER values after 12 mg of capsiate supplementation during exercise performed at 70% of the maximal aerobic speed (Rossi et al., 2022). In the second study, a meal with 10 g of hot peppers 2.5 hours prior to 1 hour of aerobic exercise at 60% $VO2_{peak}$ showed an increase in RER compared to placebo (Lim et al., 1997). An important limitation in current research is that human substrate oxidation is not usually reported in previous studies (Santos et al., 2022). For instance, 10 mg/kg of capsiate supplementation 60 min prior to aerobic exercise decreased RER in mice males (Santos et al., 2022). On the other hand, 12 mg of dihydrocapsiate supplementation has not exhibited significant differences in fat FATox, non-esterified fatty acids, energy expenditure (EE), and skin temperature in sedentary overweight men during aerobic exercise (Osuna-Prieto et al., 2022). Furthermore, most current studies do not standardize MFO measurement in the pre-test, which may bias the metabolic endpoints measured (Santos et al., 2022).

Concerning the metabolic effects of these substances on high-intensity training (i.e., resistance training and high-intensity interval training), 12 mg of capsaicin supplementation has exhibited lower lactate levels compared to placebo after four sets until failure in the SQ exercise (de Freitas, Cholewa, Freire, et al., 2018). This reduction in lactate values has also been reported in other high-intensity tasks, such as repeated sprints after 12 mg of capsaicin supplementation (de Freitas, Billaut, et al., 2019). Additionally, previous research has documented a negligible effect of capsaicin supplementation on heart rate. Overall, evidence regarding the metabolic impact of capsaicinoids and capsinoids during exercise is scarce and inconclusive. Furthermore, the effects of PC on physiological aerobic exercise variables and sport performance have not been tested yet. For this reason, if this new capsaicin analogue, which is supposed to be more bioavailable, produces an ergogenic effect on substrate oxidation, energy metabolism and aerobic capacity and if a dose threshold exists must be investigated.

Therefore, this last study of the compendium aimed to assess the effects of a LD of 0.625 mg and a HD of 2.5 mg of PC on aerobic capacity, energy metabolism, substrate oxidation and other physiological variables such as circulating lactate levels, energy expenditure, body temperature response, RPE and perceived temperature compared to placebo in active males. We hypothesized that PC supplementation increases FATox, skin body temperature,

mechanical performance and energy expenditure while reduces RPE in a dose-dependent manner.

4.1 Materials and methods

4.1.1 Participants

Sample size calculation was performed using the G* POWER software (Heinrich-Heine-Universität Düsseldorf, Germany) with an alpha of 0.05, an effect size of 0.4 and a statistical power of 0.80. Based on previous studies (Josse et al., 2010; Osuna-Prieto et al., 2022), 12 participants were required to establish statistical differences between conditions. To ensure the detection of differences 17 physically active males were enrolled in the study (**Table 9**). Participants enrolled in the study through a poster that was shared on social media. None of the participants reported any physical limitation or health condition that could compromise cycling performance. Participants were instructed not to perform any intense physical exercise during the two days preceding each visit to the laboratory and from consuming stimulant beverages or any dietary supplement within 24 h preceding each testing session. Before being included in the study, all potential participants were comprehensively informed about the study purpose, procedures and the benefits, risks, and discomforts that might result from participation. Each participant provided informed consent and was free to withdraw from the study at any time. The study protocol adhered to the tenets of the last revised Declaration of Helsinki and was approved by the Institutional Review Board (blinded for peer review).

Table 9. Characteristics of the study participants (n=17).

	Mean ± standard deviation
Age (years)	24.7 ± 6.0
Height (cm)	175.9 ± 7.6
Body mass (kg)	77.2 ± 11.7
Muscle mass (%)	41.2 ± 6.2
Fat mass (%)	6.9 ± 5.3
FATmax (W)	68.8 ± 37.7
MFO (g/min)	0.27 ± 0.09

FATmax, intensity linked to maximal fat oxidation; MFO, maximal fat oxidation.

4.1.2 Experimental design

A randomized, triple-blinded, placebo-controlled crossover trial was used to analyze the effects of PC on energy expenditure and substrate oxidation, skin body temperature, heart rate and

perceptual responses to submaximal steady-state and maximal effort cycling tests **(Figure 5).** Participants attended the laboratory four times, separated by 72-96 h to ensure a complete recovery from central and peripheral fatigue (Alix-fages et al., 2023; Carroll et al., 2017). Before the preliminary session, participants anthropometric [(i.e., height, body mass, % muscle, % body fat (Tanita BC 418 segmental, Tokyo, Japan; Seca 202 Stadiometer, Seca Ltd., Hamburg, Germany)] and sociodemographic characteristics were obtained **(see Table 9).** Then, two tests, a submaximal incremental exercise test followed by a maximal effort incremental test, were conducted in the preliminary session. The submaximal exercise test was used to determine the MFO and the cycling power values (W) at MFO (FATmax intensity). The maximal effort test assessed maximal oxygen consumption ($VO2_{max}$) and the maximal cycling power achieved during the test. The three experimental sessions were identical, only differing in the supplement (PLA, LD of PC, and HD of PC), which was only administered 45 min before the first cycling task. In each experimental session, participants performed a steady-state test (60 min at FATmax) followed by a maximal incremental effort test (25 W increments every min until volitional exhaustion). Each participant was constantly tested at the same time of the day and under similar environmental conditions (22-24 ºC and 55% humidity).

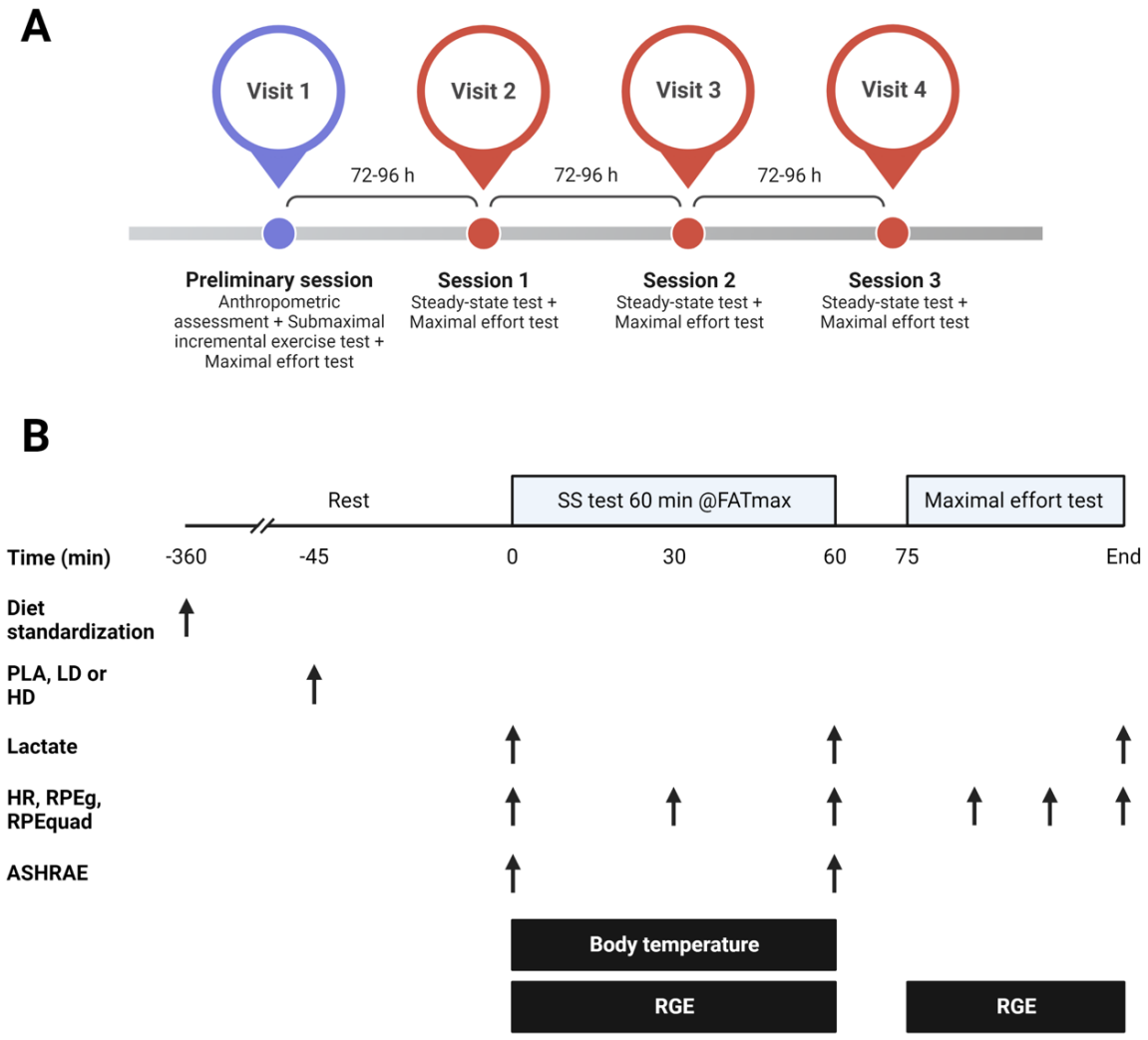

Figure 5. General overview of the study (A) and design of the experimental sessions (B) aiming to analyze the effects of two doses of phenylcapsaicin on metabolic, thermal, perceptual, and mechanical responses to steady-state and maximal cycling tests. HD, high dose; LD, low dose; PLA, placebo; HR, heart rate; RGE, respiratory gas exchange; RPE-OB, general ratings of perceived exertion; RPE-AM, ratings of perceived exertion of quadriceps; SS, steady-state. Created with BioRender.com

4.1.3 Supplementation procedures

Capsules were ingested with water. The contents of the capsules were as follows: a LD of 0.625 mg of PC, a HD of 2.5 mg HD of PC (Axivite, Malmö, Sweden), and a PLA composed of maltodextrin and excipients. According to EFSA, both PC doses are considered in the safety range proposed by its expert panel judgment (Turck, et al., 2019). Supplements and placebo were encapsulated and packaged with alphanumeric labels to ensure blinding. Accordingly, an independent technician (i.e., not involved in the study) prepared the capsules in the original

producer´s industry (Life Pro Nutrition industries, Madrid, Spain). Participants selected one capsule of the daily randomized assigned condition under the supervision of at least one researcher. To reduce possible bias, an independent researcher assigned participants to each condition with the Research Randomizer online software (www.randomizer.org). Packages and capsules were indistinguishable in appearance, smell and taste and their content was only revealed after an independent researcher performed the statistical analyses to prevent evaluators' bias. Adverse effects were not reported for any of the conditions.

4.1.4 Dietary intake standardization

To ensure intra and inter-individual reliability in metabolic variables (e.g., FATox), dietary intake was standardized at least 6 hours previous to exercise testing (Amaro-Gahete et al., 2018; Rothschild et al., 2022). For this purpose, participants performed each test with at least 6 hours of fasting prior to the start of each session. The last meal before the fasting period was standardized with 45 g of maltodextrin powder and 30 g of protein powder for all participants (Life Pro Nutrition industries, Madrid, Spain).

4.1.5 Exercise procedures

The three tests were performed employing an electronically braked cycle ergometer (Excalibur Sport; Lode, Groningen, The Netherlands). Respiratory gas exchange was monitored during all tests with a gas analyzer (Ergocard CPX, Medisoft, Belgium). The testing procedures and variables collected in each test are described below:

1. Submaximal incremental exercise test. This test was used to determine MFO and FATmax in the preliminary session. The exercise protocol started with a 3 min stage at 25 W, and the intensity was increased in steps of 25 W every 3 min until the respiratory exchange ratio (RER) was ≥ 1 for at least 30 s (Sanchez-Delgado et al., 2018). During the submaximal exercise test, VO2 and carbon dioxide production (VCO2) data were averaged over the last 60 s of each 3 min stage. Then, FATox was calculated from the aforementioned values. FATox values (g/min) from the different stages of the submaximal exercise test were plotted against the relative exercise intensity (W). Third-degree polynomial regression was built to determine the absolute MFO (g/min) (Midgley et al., 2007; Sanchez-Delgado et al., 2018). EE was estimated with Weir's abbreviated equations (Weir, 1949) and FATox and CHOox with Frayn' 's stoichiometric equations assuming a negligible urinary nitrogen excretion (Frayn, 2016;

Osuna-Prieto et al., 2022). Subsequently, RER was calculated from the CHOox and FATox obtained data. This procedure was further used for the next sessions. The equations used are presented hereunder:

EE (kcal/min) = (1.106 * VCO_2) + (3.941 * VO_2)

RER = (VCO_2/VO_2)

CHOox (g/min) = (4.55 * VCO_2) - (3.21 * VO_2)

FATox (g/min) = (1.67 * VCO_2) - (1.67 * VO_2)

2. Maximal effort test. This test was performed in the preliminary and the three experimental sessions, 15 min after the first test of each session. The exercise protocol started with a 1 min stage at 25 W, and the intensity was increased in steps of 25 W every min until (i) volitional exhaustion was reached, or (ii) participants had to stop because of peripheral fatigue. The dependent variables considered in this test were: (i) final stage completed in the protocol, (ii) $VO2_{max}$, (iii) circulating lactate concentration recorded 90 seconds after test cessation with a portable lactate analyzer (Lactate PRO2, Arkray, Kyoto, Japan) (Crotty et al., 2021), (iv) RPE-OB and quadriceps RPE-AM (RPE 0-10) and heart rate (Polar RS800, Polar Electro Inc., Woodbury, NY, USA) (Hernando et al., 2018) at an intensity representing the 30%, 60% and 90% of the maximal power attained at the final stage completed in the preliminary session. Lactate blood was extracted with a sterilized lancet after cleaning and drying the fingertip of participants before each attempt. VO2 was monitored using a galvanic fuel cell, and VCO2 was evaluated using a non-dispersive infrared sensor. The gas analyzer was calibrated following standard gas concentrations as suggested by the manufacturer.

3. Steady-state test. Participants cycled for 60 min at the intensity of FATmax determined in the preliminary session. Circulating lactate, RPE-OB, RPE-AM, and heart rate were recorded pre-exercise, in the middle of the test (30 min), and at the end (60 min). The skin body temperature was also recorded throughout the test with a set of eight DS-1922 L iButtonTM wireless thermometers (Thermochron, Dallas, TX, USA) (Smith et al., 2010; van Marken Lichtenbelt et al., 2006) attached to the participant's skin in different places: (i) forehead, (ii) right scapula, (iii) left chest, (iv) right% deltoid), (v) left elbow, (vi) left hand, (vii) right thigh and (viii) right gastrocnemius. Data were processed as mean blocks of 5 mins. Consequently, a total of 12 temperature stages were recorded and averaged for the final analysis.

The equation used to assess the effects of PC and PLA on body temperature is described hereunder:

Overall mean skin temperature = (Forehead*0.07) + (Right Scapula*0.175) + (Left Chest*0.175) + (Right Deltoid*0.07) + (Left Elbow*0.07) + (Left Hand*0.05) + (Right Thigh*0.19) + (Right Gastrocnemius*0.2) (Martinez-Tellez et al., 2017).

The mean and maximum heart rates recorded throughout the test were also compared between the experimental conditions. The substrate oxidation (fat and carbohydrates), energy expenditure, and RER during the 60 min steady-state test were also estimated and compared between the conditions. FATox, CHOox and EE were also expressed as the area under the curve (AUC) using the trapezoidal rule. For metabolic variables, the highest value achieved during the test (i.e., peak) and the individual analysis of each stage (i.e., intra-test analysis) were performed. Finally, the American Society of Heating, Refrigerating, and Air Conditioning Engineers (ASHRAE) scale was used to record thermal perception before and after the test. The scale was recorded for the following body areas: (i) clavicle, (ii) abdominal, (iii) arms, (iv) hands, (v) legs, (vi) feet, and (vii) overall body. Each item is scored with the following values: cold (−3), cool (−2), slightly cool (−1), neutral (0), slightly warm (1), warm (2), to hot (3).

4.1.6 Statistical analysis

Data are presented as means and standard deviations (Mean ± SD). The normal distribution of all the variables presented was tested with the Shapiro-Wilk test, and the homogeneity of the variances with the Levene's test ($p > 0.05$). A two-way repeated measures ANOVA (condition x time) was used to analyze the effect of the supplementation (LD, HD, PLA) across the time on each dependent metabolic, performance, and perceptual variable. F value were retrieved from each ANOVA calculation. A Bonferroni post-hoc comparison was performed when ANOVA significance was reached. A one-way repeated measures ANOVA was used to compare intra-test effects for each stage and for the maximal metabolic values of the steady-state test. The Greenhouse-Geisser correction was applied when Mauchly's sphericity test was significant ($p \leq 0.05$). For non-parametric data, Friedman`s test and post-hoc Wilcoxon corrections were used instead. Statistical analyses were performed using the software package SPSS (IBM SPSS version 25.0, Chicago, IL, USA). Statistical significance was set at $p \leq 0.05$. The magnitude of the differences was assessed with partial eta squared values (ηp^2) derived from ANOVAs and were interpreted as low (<0.04), moderate (0.04–0.13) and large (>0.13) for all the parametric outcomes. The effect size of the post-hoc comparisons

was calculated by means of Cohen's d, which was interpreted as a low (<0.50), moderate (0.50–0.80), or large effect (>0.80) (Cohen, 1988).

4.2 Results

4.2.1 Circulating lactate levels, RPE and heart rate during steady-state test

Two-way repeated measures ANOVAs did not reveal significant differences for condition in circulating lactate levels, general and local RPE, nor heart rate (p range = 0.08 to 0.56) (**Table 10**). Significant differences were reported in time for heart rate, RPE-OB, and RPE-AM ($p \leq 0.001$) (**Table 10**). Bonferroni post-hoc analyses revealed significant differences for all time comparisons (pre, '30' and '60') in heart rate, RPE-OB, and RPE-AM (p range < 0.001 to 0.004). Significant condition x time interaction was only found for RPE-OB due to higher values in LD compared to PLA (d = 27) and HD (d = 0.27) (**Table 10**).

Table 10. Two-way repeated measures analysis of variance (ANOVA) comparing circulating lactate levels, general and local RPE, and heart rate between the different experimental conditions during the steady-state test.

Variable	Time	Condition			ANOVA		
		PLA	LD	HD	Condition	Time	Condition × time
Lactate (mmol/L)	Pre	1.54 ± 0.38	2.08 ± 2.21	2.43 ± 3.49	F = 0.58, p = 0.56 ηp^2 = 0.04	F = 0.03, p = 0.86 ηp^2 = 0.002	F = 2.68, p = 0.08 ηp^2 = 0.16
	Post	3.37 ± 4.54	1.36 ± 0.43	1.63 ± 0.51			
RPE-OB (a.u.)	Pre	0.19 ± 0.40	0.06 ± 0.25	0.00 ± 0.00	F= 1.08, p = 0.33 ηp^2 = 0.06	F = 47.40, p < 0.001* ηp^2 = 0.76	F = 3.15, p = 0.02* ηp^2 = 0.17
	30'	1.94 ± 1.12	2.06 ± 1.53	2.06 ± 1.34			
	60'	2.75 ± 1.48	3.63 ± 2.31	2.94 ± 1.88			
RPE-AM (a.u.)	Pre	0.25 ± 0.58	0.25 ± 0.45	0.06 ± 0.25	F= 2.65, p = 0.08 ηp^2 = 0.15	F = 44.30, p < 0.001* ηp^2 = 0.74	F = 1.62, p = 0.20 ηp^2 = 0.10
	30'	1.94 ± 1.29	2.56 ± 1.71	2.19 ± 1.56			
	60'	3.06 ± 1.61	3.69 ± 2.18	3.19 ± 1.80			
Heart rate (b/min)	Pre	70.4 ± 10.2	66.6 ± 8.6	67.9 ± 9.6	F= 2.28, p = 0.11 ηp^2 = 0.13	F = 64.86, p < 0.001* ηp^2 = 0.81	F = 0.85, p = 0.45 ηp^2 = 0.05
	30'	105.5 ± 15.9	103.1 ± 16.59	102.2 ± 17.8			
	60'	109.3 ± 19.6	107.4 ± 18.1	103.9 ± 17.4			

Mean ± standard deviation. PLA, Placebo; HD, High dose; LD, Low dose; Pre; Before the start of the session; 30', at minute 30 of steady-state test; 60', at minute 60 of steady test; RPE-OB, general ratings of perceived effort; RPE-AM, ratings of perceived effort in quadriceps; a.u., arbitrary units. * Significant difference: $p \leq 0.05$.

4.2.2 Skin body temperature, maximal metabolic respiratory variables and mean and maximum heart rate during steady-state test

One-way repeated measures ANOVAs did not reveal significant differences in skin body temperature (p = 0.27) or mean heart rate (p = 0.24) (**Table 11**). Maximal carbohydrate oxidation, energy expenditure and RER did not differ between conditions (p ranged from 0.10 to 0.77). However, significant differences were found for maximum heart rate (p = 0.03) and MFO (p = 0.05) (**Table 11**), in favor of HD compared to PLA. However, Bonferroni post-hoc did not revealed differences between conditions in any of the outcomes (p range = 0.09 to 0.99; d range = 0.20 to 0.31).

Table 11. One-way repeated measures analysis of variance (ANOVA) comparing skin body temperature, mean and maximum heart rate, and maximal metabolic respiratory variables between the three experimental conditions during the steady-state test.

Variable	Conditions			ANOVA
	PLA	LD	HD	
Skin body temperature (ºC)	30.27 ± 1.02	30.48 ± 1.22	30.02 ± 1.22	F = 1.32, p = 0.27, ηp^2 = 0.09
Mean heart rate (bpm)	100.73 ± 14.96	99.97 ± 14.18	97.66 ± 15.22	F = 1.47, p = 0.24, ηp^2 = 0.09
Maximum heart rate (bpm)	114.13 ± 18.24	112.31 ± 18.53	108.75 ± 16.83	F = 3.23, p = 0.03*, ηp^2 = 0.19
FOpeak (g/min)	0.28 ± 0.09	0.32 ± 0.11	0.33 ± 0.11	F = 3.20, p = 0.05*, ηp^2 = 0.16
CHOOXpeak (g/min)	1.12 ± 0.60	1.03 ± 0.50	1.07 ± 0.50	F = 3.05, p = 0.10, ηp^2 = 0.15
MEEpeak (kcal/min)	5.99 ± 1.90	6.03 ± 1.85	6.10 ± 1.92	F = 0.20, p = 0.77, ηp^2 = 0.01
MRERpeak (g/min)	0.92 ± 0.05	0.91 ± 0.05	0.91 ± 0.06	F = 0.80, p = 0.45, ηp^2 = 0.05

Mean ± standard deviation. PLA, Placebo; HD, High dose; LD, Low dose; FOpeak, Peak of fat oxidation; CHOOXpeak, Peak of carbohydrate oxidation; MEEpeak, Peak of energy expenditure; MRERpeak, Peak of respiratory exchange ratio. * Significant difference: $p \leq 0.05$.

4.2.3 Thermal perception during steady-state test

Significant differences for condition were not reported for any of the ASHRAE outcomes measured (p range = 0.17 to 0.78; ηp^2 range = 0.01 to 0.12). However, significant differences were reported in time for all the variables ($p \leq 0.001$; ηp^2 range = 0.52 to 0.73). A significant condition x time interaction was found for the clavicle (p = 0.04; ηp^2 = 0.16) area due to the lower value of HD in comparison to LD an PLA. The other areas did not exhibit condition x time interactions (p range = 0.12 to 0.60; ηp^2 range = 0.04 to 0.12) (**Figure 6)**.

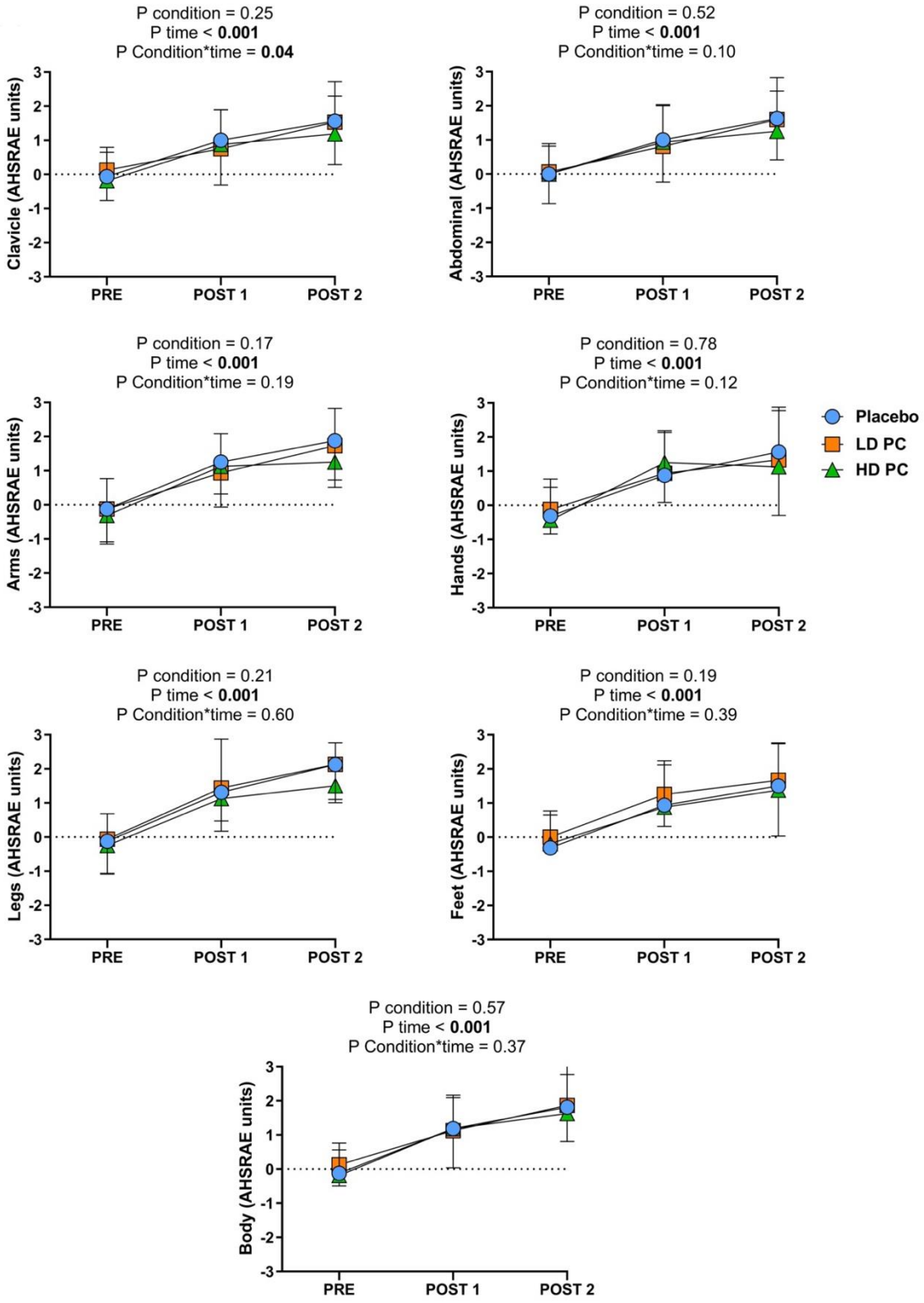

Figure 6. Two-way repeated measures analysis of variance (ANOVA) comparing the effects of consuming different dosis of phenylcapsaicin (HD and LD) or placebo (PLA) on thermal perception (ASHRAE scale) at different time points. PLA, Placebo; HD, High dose; LD, Low dose; PC, Phenylcapsaicin. PRE: Before the start of the session; Post 1: Between the steady state test and maximal effort test; Post 2: After the maximal effort test.

4.2.4 Energy expenditure and substrate oxidation during steady-state test

No significant differences were found for AUC EE, AUC FATox, and AUC CHOox (p range = 0.09 to 0.54; ηp^2 range = 0.04 to 0.18). Two-way repeated measures ANOVAs exhibited non-significant differences for condition in FAToxidation (p = 0.06; ηp^2 = 0.14), CHOox (p = 0.19; ηp^2 = 0.10), EE (p = 0.54; ηp^2 = 0.008), and RER (p = 0.21; ηp^2 = 0.10). However, two-way repeated measures ANOVA revealed significant differences for time in all the aforementioned variables ($p \leq 0.001$; ηp^2 range = 0.54 to 0.79) but not for condition x time interaction (p range = 0.17 to 0.96; ηp^2 range = 0.003 to 0.06). Intra-test one-way repeated measures ANOVA only exhibited a significant effect on FATox at min 5 (p = 0.005; ηp^2 = 0.28), 10 ($p \leq 0.001$; ηp^2 = 0.29), and 55 (p = 0.04; ηp^2 = 0.24) for HD and LD, and for CHOox at min 5 (p = 0.05; ηp^2 = 0.25) and RER (p = 0.003; ηp^2 = 0.25) at min 5 in favor of PLA but not for any variable in any other stage. Post-hoc Bonferroni reported significant differences in favor of HD and LD between PLA/ HD at 5 min (p = 0.002; d = 0.92), PLA/LD at 5 min (p = 0.002; d = 0.74), PLA/HD (p = 0.002; d = 0.66) and PLA/LD at 10 min (p = 0.002; d = 0.56) for FATox (**Figure 7**).

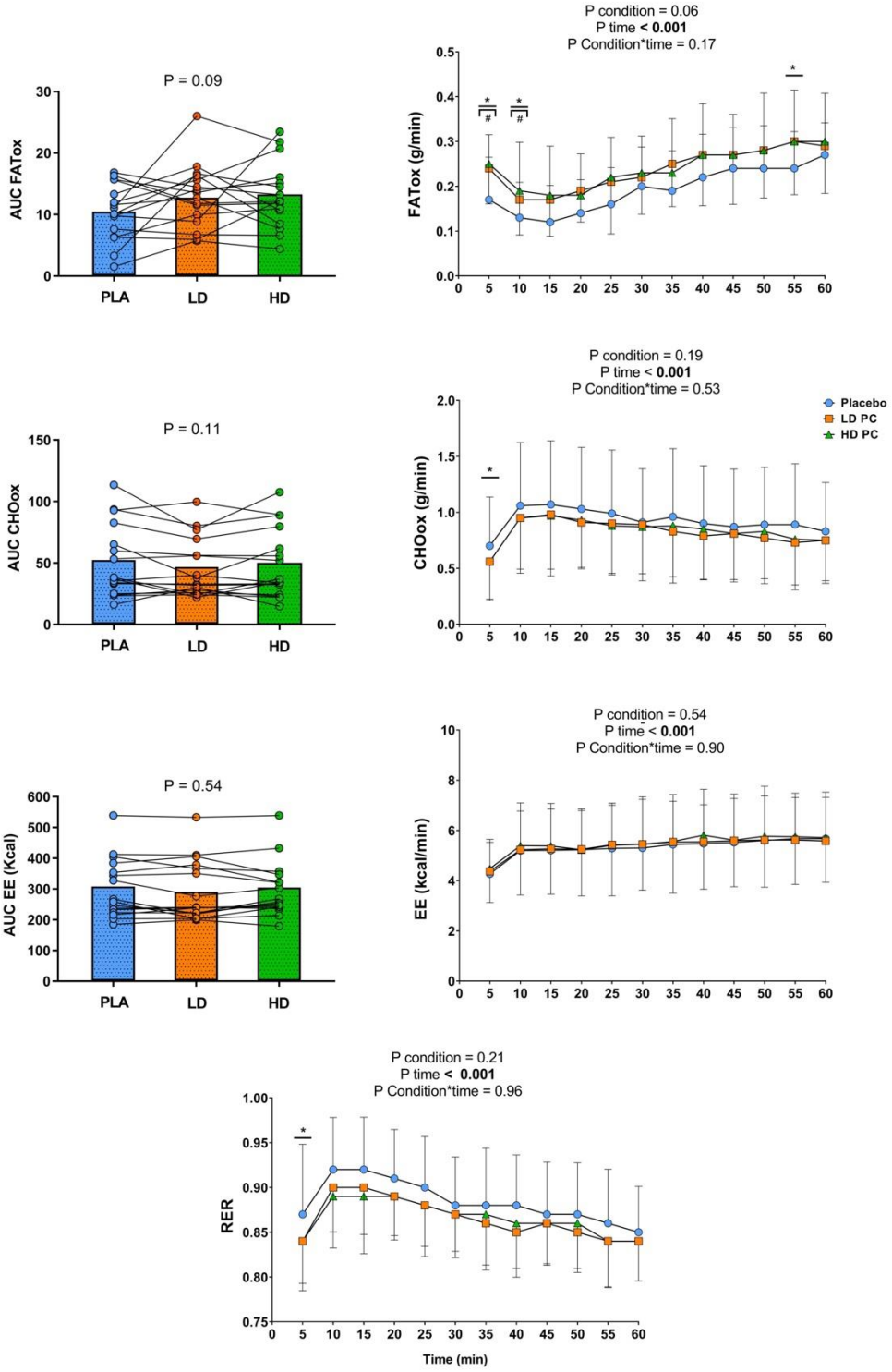

Figure 7. Two-way repeated measures analysis of variance (ANOVA) comparing the effects of phenylcapsaicin and placebo (PLA, HD, LD) across time on substrate oxidation, energy expenditure and respiratory exchange ratio during the 60 min steady-state test. AUC, Area Under the curve; FATox, Fat oxidation; CHOox, Carbohydrates oxidation; EE, Energy expenditure; RER, Respiratory exchange ratio; PLA, Placebo; HD, High dose; LD, Low dose; PC, Phenylcapsaicin. Intra-test analysis (one-way repeated measures ANOVA): * $p_{ANOVA} \leq 0.05$; # $p_{Bonferroni} \leq 0.05$.

4.2.5 Maximal effort test

None of the variables (i.e., heart rate, lactate, RPE-OB at 30 and 90%, and RPE-AM) recorded during the maximal effort test differed between the experimental conditions (p ranged from 0.011 to 0.915) except RPE-OB at 60% due to the lower values of HD compared to LD and PLA ($p = 0.05$) (**Table 12**).

Table 12. One-way repeated measures analysis of variance (ANOVA) or Friedman`s test comparing different variables obtained during the incremental test.

Variable	Conditions			ANOVA or Friedman`s
	PLA	LD	HD	
Final stage (W)	296.4 ± 43.7	291.1 ± 43.4	292.9 ± 37.2	$F = 0.48, p = 0.62, \eta p^2 = 0.05$
$VO2_{max}$ (ml/min/kg)	30.9 ± 7.3	30.5 ± 7.4	30.9 ± 5.8	$F = 0.08, p = 0.91, \eta p^2 = 0.01$
Lactate (mmol/L)	9.0 ± 3.7	8.0 ± 2.8	8.4 ± 3.3	$F = 0.51, p = 0.60, \eta p^2 = 0.04$
RPE-OB 30% (a.u.)	1.4 ± 1.3	1.7 ± 1.2	1.4 ± 0.8	$\chi = 1.08, p = 0.58$
RPE-OB 60% (a.u.)	3.8 ± 1.8	3.9 ± 2.0	3.4 ± 1.9	$\chi = 5.84, p = 0.05$*
RPE-OB 90% (a.u.)	7.1 ± 1.6	7.0 ± 1.7	6.9 ± 1.7	$\chi = 0.62, p = 0.73$
RPE-AM 30% (a.u.)	1.8 ± 1.4	2.1 ± 1.4	1.8 ± 1.3	$\chi = 0.97, p = 0.61$
RPE-AM 60% (a.u.)	4.8 ± 1.5	4.9 ± 1.5	4.4 ± 1.5	$\chi = 3.73, p = 0.15$
RPE-AM 90% (a.u.)	7.9 ± 0.9	7.9 ± 0.9	8.1 ± 1.2	$\chi = 2.60, p = 0.27$
Heart rate 30% (bpm)	106.5 ± 11.1	107.7 ± 9.4	105.8 ± 12.5	$F = 0.44, p = 0.64, \eta p^2 = 0.03$
Heart rate 60% (bpm)	140.2 ± 15.6	139.9 ± 15.0	135.9 ± 13.8	$F = 2.40, p = 0.11, \eta p^2 = 0.15$
Heart rate 90% (bpm)	169.9 ± 15.0	171.0 ± 13.7	170.1 ± 14.9	$F = 0.48, p = 0.49, \eta p^2 = 0.03$

Mean ± standard deviation. ANOVA, Analysis of variance; PLA, Placebo; LD, Low dose; HD, High dose; $VO2_{max}$, maximal oxygen consumption; RPE-OB, general rate of perceived exertion; RPE-AM, quadriceps rate of perceived exertion; a.u., arbitrary units. * Significant difference: $p \leq 0.05$.

4.3 Discussion

The study presented in this chapter aimed to evaluate for the first time the effects of two doses of PC on several metabolic and perceptual responses during a steady-state and a maximal incremental test. The main finding of this study was that LD and HD of PC increase fat oxidation during different stages of the steady-state test and the peak of fat oxidation (FOpeak) in comparison to PLA. Furthermore, both PC doses, exhibited a reduction in CHOox and RER during the first stage of the steady-state test which suggest a shift on substrate oxidation. HD of PC also reduced maximum heart rate during the steady-state test. Contrary to our hypothesis, LD elicited higher RPE-OB values compared to PLA and HD during the steady-state test. Intriguingly, although skin body temperature was not affected by PC, the thermal perception was significantly lower in the supraclavicular area for HD. Nevertheless, only RPE-OB at 60% of maximal intensity was lowered with the HD of PC supplementation during the incremental test. Therefore, the present results suggest that PC enhances fat oxidation during aerobic exercise and modulate the perceptual responses to exercise and the maximum heart rate. However, these perceptual and heart rate effects are only reported when the HD is ingested.

In the present study, the FOpeak during the steady-state test and the rate of fat oxidation during different stages of this test were higher for HD and LD in comparison to PLA (see fig.3). In addition, both PC doses also exhibited lower carbohydrates oxidation and RER values during the first stage of the steady-state test, which may suggest a shift on substrate oxidation induced by PC (de Moura e Silva, Cholewa, Billaut, et al., 2021). These results are reported for the first time with the use of PC. Furthermore, only two studies have previously documented the metabolic effects of a capsaicinoid on substrate oxidation, reporting contradictory findings between them (Lim et al., 1997; Rossi et al., 2022). The increase of fat oxidation reported in the current study might be explained by the exercise protocol employed and the type of supplement ingested (EFSA, 2012; Turck, et al., 2019). Additionally, contrary to previous research, in the present study MFO and FATmax were calculated for each participant which matched the metabolic and mechanical training intensities of the participants. This fact is essential due to different factors such as the exercise intensity, metabolic cart use, or the ergometer employed, influence the metabolic responses during exercise (Amaro-Gahete et al., 2018, 2019). Concerning the type of supplement used, previous studies revealed that capsinoids supplementation does not modulate the metabolic responses during aerobic exercise (Osuna-Prieto et al., 2022; Rossi et al., 2022). Although a MFO protocol was used by Osuna-Prieto et

al. (2022), previous research has reported that capsinoids are hydrolyzed in the gastrointestinal tract, and their metabolites are excreted rapidly after their ingestion (Bernard et al., 2008). Capsiate circulating levels are significantly much lower in comparison to capsaicin levels, and their final linkage with TRPV1 is approximately 1/10 compared to capsaicinoids such as PC (Sasahara et al., 2010). Accordingly, PC might be able to activate in a higher way TRPV1 leading to a greater increase in fatty acid oxidation and increasing glycogen sparing. This finding may be important due to the relevant link between muscle glycogen and performance in aerobic sports (Murray & Rosenbloom, 2018).

Although the TRPV1 temperature threshold is set at 43 ºC on dorsal root ganglia cells, capsaicin seems to decrease the activation threshold to 36.8ºC (Szolcsányi, 2015). In humans, topical capsaicin has been demonstrated to increase heat loss by increasing peripheral vasodilation thereby improving the skin's vasoconstrictive tone, increasing heat dissipation during exercise and increasing thermal perception and heat stress (Botonis et al., 2019). However, oral capsiate supplementation has not exhibited any of these effects on overweight participants (Osuna-Prieto et al., 2022). As our study presents an encapsulated formulation of PC, the null effect on skin body temperature may be related to the nature of the vehicle used. Accordingly, if a non-encapsulated powder formulation might have been ingested, a thermoregulatory response could be expected due to the direct contact of PC with the esophagus and gastrointestinal tissues (Szolcsányi, 2015). However, in the present study, the thermal perception was reduced in the clavicle area for the HD condition. Because PC was encapsulated and a burning reflux effect was not reported by any of the participants, this finding contrasts most literature where capsaicinoids spiciness is discussed (Naves et al., 2019). A plausible explanation for this issue may be that the digestion of the capsules did not produce an irritant effect, albeit they may have increase the internal temperature (i.e., not directly measured with skin temperature assessment) (Patcharatrakul et al., 2020). As a consequence, participants may have experienced a counterregulatory response to PC at the end of the test on the aforementioned area (see fig.2). Moreover, LD seems to be an insufficient dose to alter the thermal perception. Besides, as heat perception contributes to the development of fatigue during exercise, this finding may be helpful to counteract fatigue in aerobic sports (Willmott et al., 2020). Overall, future research should assess if this finding depends on the ingestion of PC instead of traditional capsaicin formulations, the effect of the application vehicle on capsaicin's pungency perception and the plausible existence of a counterregulatory mechanism of PC on thermal perception.

The effects of capsaicin and red peppers on cardiovascular responses are currently controversial due to the vast heterogeneity between protocols and doses used in previous literature (Shirani et al., 2021). However, recent research has shown that supplementation with purified capsaicin does not alter heart rate during an incremental exercise test in a cycloergometer until exhaustion (Giuriato et al., 2022). These studies employed intensities above 80% of $VO2_{max}$ (Grgic et al., 2022), which therefore are not comparably to lower intensities. In the present study, although the medium heart rate did not differ between conditions, HD exhibited a significantly lower maximum heart rate during the steady-state test. As in the present study the intensity was matched in the steady-state test, it is possible that under HD supplementation, participants experienced a higher mechanical efficiency, which may have reduced the relative workload, resulting in lower cardiovascular demands during the test (Mikus et al., 2009). In addition, circulating lactate response across time trended to be lower with both PC doses in the steady-state test. Previous preclinical research has shown that lactate is a potent endogenous inhibitor of TRPV1 activity (de la Roche et al., 2016). According to this possible mechanism, PC may have modulated sarcoplasmic calcium efflux channels lowering lactate levels during both tests (de la Roche et al., 2016). This finding agrees with previous research in resistance training and high-intensity running (de Freitas, Billaut, et al., 2019; de Freitas, Cholewa, Freire, et al., 2018). Furthermore, this lactate lowering effect of PC may also be partially explained by the shift on substrate oxidation produced by this substance and provides novel information about the physiological independence between this effect and the intensity of the exercise used. However, contrary to previous research where capsaicin supplementation led to lower RPE values, in our data, participants showed higher RPE scores under the LD condition, and improvements were not reported with HD compared to PLA (de Freitas, Cholewa, Freire, et al., 2018). The low intensity demanded in this task may be insufficient to report an ergogenic effect of PC on perceptual variables (Jiménez Martínez et al., 2023). However, LD of PC reported for the first time a worsening effect of a capsaicinoid on RPE. Therefore, if PC at LD in low intensity tasks produces a counterregulatory effect on perceptual performance or if the sensitivity of RPE under these novel conditions is altered, should be corroborate in further studies.

Concerning the incremental test, the HD of PC reduced RPE-OB at 60% of the maximal intensity achieved. Additionally, the reduction in RPE values during high-intensity exercise after capsaicin supplementation is well documented (Jiménez-Martínez, Ramirez-Campillo, et al., 2022). During high-intensity exercise capsaicinoids reduce perceived exertion due to TRPV1 activation. TRPV1 are linked to afferent III and IV nerve fibers (Collins et al., 2018).

These fibers influence central fatigue during exercise, which finally reduce motoneuron firing during high-intensity tasks (Jiménez Martínez et al., 2023). For this reason, HD might have produced a "desensitizer" effect leading to a lower perception of exertion in the maximal incremental test. This threshold may not be achieved by the LD. Additionally, these findings align with previous research that has reported that capsaicin supplementation does not increase $VO2_{max}$ or any other metabolic outcome during high-intensity exercise, although the time to exhaustion in interval training is increased (de Freitas, Billaut, et al., 2019).

This study presents essential strengths such as the control of the fasting conditions and the meal before each session, and the randomized, triple-blinded, crossover design. Nonetheless, some limitations should be addressed. Firstly, the study only included active males, and these results may not be extrapolated to other populations. Secondly, these results cannot be extrapolated to other exercise modalities or intensities. Finally, the chronic effects of PC on metabolic responses during exercise should not be extrapolated from this acute study.

4.4 Conclusion

The results of this last study suggest that LD and HD of PC modulate the metabolic response (FATox, CHOox and RER) to exercise and HD of PC reduces maximum heart rate values during aerobic exercise. However, PC only improves the perceptual responses (i.e., RPE-OB and clavicle thermal perception) to exercise when it is consumed in HD.

Chapter 5. Future research.

5. Future research

The findings of this doctoral thesis provide a new background to address the concerns of the use of capsaicin or its analogues on sports performance. In addition, PC has emerged as a valuable replacement of traditional purified capsaicin, eliciting a positive effect on performance while reducing the ergogenic dose required and the common side effects of higher capsaicin doses. In this line, further research is guaranteed to assess the impact of PC on resistance and aerobic exercise.

In chapters 2, 3 and 4 the mechanical and bioenergetic performance effects of PC are well documented. However, the three research projects were developed in trained young males, for this reason, how PC would impact on female athletes or different age ranges should be addressed in future research. This remains especially important on resistance training exercise due to the lack of studies addressing the effects of capsaicin or its analogues in females. Furthermore, although there is one previous study in which males' and females' responses to capsaicin supplementation in bioenergetic variables were compared, that study present important standardization limitations (see chapter 4) that guarantees future research in this topic.

Bearing in mind the procedures employed in the two resistance training studies, both evaluated lower-limb multi-joint tasks (e.g., CMJ, SQ), which makes necessary to conduct future research on upper-limbs and/or single-joint exercises. This could be of primary importance due to the higher fatigue tolerance that participants may be able to afford in these protocols in comparison to the SQ exercise.

Moreover, the evaluation of the neuromuscular properties of the skeletal muscle during resistance exercise should be conducted with the use of high-density electromyography in future research. This tool can provide a deeper overview of the individual behavior of motor units and estimate how neural drive change across conditions, its velocity and distribution in different region of the nervous system, as well as the origin of the neural impulse. In this sense, in the study presented in chapter 2 a bipolar, parallel-bar surface electromyographic wireless device was employed, which is able to detect the neural strategies of the skeletal-muscle but not to discriminate the aforementioned neuromuscular variables.

Concerning muscle damage and recovery, future research should assess other new biomarkers (e.g., creatine kinase, myoglobin, alanine aminotransferase) not previously measured during different timeslots after a strenuous resistance exercise session. In chapter 3, only urea was evaluated 40 minutes post exercise and AST 24 hours post previous session. In

this doctoral thesis only high-intensity submaximal protocols were employed due to the use of velocity as an objective measure of performance. Hence, future studies should address the effects of PC on muscle damage and protein breakdown when training volume is not matched and sets are performed until muscle failure to increase the practical applications of the findings. Furthermore, the recovery and muscle damage effects of PC should be evaluated in other tasks such as aerobic sports.

Regarding the metabolic and bioenergetic responses to exercise, there is a vast field pending to be explored about the use of PC as ergogenic aid. Accordingly, in the current thesis only FATmax and maximal incremental intensities were tested, which provide a wide range of efforts unaddressed. In addition, although it was not the main purpose of the third study, some concerns about skin temperature should be mentioned. As it is detailed in chapter 4, the clavicle thermal perception changed across time. Although skin thermometers were not used in this anatomical area, evaluating the thermal changes of clavicle could be of special interest due to the close relationship between it, brown adipose tissue distribution and its metabolic activity, which suggests that PC may be able to stimulate the acute thermal responses of brown adipose tissue.

Finally, all the studies presented in this doctoral thesis were designed as acute research projects. For this reason, the applicability of PC in the long-term remains unclear and should be evaluated to extrapolate the current findings to a greater extent in the strength and conditioning and sports performance fields.

Chapter 6. Conclusions.

6. Conclusions

The present doctoral thesis compendium includes two articles concerning the effects of PC on resistance exercise performance and recovery and one article about the bioenergetic as well as aerobic impact of PC supplementation. All the articles that composed the present doctoral thesis as well as all the scientific congress communications and publications not directly related to this thesis, but conducted and/or published during the doctoral period, can be found listed in chapter 8 (appendices).

The conclusions of each of the three studies are detailed hereunder in three sections, each one addressing each of the studies included, and presented in the development order.

6.1 Effects of phenylcapsaicin on mechanical performance and neuromuscular activity

According to the first hypothesis, the findings highlighted in chapter 2 demonstrate that acute PC supplementation at a dose of 2.5 mg is able to enhance dynamic resistance exercise performance when more than 1 exercise is completed. Consistently, mechanical fatigue after a submaximal exercise may be delayed and attenuated by PC. However, this hypothesis was only verified for the HD and not for the LD. Moreover, contrary to what it was hypothesized, PC supplementation seems not to be effective modulating the neural responses to exercise. Overall, PC can be effectively used as a "counter-fatigue" agent during resistance training sessions without impacting in the neural output.

6.2 Effects of phenylcapsaicin on muscle damage, protein breakdown, recovery and perceptual responses

The results of the second study of this doctoral thesis (chapter 3) reveal that a dose of 2.5 mg of PC ingested 45 minutes prior to resistance exercise increases SQ performance and reduces muscle damage, as well as quadriceps RPE-AM in strength-trained participants in comparison to a 0.625 mg dose and PLA. Accordingly, the initial hypothesis suggesting that PC could reduce perceived exertion was partially verified (only for HD), albeit the hypothesis presented about a higher time course of recovery was not verified, probably due to the volume of the protocol was matched. Collectively, a HD of PC could be used as a new nutritional ergogenic aid in resistance trained men.

6.3 Effects of phenylcapsaicin on substrates oxidation, energy expenditure, metabolic, perceptual and thermal responses during exercise

The last study of this doctoral thesis (chapter 4) shows that, both, 0.625 and 2.5 mg doses of PC modulate the metabolic responses (i.e., substrate oxidation) to aerobic exercise

according to the initial hypothesis presented. Moreover, the higher dose of PC reduces maximum heart rate values during aerobic tests as it was previously hypothesized. However, the third hypothesis was only partially verified because the perceptual benefits of PC (i.e., RPE-OB and clavicle thermal perception) are only obtained when it is consumed in HD. Therefore, PC can be used as a tool to maximize fat oxidation and the physiological responses to aerobic exercise.

Chapter 7. References.

7. References

AlAli, M., Alqubaisy, M., Aljaafari, M. N., AlAli, A. O., Baqais, L., Molouki, A., Abushelaibi, A., Lai, K.-S., & Lim, S.-H. E. (2021). Nutraceuticals: Transformation of conventional foods into health promoters/disease preventers and safety considerations. *Molecules*, *26*(9), 2540. https://doi.org/10.3390/molecules26092540

Alix-Fages, C., Baz-Valle, E., González-Cano, H., Jiménez-Martínez, P., & Balsalobre-Fernández, C. (2023). Mental fatigue from smartphone use or stroop task does not affect bench press force-velocity profile, one-repetition maximum, or vertical jump performance. *Motor control*, *27*(3), 631–644. https://doi.org/10.1123/mc.2022-0133

Alix-Fages, C., Del Vecchio, A., Baz-Valle, E., Santos-Concejero, J., & Balsalobre-Fernández, C. (2022). The role of the neural stimulus in regulating skeletal muscle hypertrophy. *European Journal of Applied Physiology*. https://doi.org/10.1007/s00421-022-04906-6

Alix-Fages, C., García-Ramos, A., Calderón-Nadal, G., Colomer-Poveda, D., Romero-Arenas, S., Fernández-del-Olmo, M., & Márquez, G. (2020). Anodal transcranial direct current stimulation enhances strength training volume but not the force–velocity profile. *European Journal of Applied Physiology*, *120*(8), 1881-1891. https://doi.org/10.1007/s00421-020-04417-2

Alix-Fages, C., Grgic, J., Jiménez-Martínez, P., Baz-Valle, E., & Balsalobre-Fernández, C. (2022). Effects of mental fatigue on strength endurance: A systematic review and meta-analysis. *Motor control*, *27*(2), 442–461. https://doi.org/10.1123/mc.2022-0051

Allison, G. T., & Fujiwara, T. (2002). The relationship between EMG median frequency and low frequency band amplitude changes at different levels of muscle capacity. *Clinical Biomechanics*, *17*(6), 464-469. https://doi.org/10.1016/S0268-0033(02)00033-5

Amann, M., Blain, G. M., Proctor, L. T., Sebranek, J. J., Pegelow, D. F., & Dempsey, J. A. (2010). Group III and IV muscle afferents contribute to ventilatory and cardiovascular response to rhythmic exercise in humans. *Journal of Applied Physiology*, *109*(4), 966-976. https://doi.org/10.1152/japplphysiol.00462.2010

Amaro-Gahete, F. J., Sanchez-Delgado, G., Jurado-Fasoli, L., De-la-O, A., Castillo, M. J., Helge, J. W., & Ruiz, J. R. (2019). Assessment of maximal fat oxidation during exercise: A systematic review. *Scandinavian Journal of Medicine & Science in Sports*, *29*(7), 910-921. https://doi.org/10.1111/sms.13424

Amaro-Gahete, F. J., Sanchez-Delgado, G., & Ruiz, J. R. (2018). Commentary: Contextualising maximal fat oxidation during exercise: Determinants and normative values.

Frontiers in Physiology, *9*, 1460. https://doi.org/10.3389/fphys.2018.01460

Arora, V., Campbell, J. N., & Chung, M.K. (2021). Fight fire with fire: Neurobiology of capsaicin-induced analgesia for chronic pain. *Pharmacology & Therapeutics*, *220*, 107743. https://doi.org/10.1016/j.pharmthera.2020.107743

Baamonde, A., Lastra, A., Juarez, L., Hidalgo, A., & Menéndez, L. (2005). TRPV1 desensitisation and endogenous vanilloid involvement in the enhanced analgesia induced by capsaicin in inflamed tissues. *Brain Research Bulletin*, *67*(6), 476-481. https://doi.org/10.1016/j.brainresbull.2005.07.001

Baird, M. F., Graham, S. M., Baker, J. S., & Bickerstaff, G. F. (2012). Creatine-kinase- and exercise-related muscle damage implications for muscle performance and recovery. *Journal of Nutrition and Metabolism*, *2012*, 1-13. https://doi.org/10.1155/2012/960363

Basith, S., Cui, M., Hong, S., & Choi, S. (2016). Harnessing the therapeutic potential of capsaicin and its analogues in pain and other diseases. *Molecules*, *21*(8), 966. https://doi.org/10.3390/molecules21080966

Bauer, P., Sansone, P., Mitter, B., Makivic, B., Seitz, L. B., & Tschan, H. (2019). Acute effects of back squats on countermovement jump performance across multiple sets of a contrast training protocol in resistance-trained men. *Journal of Strength and Conditioning Research*, *33*(4), 995-1000. https://doi.org/10.1519/JSC.0000000000002422

Bernard, B. K., Watanabe, E., Kodama, T., Tsubuku, S., Otabe, A., Nakajima, M., Masumori, S., Shimada, S., Tanaka, J., & Masuyama, T. (2008). Studies of the toxicological potential of capsinoids: V. Genotoxicity studies of dihydrocapsiate. *International Journal of Toxicology*, *27*(SUPPL. 3), 59-72. https://doi.org/10.1080/10915810802513536

Bessa, A. L., Oliveira, V. N., Agostini, G. G., Oliveira, R. J., Oliveira, A. C., White, G. E., Wells, G. D., Teixeira, D. N., & Espindola, F. S. (2016). Exercise intensity and recovery: biomarkers of injury, inflammation, and oxidative stress. *Journal of strength and conditioning research*, *30*(2), 311–319. https://doi.org/10.1519/JSC.0b013e31828f1ee9

Bigland-Ritchie, B. R., Dawson, N. J., Johansson, R. S., & Lippold, O. C. (1986). Reflex origin for the slowing of motoneurone firing rates in fatigue of human voluntary contractions. *The Journal of Physiology*, *379*(1), 451-459. https://doi.org/10.1113/jphysiol.1986.sp016263

Blain, G. M., Mangum, T. S., Sidhu, S. K., Weavil, J. C., Hureau, T. J., Jessop, J. E., Bledsoe, A. D., Richardson, R. S., & Amann, M. (2016). Group III/IV muscle afferents limit the intramuscular metabolic perturbation during whole body exercise in humans. *Journal of Physiology*, *594*(18), 5303-5315. https://doi.org/10.1113/JP272283

Boat, R., & Taylor, I. M. (2017). Prior self-control exertion and perceptions of pain

during a physically demanding task. *Psychology of Sport and Exercise*, *33*, 1-6. https://doi.org/10.1016/j.psychsport.2017.07.005

Botonis, P. G., Miliotis, P. G., Kounalakis, S. N., Koskolou, M. D., & Geladas, N. D. (2019). Thermoregulatory and cardiovascular effects of capsaicin application on human skin during dynamic exercise to temperate and warm conditions. *Physiological Reports*, *7*(24). https://doi.org/10.14814/phy2.14325

Broxterman, R. M., Hureau, T. J., Layec, G., Morgan, D. E., Bledsoe, A. D., Jessop, J. E., Amann, M., & Richardson, R. S. (2018). Influence of group III/IV muscle afferents on small muscle mass exercise performance: A bioenergetics perspective. *Journal of Physiology*, *596*(12), 2301-2314. https://doi.org/10.1113/JP275817

Carroll, T. J., Taylor, J. L., & Gandevia, S. C. (2017). Recovery of central and peripheral neuromuscular fatigue after exercise. *Journal of Applied Physiology*, *122*(5), 1068-1076. https://doi.org/10.1152/japplphysiol.00775.2016

Cattozzo, G., Franzini, C., Hubbuch, A., & Tritschler, W. (1988). Evaluation of determination of uric acid in serum and whole blood with the Reflotron. *Clinical Chemistry*, *34*(2), 414-416. https://doi.org/10.1093/clinchem/34.2.414

Chait, A., & den Hartigh, L. J. (2020). Adipose tissue distribution, inflammation and its metabolic consequences, including diabetes and cardiovascular disease. *Frontiers in Cardiovascular Medicine*, *7*, 22. https://doi.org/10.3389/fcvm.2020.00022

Chenevière, X., Borrani, F., Sangsue, D., Gojanovic, B., & Malatesta, D. (2011). Gender differences in whole-body fat oxidation kinetics during exercise. *Applied Physiology, Nutrition, and Metabolism*, *36*(1), 88-95. https://doi.org/10.1139/H10-086

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences* (2nd ed.). Lawrence Erlbaum Associates. https://doi.org/10.1234/12345678

Colado, J. C., Pedrosa, F. M., Juesas, A., Gargallo, P., Carrasco, J. J., Flandez, J., Chupel, M. U., Teixeira, A. M., & Naclerio, F. (2018). Concurrent validation of the OMNI-Resistance Exercise Scale of perceived exertion with elastic bands in the elderly. *Experimental Gerontology, 103,* 11-16. https://doi.org/10.1016/j.exger.2017.12.009

Collins, B. W., Pearcey, G. E. P., Buckle, N. C. M., Power, K. E., & Button, D. C. (2018). Neuromuscular fatigue during repeated sprint exercise: Underlying physiology and methodological considerations. *Applied Physiology, Nutrition, and Metabolism*, *43*(11), 1166-1175. https://doi.org/10.1139/apnm-2018-0080

Cross, B. L., Parker, D., Langan, S. P., & Grosicki, G. J. (2020). Effect of a commercially available low-Dose capsaicin supplement on knee extensor contractile

function. *International journal of exercise science*, *13*(2), 312–318.

Crotty, N. M., Boland, M., Mahony, N., Donne, B., & Fleming, N. (2021). Reliability and validity of the Lactate Pro 2 Analyzer. *Measurement in Physical Education and Exercise Science*, *25*(3), 202-211. https://doi.org/10.1080/1091367X.2020.1865966

da Silva, B. V. C., Mota, G. R., Marocolo, M., Martin, J. S., & Prado, L. S. (2022). Acute supplementation with capsaicin enhances upper-limb performance in male jiu-jitsu athletes. *Sports*, *10*(8), 120. https://doi.org/10.3390/sports10080120

Daher, J., Mallick, M., & El Khoury, D. (2022). Prevalence of dietary supplement use among athletes worldwide: A scoping review. *Nutrients*, *14*(19), 4109. https://doi.org/10.3390/nu14194109

de Freitas, M. C., Billaut, F., Panissa, V. L. G., Rossi, F. E., Figueiredo, C., Caperuto, E. C., & Lira, F. S. (2019). Capsaicin supplementation increases time to exhaustion in high-intensity intermittent exercise without modifying metabolic responses in physically active men. *European Journal of Applied Physiology*, *119*(4), 971-979. https://doi.org/10.1007/s00421-019-04086-w

de Freitas, M. C., Cholewa, J. M., Freire, R. V., Carmo, B. A., Bottan, J., Bratfich, M., Della Bandeira, M. P., Gonçalves, D. C., Caperuto, E. C., Lira, F. S., & Rossi, F. E. (2018). Acute capsaicin supplementation improves resistance training performance in trained men. *Journal of Strength and Conditioning Research*, *32*(8), 2227-2232. https://doi.org/10.1519/JSC.0000000000002109

de Freitas, M. C., Cholewa, J. M., Gobbo, L. A., de Oliveira, J. V. N. S., Lira, F. S., & Rossi, F. E. (2018). Acute capsaicin supplementation improves 1,500-m running time-trial performance and rate of perceived exertion in physically active adults. *Journal of Strength and Conditioning Research*, *32*(2), 572-577. https://doi.org/10.1519/JSC.0000000000002329

de Freitas, M. C., Cholewa, J. M., Panissa, V. L. G., Toloi, G. G., Netto, H. C., Zanini de Freitas, C., Freire, R. V., Lira, F. S., & Rossi, F. E. (2019). Acute capsaicin supplementation improved resistance exercise performance performed after a high-intensity intermittent running in resistance-trained men. *Journal of Strength and Conditioning Research, Publish Ahead of Print*. https://doi.org/10.1519/JSC.0000000000003431

de la Roche, J., Walther, I., Leonow, W., Hage, A., Eberhardt, M., Fischer, M., Reeh, P. W., Sauer, S., & Leffler, A. (2016). Lactate is a potent inhibitor of the capsaicin receptor TRPV1. *Scientific Reports*, *6*(1), 36740. https://doi.org/10.1038/srep36740

De Luca, C. J., & Contessa, P. (2012). Hierarchical control of motor units in voluntary contractions. *Journal of Neurophysiology*, *107*(1), 178-195.

https://doi.org/10.1152/jn.00961.2010

de Morton, N. A. (2009). The PEDro scale is a valid measure of the methodological quality of clinical trials: A demographic study. *Australian Journal of Physiotherapy*, *55*(2), 129-133. https://doi.org/10.1016/S0004-9514(09)70043-1

de Moura e Silva, V. E. L., Cholewa, J. M., Billaut, F., Jäger, R., de Freitas, M. C., Lira, F. S., & Rossi, F. E. (2021). Capsaicinoid and capsinoids as an ergogenic aid: A systematic review and the potential mechanisms involved. *International Journal of Sports Physiology and Performance*, *16*(4), 464-473. https://doi.org/10.1123/ijspp.2020-0677

de Moura e Silva, V. E. L., Cholewa, J. M., Jäger, R., Zanchi, N. E., de Freitas, M. C., de Moura, R. C., Barros, E. M. L., Antunes, B. M., Caperuto, E. C., Ribeiro, S. L. G., Lira, F. S., Pereira dos Santos, M. A., & Rossi, F. E. (2021). Chronic capsiate supplementation increases fat-free mass and upper body strength but not the inflammatory response to resistance exercise in young untrained men: A randomized, placebo-controlled and double-blind study. *Journal of the International Society of Sports Nutrition*, *18*(1), 50. https://doi.org/10.1186/s12970-021-00446-0

Del Vecchio, A., Casolo, A., Negro, F., Scorcelletti, M., Bazzucchi, I., Enoka, R., Felici, F., & Farina, D. (2019). The increase in muscle force after 4 weeks of strength training is mediated by adaptations in motor unit recruitment and rate coding. *The Journal of Physiology*, *597*(7), 1873-1887. https://doi.org/10.1113/JP277250

Del Vecchio, A., Úbeda, A., Sartori, M., Azorín, J. M., Felici, F., & Farina, D. (2018). Central nervous system modulates the neuromechanical delay in a broad range for the control of muscle force. *Journal of Applied Physiology*, *125*(5), 1404-1410. https://doi.org/10.1152/japplphysiol.00135.2018

Duncan, R. E., Ahmadian, M., Jaworski, K., Sarkadi-Nagy, E., & Sul, H. S. (2007). Regulation of lipolysis in adipocytes. *Annual Review of Nutrition*, *27*(1), 79-101. https://doi.org/10.1146/annurev.nutr.27.061406.093734

EFSA. (2012). Scientific opinion on dihydrocapsiate. *EFSA Journal*, *10*(7), 1-28. https://doi.org/10.2903/j.efsa.2012.2812

Evans, V., Koh, R. G. L., Duarte, F. C. K., Linde, L., Amiri, M., & Kumbhare, D. (2021). A randomized double blinded placebo controlled study to evaluate motor unit abnormalities after experimentally induced sensitization using capsaicin. *Scientific Reports*, *11*(1), 13793. https://doi.org/10.1038/s41598-021-93188-7

Fattori, V., Hohmann, M., Rossaneis, A., Pinho-Ribeiro, F., & Verri, W. (2016). Capsaicin: Current understanding of its mechanisms and therapy of pain and other pre-clinical

and clinical uses. *Molecules*, *21*(7), 844. https://doi.org/10.3390/molecules21070844

Fernandes, A. I., & Jozala, A. F. (2022). Polymers enhancing bioavailability in drug delivery. *Pharmaceutics*, *14*(10), 2199. https://doi.org/10.3390/pharmaceutics14102199

Framroze, Z. (2022). Placebo controlled study to investigate the relationship between lowering serum zonulin levels and improved body weight composition using a daily oral dose of phenylcapsaicin. *International Journal of Clinical Skills*, 7.

Frayn, K. N. (2016). Calculation of substrate oxidation rates in vivo from gaseous exchange. *Journal of Applied Physiology*, *121*(6), 628-634.

Giuriato, G., Venturelli, M., Matias, A., Soares, E. M. K. V. K., Gaetgens, J., Frederick, K. A., & Ives, S. J. (2022). Capsaicin and its effect on exercise performance, fatigue and inflammation after exercise. *Nutrients*, *14*(2). https://doi.org/10.3390/nu14020232

Glatthorn, J. F., Gouge, S., Nussbaumer, S., Stauffacher, S., Impellizzeri, F. M., & Maffiuletti, N. A. (2011). Validity and reliability of optojump photoelectric cells for estimating vertical jump height. *Journal of Strength and Conditioning Research*, *25*(2), 556-560. https://doi.org/10.1519/JSC.0b013e3181ccb18d

Gomez-Orellana, I. (2005). Strategies to improve oral drug bioavailability. *Expert Opinion on Drug Delivery*, *2*(3), 419-433. https://doi.org/10.1517/17425247.2.3.419

González-Badillo, J. J., & Gorostiaga, E. (1995). *Fundamentos del entrenamiento de la fuerza. Barcelona: Inde. Pp. 53-63.* Inde.

González-Badillo, J. J., & Sánchez-Medina, L. (2010). Movement velocity as a measure of loading intensity in resistance training. *International Journal of Sports Medicine*, *31*(05), 347-352. https://doi.org/10.1055/s-0030-1248333

González-Badillo, J. J., Yañez-García, J. M., Mora-Custodio, R., & Rodríguez-Rosell, D. (2017). Velocity loss as a variable for monitoring resistance exercise. *International Journal of Sports Medicine*, *38*(03), 217-225. https://doi.org/10.1055/s-0042-120324

González-Badillo, J., Rodríguez-Rosell, D., Sánchez-Medina, L., Ribas, J., López-López, C., Mora-Custodio, R., Yañez-García, J., & Pareja-Blanco, F. (2015). Short-term recovery following resistance exercise leading or not to failure. *International Journal of Sports Medicine*, *37*(04), 295-304. https://doi.org/10.1055/s-0035-1564254

González-Cano H, Jiménez-Martínez P, Baz-Valle E, Contreras C, Colado J.C., Alix-Fages C. (2023). Nutritional and supplementation strategies of Spanish natural elite bodybuilders in precontest. *Gazz Med Ital - Arch Sci Med*, 182:32-42. DOI: 10.23736/S0393-3660.22.04877-X

Grgic, J., Memon, A. R., Chen, S., Ramirez-Campillo, R., Barreto, G., Haugen, M. E.,

& Schoenfeld, B. J. (2022). Effects of capsaicin and capsiate on endurance performance: A meta-analysis. *Nutrients* (Vol. 14, Número 21). https://doi.org/10.3390/nu14214531

Gutiérrez-Hellín, J., & Del Coso, J. (2016). Acute p-synephrine ingestion increases fat oxidation rate during exercise: P-Synephrine and fat oxidation during exercise. *British Journal of Clinical Pharmacology*, *82*(2), 362-368. https://doi.org/10.1111/bcp.12952

Gutiérrez-Hellín, J., Ruiz-Moreno, C., & Del Coso, J. (2020). Acute p-synephrine ingestion increases whole-body fat oxidation during 1-h of cycling at Fatmax. *European Journal of Nutrition*, *59*(7), 3341-3345. https://doi.org/10.1007/s00394-019-02101-6

Hayman, M., & Kam, P. C. A. (2008). Capsaicin: A review of its pharmacology and clinical applications. *Current Anaesthesia & Critical Care*, *19*(5-6), 338-343. https://doi.org/10.1016/j.cacc.2008.07.003

Heckman, C. J., & Enoka, R. M. (2004). Physiology of the motor neuron and the motor unit. En *Handbook of Clinical Neurophysiology* (Vol. 4, pp. 119-147). Elsevier. https://doi.org/10.1016/S1567-4231(04)04006-7

Hermens, H. J., Freriks, B., Disselhorst-Klug, C., & Rau, G. (2000). Development of recommendations for SEMG sensors and sensor placement procedures. *Journal of Electromyography and Kinesiology*, *10*(5), 361-374. https://doi.org/10.1016/S1050-6411(00)00027-4

Hernando, D., Garatachea, N., Almeida, R., Casajús, J. A., & Bailón, R. (2018). Validation of heart rate Monitor Polar RS800 for heart rate variability analysis during exercise. *Journal of Strength and Conditioning Research*, *32*(3), 716-725. https://doi.org/10.1519/JSC.0000000000001662

Hunter, S. K., Duchateau, J., & Enoka, R. M. (2004). Muscle fatigue and the mechanisms of task failure. *Exercise and Sport Sciences Reviews*, *32*(2), 44-49. https://doi.org/10.1097/00003677-200404000-00002

Ito, N., Ruegg, U. T., Kudo, A., Miyagoe-Suzuki, Y., & Takeda, S. (2013). Capsaicin mimics mechanical load-induced intracellular signaling events: Involvement of TRPV1-mediated calcium signaling in induction of skeletal muscle hypertrophy. *Channels*, *7*(3), 221-224. https://doi.org/10.4161/chan.24583

Izquierdo, M., González-Izal, M., Navarro-Amezqueta, I., Calbet, J. A., Ibañez, J., Malanda, A., Mallor, F., Häkkinen, K., Kraemer, W. J., & Gorostiaga, E. M. (2011). Effects of strength training on muscle fatigue mapping from surface EMG and blood metabolites. *Medicine and science in sports and exercise*, *43*(2), 303–311. https://doi.org/10.1249/MSS.0b013e3181edfa96

Jiménez Martínez, P., Cornejo Daza, P. J., Sánchez-Valdepeñas, J., Asín-Izquierdo, I., Cano-Castillo, C., Alix-Fages, C., Pareja-blanco, F., & Colado, J. C. (2023). Effects of different phenylcapsaicin doses on resistance training performance, muscle damage, protein breakdown, metabolic response and recovery: A randomized, triple-blinded, placebo-controlled, crossover trial. *Journal of the International Society of Sports Nutrition*, *20*(1). https://doi.org/10.1080/15502783.2023.2204083

Jiménez-Martínez, P., Alix-Fages, C., Janicijevic, D., Miras-Moreno, S., Chacón-Ventura, S., Martín-Olmedo, J. J., De La Cruz-Márquez, J. C., Osuna-Prieto, F. J., Jurado-Fasoli, L., Amaro-Gahete, F. J., García-Ramos, A., & Colado, J. C. (2023). Effects of phenylcapsaicin on aerobic capacity and physiological parameters in active young males: A randomized, triple-blinded, placebo-controlled, crossover trial. *Frontiers in Physiology*, *14*, 1190345. https://doi.org/10.3389/fphys.2023.1190345

Jiménez-Martínez, P., Ramirez-Campillo, R., Flandez, J., Alix-Fages, C., Baz-Valle, E., & Colado, J. C. (2022). Effects of oral capsaicinoids and capsinoids supplementation on resistance and high intensity interval training: A systematic review of randomized controlled trials Effects of oral capsaicinoids and capsinoids supplementation on resistance and high intens. *Journal of Human Sport & Exercise*, 75-91. https://doi.org/10.14198/jhse.2023.182.09

Jiménez-Martínez, P., Sánchez-Valdepeñas, J., Cornejo-Daza, P. J., Cano-Castillo, C., Asín-Izquierdo, I., Alix-Fages, C., Pareja-Blanco, F., & Colado, J. C. (2023). Effects of different phenylcapsaicin doses on neuromuscular activity and mechanical performance in trained male subjects: A randomized, triple-blinded, crossover, placebo-controlled trial. *Frontiers in Physiology*, 14. https://doi.org/10.3389/fphys.2023.1215644

Josse, A. R., Sherriffs, S. S., Holwerda, A. M., Andrews, R., Staples, A. W., & Phillips, S. M. (2010). Effects of capsinoid ingestion on energy expenditure and lipid oxidation at rest and during exercise. *Nutrition & Metabolism*, *7*, 65. https://doi.org/10.1186/1743-7075-7-65

Kamiński, M., Kręgielska-Narożna, M., & Bogdański, P. (2020). Determination of the popularity of dietary supplements using google search rankings. *Nutrients*, *12*(4), 908. https://doi.org/10.3390/nu12040908

Kattla, S., & Lowery, M. M. (2010). Fatigue related changes in electromyographic coherence between synergistic hand muscles. *Experimental Brain Research*, *202*(1), 89-99. https://doi.org/10.1007/s00221-009-2110-0

Kaufman, M. P., Iwamoto, G. A., Longhurst, J. C., & Mitchell, J. H. (1982). Effects of capsaicin and bradykinin on afferent fibers with ending in skeletal muscle. *Circulation Research*, *50*(1), 133-139. https://doi.org/10.1161/01.RES.50.1.133

Knotkova, H., Pappagallo, M., & Szallasi, A. (2008). Capsaicin (TRPV1 agonist) therapy for pain relief: Farewell or revival? *The Clinical Journal of Pain*, *24*(2), 142-154. https://doi.org/10.1097/AJP.0b013e318158ed9e

Lang, Y., Kisaka, H., Sugiyama, R., Nomura, K., Morita, A., Watanabe, T., Tanaka, Y., Yazawa, S., & Miwa, T. (2009). Functional loss of pAMT results in biosynthesis of capsinoids, capsaicinoid analogs, in *Capsicum annuum* cv. CH-19 Sweet. *The Plant Journal*, *59*(6), 953-961. https://doi.org/10.1111/j.1365-313X.2009.03921.x

Laurent, C. M., Green, J. M., Bishop, P. A., Sjökvist, J., Schumacker, R. E., Richardson, M. T., & Curtner-Smith, M. (2011). A practical approach to monitoring recovery: development of a perceived recovery status scale. *Journal of strength and conditioning research*, *25*(3), 620–628. https://doi.org/10.1519/JSC.0b013e3181c69ec6

Lebovitz, E. E., Keller, J. M., Kominsky, H., Kaszas, K., Maric, D., & Iadarola, M. J. (2012). Positive allosteric modulation of TRPV1 as a novel analgesic mechanism. *Molecular pain*, *8*, 70. https://doi.org/10.1186/1744-8069-8-70

Lejeune, M. P. G. M., Kovacs, E. M. R., & Westerterp-Plantenga, M. S. (2003). Effect of capsaicin on substrate oxidation and weight maintenance after modest body-weight loss in human subjects. *The British Journal of Nutrition*, *90*(3), 651-659. https://doi.org/10.1079/bjn2003938

Lim, K., Yoshioka, M., Kikuza, S., Kiyonaga, A., Tanaka, H., Shindo, M., & Suzuk, M. (1997). Dietary red pepper ingestion increases carbohydrate oxidation at rest and during exercise in runners. *Medicine & Science in Sports & Exercise*, *29*(3).

Luo, X. J., Peng, J., & Li, Y. J. (2011). Recent advances in the study on capsaicinoids and capsinoids. *European Journal of Pharmacology*, *650*(1), 1-7. https://doi.org/10.1016/j.ejphar.2010.09.074

Martinez-Tellez, B., Sanchez-Delgado, G., Acosta, F. M., Alcantara, J. M. A., Boon, M. R., Rensen, P. C. N., & Ruiz, J. R. (2017). Differences between the most used equations in BAT-human studies to estimate parameters of skin temperature in young lean men. *Scientific Reports*, *7*(1), 10530. https://doi.org/10.1038/s41598-017-10444-5

Martinopoulou, K., Donti, O., Sands, W. A., Terzis, G., & Bogdanis, G. C. (2022). Evaluation of the isometric and dynamic rates of force development in multi-Joint muscle actions. *Journal of Human Kinetics*, *81*(1), 135-148. https://doi.org/10.2478/hukin-2021-0130

Maughan, R. J., Burke, L. M., Dvorak, J., Larson-Meyer, D. E., Peeling, P., Phillips, S. M., Rawson, E. S., Walsh, N. P., Garthe, I., Geyer, H., Meeusen, R., van Loon, L. J. C., Shirreffs, S. M., Spriet, L. L., Stuart, M., Vernec, A., Currell, K., Ali, V. M., Budgett, R. G.,

Ljungqvist, A., … Engebretsen, L. (2018). IOC consensus statement: dietary supplements and the high-performance athlete. *British journal of sports medicine*, *52*(7), 439–455. https://doi.org/10.1136/bjsports-2018-099027

Maunder, E., Plews, D. J., & Kilding, A. E. (2018). Contextualising maximal fat oxidation during exercise: Determinants and normative values. *Frontiers in Physiology, 9*, 599. https://doi.org/10.3389/fphys.2018.00599

Mendez-Villanueva, A., Hamer, P., & Bishop, D. (2008). Fatigue in repeated-sprint exercise is related to muscle power factors and reduced neuromuscular activity. *European journal of applied physiology*, *103*(4), 411–419. https://doi.org/10.1007/s00421-008-0723-9

Midgley, A. W., McNaughton, L. R., Polman, R., & Marchant, D. (2007). Criteria for determination of maximal oxygen uptake: A brief critique and recommendations for future research. *Sports Medicine,* 37(12), 1019-1028. https://doi.org/10.2165/00007256-200737120-00002

Mikus, C. R., Earnest, C. P., Blair, S. N., & Church, T. S. (2009). Heart rate and exercise intensity during training: Observations from the DREW Study. *British Journal of Sports Medicine*, *43*(10), 750-755. https://doi.org/10.1136/bjsm.2008.046342

Montuori, P., Loperto, I., Paolo, C., Castrianni, D., Nubi, R., De Rosa, E., Palladino, R., & Triassi, M. (2021). Bodybuilding, dietary supplements and hormones use: Behaviour and determinant analysis in young bodybuilders. *BMC Sports Science, Medicine and Rehabilitation*, *13*(1), 147. https://doi.org/10.1186/s13102-021-00378-x

Mora-Custodio, R., Rodríguez-Rosell, D., Yáñez-García, J. M., Sánchez-Moreno, M., Pareja-Blanco, F., & González-Badillo, J. J. (2018). Effect of different inter-repetition rest intervals across four load intensities on velocity loss and blood lactate concentration during full squat exercise. *Journal of sports sciences*, *36*(24), 2856–2864. https://doi.org/10.1080/02640414.2018.1480052

Morán-Navarro, R., Martínez-Cava, A., Sánchez-Medina, L., Mora-Rodríguez, R., González-Badillo, J. J., & Pallarés, J. G. (2019). Movement velocity as a measure of level of effort during resistance exercise. *Journal of Strength and Conditioning Research*, *33*(6), 1496-1504. https://doi.org/10.1519/JSC.0000000000002017

Murray, B., & Rosenbloom, C. (2018). Fundamentals of glycogen metabolism for coaches and athletes. *Nutrition Reviews*, *76*(4), 243-259. https://doi.org/10.1093/NUTRIT/NUY001

Naves, E. R., de Ávila Silva, L., Sulpice, R., Araújo, W. L., Nunes-Nesi, A., Peres, L. E. P., & Zsögön, A. (2019). Capsaicinoids: Pungency beyond *Capsicum*. *Trends in Plant*

Science, *24*(2), 109-120. https://doi.org/10.1016/j.tplants.2018.11.001

Nuzzo, J. L., McBride, J. M., Cormie, P., & McCaulley, G. O. (2008). Relationship between countermovement jump performance and multijoint isometric and dynamic tests of strength. *Journal of Strength and Conditioning Research*, *22*(3), 699-707. https://doi.org/10.1519/JSC.0b013e31816d5eda

Okano, A. H., Fontes, E. B., Montenegro, R. A., Farinatti, P. de T. V., Cyrino, E. S., Li, L. M., Bikson, M., & Noakes, T. D. (2015). Brain stimulation modulates the autonomic nervous system, rating of perceived exertion and performance during maximal exercise. *British Journal of Sports Medicine*, *49*(18), 1213-1218. https://doi.org/10.1136/bjsports-2012-091658

Opheim, M. N., & Rankin, J. W. (2012). Effect of capsaicin supplementation on repeated sprinting performance. *Journal of Strength and Conditioning Research*, *26*(2), 319-326. https://doi.org/10.1519/JSC.0b013e3182429ae5

Ortega-Becerra, M., Sánchez-Moreno, M., & Pareja-Blanco, F. (2021). Effects of cluster set configuration on mechanical performance and neuromuscular activity. *Journal of Strength and Conditioning Research*, *35*(2), 310-317. https://doi.org/10.1519/JSC.0000000000003907

Osuna-Prieto, F. J., Acosta, F. M., Perez de Arrilucea Le Floc'h, U. A., Riquelme-Gallego, B., Merchan-Ramirez, E., Xu, H., De La Cruz-Márquez, J. C., Amaro-Gahete, F. J., Llamas-Elvira, J. A., Triviño-Ibáñez, E. M., Segura-Carretero, A., & Ruiz, J. R. (2022). Dihydrocapsiate does not increase energy expenditure nor fat oxidation during aerobic exercise in men with overweight/obesity: a randomized, triple-blinded, placebo-controlled, crossover trial. *Journal of the International Society of Sports Nutrition*, *19*(1), 417–436. https://doi.org/10.1080/15502783.2022.2099757

Pal, S., Chaki, B., Chattopadhyay, S., & Bandyopadhyay, A. (2018). High-intensity exercise induced oxidative stress and skeletal muscle damage in postpubertal boys and girls: A comparative study. *Journal of Strength and Conditioning Research*, *32*(4), 1045-1052. https://doi.org/10.1519/JSC.0000000000002167

Pareja-Blanco, F., Alcazar, J., Sánchez-Valdepeñas, J., Cornejo-Daza, P. J., Piqueras-Sanchiz, F., Mora-Vela, R., Sánchez-Moreno, M., Bachero-Mena, B., Ortega-Becerra, M., & Alegre, L. M. (2020). Velocity loss as a critical variable determining the adaptations to strength training. *Medicine & Science in Sports & Exercise*, *52*(8), 1752-1762. https://doi.org/10.1249/MSS.0000000000002295

Pareja-Blanco, F., Rodríguez-Rosell, D., Aagaard, P., Sánchez-Medina, L., Ribas-Serna, J., Mora-Custodio, R., Otero-Esquina, C., Yáñez-García, J. M., & González-Badillo, J.

J. (2020). Time course of recovery from resistance exercise with different set configurations. *Journal of Strength and Conditioning Research*, *34*(10), 2867-2876. https://doi.org/10.1519/JSC.0000000000002756

Pareja-Blanco, F., Rodríguez-Rosell, D., Sánchez-Medina, L., Gorostiaga, E., & González-Badillo, J. (2014). Effect of movement velocity during resistance training on neuromuscular performance. *International Journal of Sports Medicine*, *35*(11), 916-924. https://doi.org/10.1055/s-0033-1363985

Pareja-Blanco, F., Villalba-Fernández, A., Cornejo-Daza, P., Sánchez-Valdepeñas, J., & González-Badillo, J. (2019). Time course of recovery following resistance exercise with different loading magnitudes and velocity loss in the set. *Sports*, *7*(3), 59. https://doi.org/10.3390/sports7030059

Patcharatrakul, T., Kriengkirakul, C., Chaiwatanarat, T., & Gonlachanvit, S. (2020). Acute effects of red chili, a natural capsaicin receptor agonist, on gastric accommodation and upper gastrointestinal symptoms in healthy volunteers and gastroesophageal reflux disease patients. *Nutrients*, *12*(12). https://doi.org/10.3390/nu12123740

Pearson, J. R., Dusenbury, L. J., Bakes-Martin, R., Loverde, M., Johnson, J., & Byyny, R. L. (1988). Evaluation of a simple method for measuring blood cholesterol levels using non-laboratory observers. *The American Journal of Medicine*, *85*(3), 369-374. https://doi.org/10.1016/0002-9343(88)90589-X

Piccolella, S., Crescente, G., Candela, L., & Pacifico, S. (2019). Nutraceutical polyphenols: New analytical challenges and opportunities. *Journal of Pharmaceutical and Biomedical Analysis*, *175*, 112774. https://doi.org/10.1016/j.jpba.2019.07.022

Piqueras-Sanchiz, F., Cornejo-Daza, P. J., Sánchez-Valdepeñas, J., Bachero-Mena, B., Sánchez-Moreno, M., Martín-Rodríguez, S., García-García, Ó., & Pareja-Blanco, F. (2021). Acute mechanical, neuromuscular, and metabolic responses to different set configurations in resistance training. *Journal of Strength and Conditioning Research, Publish Ahead of Print*. https://doi.org/10.1519/JSC.0000000000004068

Poitras, I., Bielmann, M., Campeau-Lecours, A., Mercier, C., Bouyer, L. J., & Roy, J.-S. (2019). Validity of wearable sensors at the shoulder joint: Combining wireless electromyography sensors and inertial measurement units to perform physical workplace Assessments. *Sensors*, *19*(8), 1885. https://doi.org/10.3390/s19081885

Purdom, T., Kravitz, L., Dokladny, K., & Mermier, C. (2018). Understanding the factors that effect maximal fat oxidation. *Journal of the International Society of Sports Nutrition*, *15*(1), 3. https://doi.org/10.1186/s12970-018-0207-1

Pyne, D. B., Boston, T., Martin, D. T., & Logan, A. (2000). Evaluation of the lactate Pro blood lactate analyser. *European Journal of Applied Physiology*, *82*(1-2), 112-116. https://doi.org/10.1007/s004210050659

Rossi, P. A. Q., Lira, F. S., Bezerra, V. R., Clark, N. W., Fukuda, D. H., & Panissa, V. L. G. (2022). Acute response to capsiate supplementation at rest and during exercise on energy intake, appetite, metabolism, and autonomic function: A randomized trial. *Journal of the American Nutrition Association*, *41*(6), 541-550. https://doi.org/10.1080/07315724.2021.1938294

Rothschild, J. A., Kilding, A. E., Stewart, T., & Plews, D. J. (2022). Factors influencing substrate oxidation during submaximal cycling: A modelling analysis. *Sports Medicine*, *52*(11), 2775-2795. https://doi.org/10.1007/s40279-022-01727-7

Sanchez-Delgado, G., Alcantara, J. M. A., Ortiz-Alvarez, L., Xu, H., Martinez-Tellez, B., Labayen, I., & Ruiz, J. R. (2018). Reliability of resting metabolic rate measurements in young adults: Impact of methods for data analysis. *Clinical Nutrition*, *37*(5), 1618-1624. https://doi.org/10.1016/j.clnu.2017.07.026

Sánchez-Medina, L., & González-Badillo, J. J. (2011). Velocity loss as an indicator of neuromuscular fatigue during resistance training. *Medicine & Science in Sports & Exercise*, *43*(9), 1725-1734. https://doi.org/10.1249/MSS.0b013e318213f880

Sánchez-Medina, L., Pallarés, J., Pérez, C., Morán-Navarro, R., & González-Badillo, J. (2017). Estimation of relative load from bar velocity in the full back squat exercise. *Sports Medicine International Open*, *01*(02), E80-E88. https://doi.org/10.1055/s-0043-102933

Sánchez-Moreno, M., Cornejo-Daza, P. J., González-Badillo, J. J., & Pareja-Blanco, F. (2020). Effects of velocity loss during body mass prone-grip pull-up training on strength and endurance performance. *Journal of Strength and Conditioning Research*, *34*(4), 911-917. https://doi.org/10.1519/JSC.0000000000003500

San-Millán, I., & Brooks, G. A. (2018). Assessment of metabolic flexibility by means of measuring blood lactate, fat, and carbohydrate oxidation responses to exercise in professional endurance athletes and less-Fit individuals. *Sports Medicine*, *48*(2), 467-479. https://doi.org/10.1007/s40279-017-0751-x

Santos, F., Eduardo, F., Caetano, D., Clark, N., & Panissa, G. (2022). Can capsaicinoids and their analogs affect energy expenditure and substrate oxidation during exercise? A systematic review with acute studies. *Human Nutrition & Metabolism*, *29*(3). https://doi.org/10.1016/j.hnm.2022.200156

Sanz-Salvador, L., Andrés-Borderia, A., Ferrer-Montiel, A., & Planells-Cases, R.

(2012). Agonist- and Ca2+-dependent desensitization of TRPV1 channel targets the teceptor to lysosomes for degradation. *Journal of Biological Chemistry*, *287*(23), 19462-19471. https://doi.org/10.1074/jbc.M111.289751

Sasahara, I., Furuhata, Y., Iwasaki, Y., Inoue, N., Sato, H., Watanabe, T., & Takahashi, M. (2010). Assessment of the biological similarity of three capsaicin analogs (Capsinoids) found in non-pungent chili pepper (CH-19 Sweet) fruits. *Bioscience, Biotechnology and Biochemistry*, *74*(2), 274-278. https://doi.org/10.1271/bbb.90570

Sharma, S. K., Vij, A. S., & Sharma, M. (2013). Mechanisms and clinical uses of capsaicin. *European Journal of Pharmacology*, *720*(1-3), 55-62. https://doi.org/10.1016/j.ejphar.2013.10.053

Shirani, F., Foshati, S., Tavassoly, M., Clark, C. C. T., & Rouhani, M. H. (2021). The effect of red pepper/capsaicin on blood pressure and heart rate: A systematic review and meta-analysis of clinical trials. *Phytotherapy Research : PTR*, *35*(11), 6080-6088. https://doi.org/10.1002/ptr.7217

Sidhu, S. K., Weavil, J. C., Mangum, T. S., Jessop, J. E., Richardson, R. S., Morgan, D. E., & Amann, M. (2017). Group III/IV locomotor muscle afferents alter motor cortical and corticospinal excitability and promote central fatigue during cycling exercise. *Clinical Neurophysiology*, *128*(1), 44-55. https://doi.org/10.1016/j.clinph.2016.10.008

Smith, A. D. H., Crabtree, D. R., Bilzon, J. L. J., & Walsh, N. P. (2010). The validity of wireless iButtons and thermistors for human skin temperature measurement. *Physiological Measurement*, *31*(1), 95-114. https://doi.org/10.1088/0967-3334/31/1/007

Sutton, J. R., Toews, C. J., Ward, G. R., & Fox, I. H. (1980). Purine metabolism during strenuous muscular exercise in man. *Metabolism*, *29*(3), 254-260. https://doi.org/10.1016/0026-0495(80)90067-0

Swank, A., & Robertson, R. J. (1989). Effect of induced alkalosis on perception of exertion during intermittent exercise. *Journal of applied physiology*, *67*(5), 1862–1867. https://doi.org/10.1152/jappl.1989.67.5.1862

Szolcsányi, J. (2015). Effect of capsaicin on thermoregulation: An update with new aspects. *Temperature*, *2*(2), 277-296. https://doi.org/10.1080/23328940.2015.1048928

Taylor, J. L., Amann, M., Duchateau, J., Meeusen, R., & Rice, C. L. (2016). Neural contributions to muscle fatigue: From the brain to the muscle and back again. *Medicine & Science in Sports & Exercise*, *48*(11), 2294-2306. https://doi.org/10.1249/MSS.0000000000000923

Tominaga, M., & Tominaga, T. (2005). Structure and function of TRPV1. *European*

Journal of Physiology, *451*(1), 143-150. https://doi.org/10.1007/s00424-005-1457-8

Turck, D., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K. I., Kearney, J., Maciuk, A., Mangelsdorf, I., McArdle, H. J., Naska, A., Pelaez, C., Pentieva, K., Siani, A., Thies, F., Tsabouri, S., Vinceti, M., Cubadda, F., Engel, K. H., Frenzel, T., Heinonen, M., … Knutsen, H. K. (2019). Safety of phenylcapsaicin as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal*, *17*(6). https://doi.org/10.2903/j.efsa.2019.5718

van Marken Lichtenbelt, W. D., Daanen, H. A. M., Wouters, L., Fronczek, R., Raymann, R. J. E. M., Severens, N. M. W., & Van Someren, E. J. W. (2006). Evaluation of wireless determination of skin temperature using iButtons. *Physiology & Behavior*, *88*(4-5), 489-497. https://doi.org/10.1016/j.physbeh.2006.04.026

Villavicencio-Kim, J., & Wu, G. Y. (2020). Body building and aminotransferase elevations: A review. *Journal of Clinical and Translational Hepatology*, *8*(2), 161-167. https://doi.org/10.14218/JCTH.2020.00005

Volek, J. S., Noakes, T., & Phinney, S. D. (2015). Rethinking fat as a fuel for endurance exercise. *European Journal of Sport Science*, *15*(1), 13-20. https://doi.org/10.1080/17461391.2014.959564

Walter, A. A., Herda, T. J., Ryan, E. D., Costa, P. B., Hoge, K. M., Beck, T. W., Stout, J. R., & Cramer, J. T. (2009). Acute effects of a thermogenic nutritional supplement on cycling time to exhaustion and muscular strength in college-aged men. *Journal of the International Society of Sports Nutrition*, *6*(1), 15. https://doi.org/10.1186/1550-2783-6-15

Warburton, D. E. R. (2002). Biochemical changes as a result of prolonged strenuous exercise. *British Journal of Sports Medicine*, *36*(4), 301-303. https://doi.org/10.1136/bjsm.36.4.301

Weir, J. D. (1949). New methods for calculating metabolic rate with special reference to protein metabolism. *The Journal of Physiology*, *109*(1-2), 1-9. https://doi.org/10.1113/jphysiol.1949.sp004363

Wells, G. D., Selvadurai, H., & Tein, I. (2009). Bioenergetic provision of energy for muscular activity. *Paediatric Respiratory Reviews*, *10*(3), 83-90. https://doi.org/10.1016/j.prrv.2009.04.005

Willmott, A. G. B., Hayes, M., James, C. A., Gibson, O. R., & Maxwell, N. S. (2020). Heat acclimation attenuates the increased sensations of fatigue reported during acute exercise-heat stress. *Temperature*, *7*(2), 178-190. https://doi.org/10.1080/23328940.2019.1664370

Wirtz, N., Wahl, P., Kleinöder, H., & Mester, J. (2014). Lactate kinetics during multiple set resistance exercise. *Journal of Sports Science & Medicine*, *13*(1), 73-77.

Yeo, W. K., Carey, A. L., Burke, L., Spriet, L. L., & Hawley, J. A. (2011). Fat adaptation in well-trained athletes: Effects on cell metabolism. *Applied Physiology, Nutrition, and Metabolism*, *36*(1), 12-22. https://doi.org/10.1139/H10-089

Zhai, K., Liskova, A., Kubatka, P., & Büsselberg, D. (2020). Calcium entry through TRPV1: A potential target for the regulation of proliferation and apoptosis in cancerous and healthy cells. *International Journal of Molecular Sciences*, *21*(11), 4177. https://doi.org/10.3390/ijms21114177

Chapter 8. Appendices.

8. Appendices

8.1 Information about the articles included in the compendium

1. **Jiménez-Martínez, P.**, Sánchez-Valdepeñas, J., Cornejo-Daza, P. J., Cano-Castillo, C., Asín-Izquierdo, I., Alix-Fages, C., Pareja-Blanco, F., & Colado, J. C. (2023). Effects of different phenylcapsaicin doses on neuromuscular activity and mechanical performance in trained male subjects: A randomized, triple-blinded, crossover, placebo-controlled trial. *Frontiers in Physiology*, 14. https://doi.org/10.3389/fphys.2023.1215644

2. **Jiménez-Martínez, P.**, Cornejo-Daza, P. J., Sánchez-Valdepeñas, J., Asín-Izquierdo, I., Cano-Castillo, C., Alix-Fages, C., Pareja-Blanco, F., & Colado, J. C. (2023). Effects of different phenylcapsaicin doses on resistance training performance, muscle damage, protein breakdown, metabolic response, ratings of perceived exertion, and recovery: a randomized, triple-blinded, placebo-controlled, crossover trial. *Journal of the International Society of Sports Nutrition*, *20*(1), 2204083. https://doi.org/10.1080/15502783.2023.2204083

3. **Jiménez-Martínez, P.**, Alix-Fages, C., Janicijevic, D., Miras-Moreno, S., Chacón-Ventura, S., Martín-Olmedo, J. J., De La Cruz-Márquez, J. C., Osuna-Prieto, F. J., Jurado-Fasoli, L., Amaro-Gahete, F. J., García-Ramos, A., & Colado, J. C. (2023). Effects of phenylcapsaicin on aerobic capacity and physiological parameters in active young males: a randomized, triple-blinded, placebo-controlled, crossover trial. *Frontiers in physiology*, *14*, 1190345. https://doi.org/10.3389/fphys.2023.1190345

Article	Journal	Impact factor*	Citation indicator	Area	Rank	Quartile
1	Frontiers in Physiology	4.0	1.0	Physiology	20/79	Q2
2	Journal of the International Society of Sports Nutrition	6.2	1.34	Sport Sciences	8/87	Q1
3	Frontiers in Physiology	4.0	1.0	Physiology	20/79	Q2

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8.1.1 Article one

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Effects of different phenylcapsaicin doses on neuromuscular activity and mechanical performance in trained male subjects: a randomized, triple-blinded, crossover, placebo-controlled trial

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Objective: This study aimed to examine the effects of phenylcapsaicin (PC) supplementation on strength performance and neuromuscular activity in young trained male subjects.

Materials and methods: A total of 25 trained subjects [full-squat (SQ) one repetition maximum (1RM) = 125.6 ± 21.0 kg] were enrolled in this randomized, triple-blinded, crossover, placebo-controlled trial. The subjects performed a first session and a post-24 h session for each condition. In the first session, the subjects ingested a high dose of PC (HD, 2.5 mg), a low dose (LD, 0.625 mg), or a placebo (PLA). Their performance in SQ was assessed under a 3% × 8 × 70% 1RM protocol in the first session. Their performances in countermovement jump (CMJ), SQ with 60% 1RM, and isometric squat were measured before and after the SQ protocol in both sessions. The neural activity of the vastus lateralis (VL) and vastus medialis (VM) was recorded via surface electromyography (EMG) and averaged in both sessions.

Results: Significant differences between the conditions were reported for lifting velocity, velocity loss, and the 60% load in dynamic SQ (p range = 0.02–0.04). Electrical changes were not identified for any outcome, although neural activity changed across time (p range ≤0.001–0.006). A significant condition × time effect was observed in CMJ compared to PLA (p ≤0.001) and LD (p ≤0.001). Intra-set analyses revealed higher velocities in HD compared to those in LD (p = 0.01) and PLA (p range = 0.004–0.008).

Conclusion: Therefore, PC may improve the strength performance and attenuate the mechanical fatigue induced by resistance training in SQ and CMJ exercises.

KEYWORDS

electromyography, neuromuscular physiology, resistance training, ergogenic aid, velocity-based training, strength endurance

Introduction

Human voluntary movement and force production are determined by the nervous system behavior (Alix-Fages et al., 2022b). During strenuous exercise, changes in neuronal circuitry (e.g., supraspinal structure excitability) lead to a decline in fatigue tolerance and sports performance (Alix-Fages et al., 2022a). In this regard, a heterogeneous group of substances known as capsaicinoids, which are found in chili peppers, has emerged as plausible ergogenic nervous system modulators (Jiménez-Martínez et al., 2022). Previously, researchers have focused on the impact of capsaicin (i.e., the main active principle of spicy peppers) on pain relief, weight loss, and performance (Arora et al., 2021; de Moura e Silva et al., 2021).

As a vanilloid-structured substance, capsaicin interacts with the transient receptor vanilloid 1 (TRPV1) (Hayman and Kam, 2008). TRPV1 are receptors related to afferent feedback from III and IV nerve fibers, a type of peripheral afferent fibers that are linked to the detection of pain and the development of central fatigue by affecting both supraspinal and spinal levels of the nervous system (Okano et al., 2015; Alix-Fages et al., 2022a). An exercise experience modulates the nervous system behavior, eliciting a higher tolerance to fatigue and discomfort in high-intensity efforts (i.e., near exhaustion) (Alix-Fages et al., 2022a). However, capsaicin and its analogs have been shown to modulate the mechanical responses to exercise during different intensities and under neural fatiguing conditions (Jiménez-Martínez et al., 2022). In this regard, TRPV1 agonists display their main physiological functions through reducing inflammatory hyperalgesia, downregulating voltage-activated calcium channels, and influencing thermoception (Baamonde et al., 2005; Hayman and Kam, 2008; Fattori et al., 2016). Accordingly, some discomfort-related responses to exercise, such as metabolite accumulation and calcium overload, are linked to III and IV afferent nerve fiber activity during exercise (Taylor et al., 2016; de Moura e Silva et al., 2021). Capsaicin supplementation is able to reduce the afferent signals of pain that are driven from the peripheral to the central nervous system, delaying the onset of fatigue in the neuromuscular junction (Taylor et al., 2016; Jiménez-Martínez et al., 2022). Consequently, the upregulation of TRPV1 leads to a decline in the rate of perceived exertion (RPE) and the perception of pain, as well as discomfort, during exercise (Jiménez-Martínez et al., 2022). For instance, recent evidence highlights that lumbar intrathecal fentanyl (afferent blockage) effectively attenuates the group III and IV afferent feedback during intermittent knee-extensor all-out exercise, resulting in improved physical performance and reduced RPE (Broxterman et al., 2018). Therefore, the increased firing of group III and IV muscle afferents, caused by mechanical forces and metabolite accumulation, has been well-documented to contribute to the development of central fatigue, affecting both spinal and supraspinal levels (Amann et al., 2011; Blain et al., 2016; Sidhu et al., 2017).

Recently, new analogs of capsaicin have been formulated to ensure higher bioavailability and gastrointestinal tolerance (Cross et al., 2020). Phenylcapsaicin (PC) is a synthetic analog of capsaicin composed of 98% of PC and its excipients (Turck et al., 2019). PC pharmacokinetics has shown a fast metabolism (i.e., 30 min) after administration (Turck et al., 2019). For these reasons, the PC ergogenic dose, although lower than that of traditional purified capsaicin, remains to be verified. On the other hand, capsaicin has demonstrated a positive influence on repetitions until failure, total volume load, and the rate of perceived exertion during resistance exercise interventions (de Freitas et al., 2018). Previous research has elucidated the effects of capsaicin on dynamic exercise (Jiménez-Martínez et al., 2022). In this regard, de Freitas et al. (2018) observed that 12 mg of capsaicin enhanced repetitions until exhaustion, total mass lifted, and the rate of perceived effort of four sets at 70% of maximum repetition (1RM) in the squat exercise (SQ) of a double-blinded, randomized, placebo-controlled intervention. However, these previous studies have not addressed different concerns that are still present in the literature. First, previous research studies are based on strength endurance protocols (Jiménez-Martínez et al., 2022), which may not be extrapolated to other types of exercise tasks, such as isometric contractions. In addition, the electromyographical mechanisms underlying the effects of capsaicin on resistance training performance and most of the mechanical outcomes used in the strength and conditioning field, which include the assessment of mechanical fatigue, have not been evaluated yet (de Moura e Silva et al., 2021). Moreover, in these previous studies, submaximal (i.e., not performed until failure) intensities were not assessed (Jiménez-Martínez et al., 2022), which reduces the real applicability of capsaicin on physical conditioning because the induction of excessive fatigue can disturb training adaptations (Pareja-Blanco et al., 2020a). Furthermore, most of the current research studies have not used objective measures of performance (e.g., infrared detection of jump height or linear velocity), which may hinder the estimate of the real impact of this substance on mechanical performance and fatigue (e.g., linear velocity loss) (Sánchez-Medina and González-Badillo, 2011).

In addition, as capsaicin may produce an analgesic effect, its impact on direct muscle force production and electromyographical outcomes is relevant. Currently, there are no previous data evaluating the influence of oral capsaicin on force production and neural responses during exercise. In addition, the electromyographical effects of capsaicin have only been evaluated for topical administration (Evans et al., 2021). In this study, topical capsaicin elicited significant changes in the motor unit recruitment pattern, which violated Henneman's size principle in free-of-pain adults during voluntary trapezius and infraspinatus contractions

(Evans et al., 2021). However, these neural responses have not been assessed during dynamic exercise after the ingestion of capsaicin, which makes it difficult to extrapolate this finding to sports performance. In addition, this issue must be highlighted because the neural strategies of the nervous system during exercise are reflected in force production during sports and in the induction of fatigue (Alix-Fages et al., 2022a). None of the previous studies has directly evaluated the effects of capsaicin on force production. Although this can be easily addressed with the use of a force platform (Piqueras-Sanchiz et al., 2021), the information obtained from this approach can be useful in determining whether this substance alters the amount, slope, or time of force production. On the other hand, the "desensitizer" effect of capsaicin on a sports task may lead to a higher degree of fatigue in the post-exercise window. Nevertheless, the effects of capsaicin on mechanical recovery outcomes have not been assessed yet (Jiménez-Martínez et al., 2022). This issue must be pointed out because an acute increase in fatigue could lead to a reduction in force production and sports performance during the subsequent training sessions or competitions (Pareja-Blanco et al., 2020b). Overall, the information about how capsaicin may modulate resistance training performance and fatigue, as well as the neural and mechanical mechanisms underlying these effects, is still lacking in current research studies.

Therefore, this study aims to examine the effects of two different doses of PC on fatigue and short-term mechanical responses, by measuring the isometric and dynamic performance, as well as neural activity, in CMJ and SQ exercises. For the first time, this study included the confluence of neural responses, dynamic and isometric exercise performances, and mechanical fatigue after capsaicinoid ingestion. It was hypothesized that PC may acutely increase velocity in the SQ exercise, neural excitability, and CMJ height. However, due to the improvements in performance, a higher degree of fatigue and a detrimental effect on recovery in post-test measurements were also expected.

Materials and methods

Experimental design

This study was conceived as a randomized, triple-blinded, crossover, placebo-controlled trial. Two weeks before the beginning of the study, the subjects were tested for anthropometrical measures (body mass and height), one-repetition maximum (1RM), and load–velocity relationship in SQ (see the Dynamic full-squat test section). Then, the subjects completed three experimental conditions, each one composed of a main session and a 24-h second session (post 24 h). The three experimental conditions were identical with the only difference in the supplement dose ingested. In addition, the subjects randomly ingested either a placebo (PLA) or a low (LD) or high (HD) dose of PC before the first weekly session. During the first weekly session, the subjects completed a SQ protocol that consisted of three sets of eight repetitions at 70% 1RM. Before (pre), immediately after (post), and 24 h after (post 24 h) the SQ protocol, a battery of tests was conducted to analyze the fatigue induced by each condition: CMJ, two SQ repetitions with 60% 1RM, and maximal isometric SQ at 90°, respectively. Since it is important to standardize temporalization to measure acute responses, the tests were conducted at the following time points: post-CMJ (1 min post-exercise), SQ with 60% 1RM (2 min post exercise), and isometric SQ (3 min post exercise). The electromyographical assessment of each session was recorded while the subjects were performing the SQ tests. The subjects performed each session at the same individual time of the day under stable environmental conditions (22°C–24°C and 55% humidity). The overall design of the study is depicted in Figure 1.

Subjects

Sample size calculation was performed using G*POWER software (Heinrich-Heine-Universität Düsseldorf, Germany) with an alpha value of 0.05. Statistical power was fixed at 0.80 and the effect size at 0.60. Based on the total volume in the SQ exercise of the previous research (de Freitas et al., 2018), at least 21 subjects were required for this study. Finally, 25 healthy men (age = 21.7 ± 3.7 years, body mass = 77.4 ± 9.1 kg, height = 176.7 ± 7.2 cm, 1RM in SQ = 125.6 ± 21.0 kg, and 1RM normalized to body mass = 1.64 ± 0.22) with at least 2 years of experience in resistance training (range = 2–5 years) were enrolled in this study. If the subjects suffered from any cardiovascular, muscular, neurological, and/or metabolic disorder, they were directly excluded. Once the subjects were informed about the aim of the study, procedures, and possible risks, the subjects freely signed the informed consent sheet. The present research was approved by the Local Research Ethics Committee of Junta de Andalucía (Code: 0513-N-22) in accordance with the tenets of the Declaration of Helsinki. Each condition was established under the safety ranges proposed by the European Food Safety Authority (EFSA) expert panel (Turck et al., 2019).

The subjects were asked not to ingest stimulants (e.g., caffeine) or other ergogenic aids prior to each session, not to perform strenuous physical activity, and not to modify their dietary intake 2 days before the tests. One of the researchers reminded the subjects to maintain their normal dietary intake and not to train 2 days before the weekly testing sessions with a text message to each one separately. During 3 weeks of the study, two subjects withdrew from the study, one of them due to injury and the other because of missing a session.

Procedures

Supplementation procedures

Supplements and placebo were prepared and packaged by a non-involved researcher in independent installations (Life Pro Nutrition industries, Madrid, Spain). To ensure triple blinding, each package was encoded with a number from one to three. The packages were not unblinded until a third-party researcher performed all the analyses. Packages and capsules were identical in appearance, color, and taste, and their content was only revealed after the independent researcher completed all the analyses. The capsule composition included the following: 1) PLA, a maltodextrin and excipient placebo with a red dye; 2) HD, 2.5 mg of PC (aXivite, Malmö, Sweden); and 3) LD, 0.625 mg of PC. Randomization and

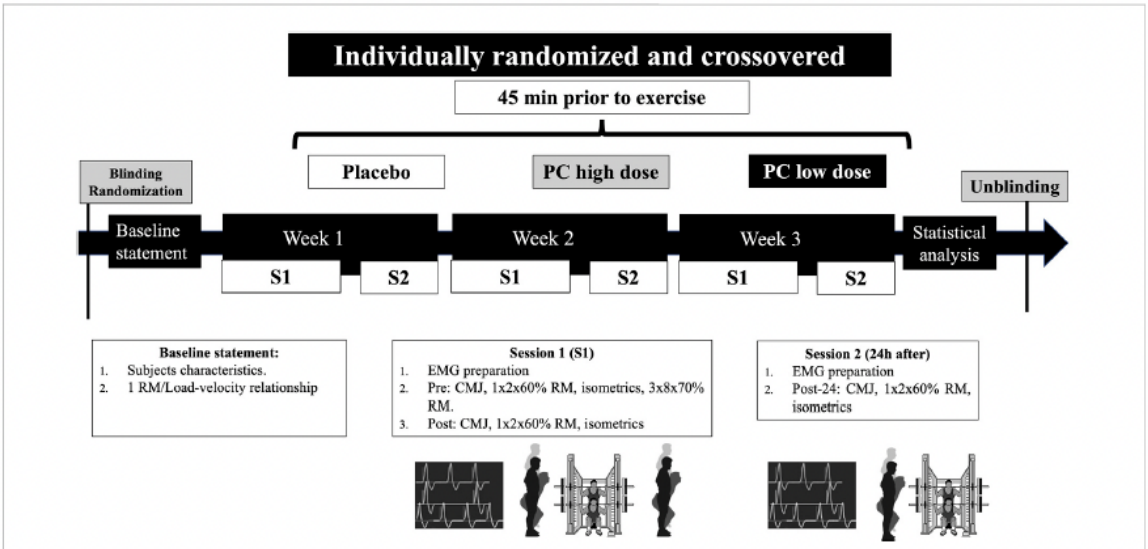

FIGURE 1
Timeline of the experimental protocol. PC, phenylcapsaicin; CMJ, countermovement jump; EMG, electromyography; 1RM, one-repetition maximum; S1/S2, session 1/2.

crossover were performed 2 weeks before the beginning of the study. To reduce possible bias, a third-party researcher assigned the subjects to each condition in the Research Randomizer website (www.randomizer.org). Each subject consumed one condition per week during the three total weeks of the study. PC doses or placebo was ingested 45 min prior to the first exercise session. Researchers encouraged the subjects to freely select a capsule from the daily assigned condition package. The capsules were taken with water under the supervision of at least one researcher. The randomized, counterbalanced, crossover sequences of each week of the study were eight HD, eight LD, and nine PLA in week 1; seven HD, 10 LD, and eight PLA in week 2; and 10 HD, seven LD, and eight PLA in week 3. This information was pooled after unblinding. The adverse effects were not reported for any of the supplementation conditions. Additionally, to ensure the absence of a maturation effect, order effect statistical analyses were also performed (Supplementary Tables S1–S3 of the Supplementary Material).

Electromyography

Before electromyography (EMG) recording, a researcher checked whether the subjects were properly shaved. Then, a black permanent marker was used to ensure consistency in the electrode position across conditions (Pareja-Blanco et al., 2020a). Surface EMG electrodes were placed over the vastus medialis (VM) and vastus lateralis (VL) of the right leg according to SENIAM criteria (Hermens et al., 2000) and the previous research of the field (Ortega-Becerra et al., 2021; Piqueras-Sanchiz et al., 2021). EMG signals were evaluated for 60% and 70% 1RM sets and isometric tests in both sessions. EMG signals were recorded continuously using a bipolar, parallel-bar surface electromyographic Wireless Trigno™ sensor (Delsys Inc., MA, United States) (r range = 0.92–0.99) (Poitras et al., 2019). Baseline noise was established at <5 μV peak-to-peak, and the sampling rate was 1926 Hz. The EMG system was set at an inter-electrode distance of 10 mm, common mode rejection ratio >80 dB, and bandwidth filter between 20 and 450 Hz ± 10% (Delsys Inc., MA, United States). Data were stored using EMGworks Acquisition software (Delsys Inc., MA, United States). For each measure, the median frequency (MDF) [ICC (95% CI): 0.95 (0.90–0.98) and CV: 5.3%] and root mean square (RMS) [ICC (95% CI): 0.95 (0.90–0.98) and CV: 7.4%] were individually calculated for VM and VL as excitatory muscle activity assessments and averaged for further analyses as described in the previous research (Kattla and Lowery, 2010; Piqueras-Sanchiz et al., 2021). All the outcomes measured were recorded for each repetition (over sliding windows of 500 ms with an overlap of 499 ms) and averaged for further analysis for dynamic and isometric tests. According to previous research, data were normalized under the daily maximal value of the first isometric signal (Piqueras-Sanchiz et al., 2021). Thus, EMG values were expressed as a percentage of the maximal daily value obtained (Hermens et al., 2000).

Resistance exercise protocol

Warm-up

A standardized warm-up was performed 30 min after capsule ingestion in the first session and immediately after the subjects arrived at the laboratory in the second session. All the subjects were inspected regarding EMG marks before the warm-up. The general warm-up consisted of 5 min of continuous running at 9 $km \cdot h^{-1}$. Then, a specific warm-up was conducted before each test. The specific warm-up consisted of three sets of 10 repetitions of bodyweight SQs followed by three progressive CMJs and two

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maximal CMJs. Then, three SQ sets of two repetitions with 40%, 50%, and 60% 1RM were performed before the main SQ test.

Countermovement jump test

The CMJ height was determined using an infrared timing system (OptoJump Next, Microgate, Bolzano, Italy) (r = 0.99) (Glatthorn et al., 2011). The subjects were instructed to perform CMJs with their arms akimbo during eccentric and concentric phases. Accordingly, the CMJ technique was established at approximately 90° of knee flexion, followed by a maximal vertical jump. For each attempt, the landing was required to be in an upright position without bending the knees until the movement was completed. For each measurement, the subjects were required to perform two attempts with an interval of 10 s, and the mean value was calculated for further analyses [ICC (95% CI): 0.99 (0.97–0.99) and CV: 1.9%] (Piqueras-Sanchiz et al., 2021). If the jump height difference was greater than 2 cm between trials, a third measurement was made, and the two nearest values were averaged.

Dynamic full-squat test

An initial test with increasing loads was performed before the start of the study for the individual calculation of the 1RM and load–velocity relationship in the full-SQ exercises (González-Badillo et al., 2017). A Smith machine with no counterweight mechanism was used (Multipower Fitness Line, Peroga, Murcia, Spain). The mean propulsive velocity (MPV) was directly measured for each repetition using a linear velocity transducer (T-Force System, Ergotech, Murcia, Spain) (r = 0.99) (Sánchez-Medina and González-Badillo, 2011) attached perpendicularly to the barbell (González-Badillo and Sánchez-Medina, 2010). Although all the subjects were experienced in all the tests performed, as they had participated in at least two previous studies where these mechanical variables were assessed in the previous 3 months, the initial session was also used as familiarization. Accordingly, all the subjects performed isometric and CMJ tests after the progressive loading test.

Regarding the progressive loading test, the initial load used was 30 kg, and it was progressively increased to 10 kg until it reached a mean propulsive velocity of 0.50 m s^{-1} or lower. Then, the load was gradually increased (2.5–5.0 kg) until the repetition could not be completed. Three repetitions were completed for light loads (≥1.00 m s^{-1}), two for medium loads (1.00–0.80 m s^{-1}), and one for the heaviest loads (≤0.80 m s^{-1}). Rest periods were set at 3 minutes for light and medium loads and 5 min for heavy loads. The load–velocity relationship was calculated with the best repetition of each attempt (i.e., highest MPV) (Piqueras-Sanchiz et al., 2021).

For the dynamic full-SQ evaluation, two SQ repetitions with 60% 1RM were performed. SQ was performed using a Smith machine (Fitness Line, Peroga, Murcia, Spain) with the subjects starting from the upright position with their knees and hips fully extended, parallel feet and their stance approximately shoulder-width apart, and the barbell resting across the back at the level of the acromion. Each subject descended in a continuous motion, until the top of their thighs was below the horizontal plane and the posterior thighs and shanks making contact with each other (~35°–40° knee flexion), and then immediately reversed the motion and rose back to the upright position. Unlike the eccentric phase that was performed at a controlled mean velocity (~0.50–0.65 m s^{-1}), the subjects were encouraged to always perform the concentric phase of SQ at the maximal intended velocity (Pareja-Blanco et al., 2014). The mean propulsive values of velocity were acquired using a linear velocity transducer (T-Force System, Ergotech, Murcia, Spain) attached perpendicularly to the barbell. The highest values of each variable were recorded for further analyses. Resting between sets was set at 2 min.

Isometric squat test

The maximal isometric SQ test was performed at 90° of the knee flexion position (180° = full extension) for elucidating the effects of PC on the maximal isometric force (MIF) [ICC (95% CI): 0.99 (0.97–0.99) and CV: 3.4%] and the maximal rate of force development (RFDmax) [ICC (95% CI): 0.94 (0.86–0.97) and CV: 13.8%] (Piqueras-Sanchiz et al., 2021; Martinopoulou et al., 2022). For this purpose, a Smith machine with customizable height supports was equipped with an 80 × 80 cm dynamometric platform (FP-500, Ergotech, Murcia, Spain). The subjects were instructed to push with their legs against the floor of the platform as hard as possible after the cue "ready, set, go!" The subjects were required to execute two 5-s attempts separated by 1 min of rest per test. The external forces of each attempt were collected at a sampling rate of 1,000 Hz and processed using a specific software (T-Force System, Ergotech, Murcia, Spain) (r = 0.99) (Sánchez-Medina and González-Badillo, 2011). For the RFDmax assessment, the maximum slope in the force–time curve in 20-ms time intervals was selected. Furthermore, as RFD data were represented for different discriminable time gaps, RFD was calculated for the 0–50, 0–100, 0–150, 0–200, and 0–400 ms intervals. RFD and MIF outcomes were both averaged for further analyses. MIF was presented as the percentage of change from the pre-values. The specific warm-up consisted of two submaximal attempts at 70% and 90% of the maximal perceived effort.

Full-squat protocol

The execution technique and setting have been described in the "Dynamic full-squat test" section. The SQ protocol consisted of three sets of eight repetitions with 70% 1RM with a 2-min rest period between sets. According to warm-up sets and individual load–velocity relationships, a 70% 1RM load was established daily for each subject. The MPV values for every repetition were recorded. The velocity loss (VLoss) induced within the set was calculated as the relative difference between the fastest repetition velocity and the last repetition velocity of each set (Sánchez-Medina and González-Badillo, 2011). The total volume load accumulated within the session was calculated using the following formula: absolute load lifted (kg) × total repetitions.

Statistical analysis

The normal distribution of the variables and homoscedasticity were tested using the Shapiro–Wilk and Levene's tests, respectively (p >0.05). A two-way repeated measures analysis of variance (ANOVA) (condition × time) with the Bonferroni *post hoc* test

TABLE 1 Comparison of electromyographical responses to the three different supplementation conditions using two-way repeated measures analysis of variance.

Variable	Time	Condition			ANOVA		
		PLA	LD	HD	Condition	Time	Condition × time
Isometric RMS (%)	Post	100.99 ± 23.72	101.31 ± 20.39	97.97 ± 35.00	$F = 1.13$; $p = 0.33$	$F = 2.64$; $p = 0.11$	$F = 0.37$; $p = 0.69$
	Post-24	105.59 ± 19.10	127.49 ± 34.42	107.19 ± 24.37	$\eta p^2 = 0.05$	$\eta p^2 = 0.12$	$\eta p^2 = 0.02$
60% 1RM load RMS (%)	Pre	119.77 ± 24.11	125.20 ± 25.01	129.27 ± 39.11	$F = 1.095$; $p = 0.35$	$F = 10.67$; $p < 0.001$*	$F = 1.14$; $p = 0.34$
	Post	102.82 ± 29.83	123.39 ± 40.81	102.84 ± 35.18	$\eta p^2 = 0.06$	$\eta p^2 = 0.40$	$\eta p^2 = 0.07$
	Post-24	120.41 ± 31.13	132.88 ± 33.33	131.66 ± 26.60			
Isometric MDF (%)	Post	98.09 ± 10.34	95.87 ± 6.93	97.04 ± 8.23	$F = 1.16$; $p = 0.32$	$F = 9.51$; $p = 0.006$*	$F = 0.49$; $p = 0.62$
	Post-24	102.96 ± 15.81	95.95 ± 7.30	99.94 ± 8.14	$\eta p^2 = 0.05$	$\eta p^2 = 0.32$	$\eta p^2 = 0.02$
60% 1RM load MDF (%)	Pre	88.22 ± 8.42	91.72 ± 9.65	89.81 ± 9.45	$F = 1.20$; $p = 0.88$	$F = 3.52$; $p = 0.006$*	$F = 1.35$; $p = 0.26$
	Post	87.50 ± 11.17	83.64 ± 6.36	88.98 ± 10.57	$\eta p^2 = 0.01$	$\eta p^2 = 0.18$	$\eta p^2 = 0.08$
	Post-24	95.51 ± 16.42	89.16 ± 10.84	90.93 ± 11.88			
SQ protocol RMS (%)	Set 1	88.89 ± 8.27	89.47 ± 10.05	90.69 ± 10.80	$F = 0.85$; $p = 0.36$	$F = 3.42$; $p = 0.07$	$F = 0.43$; $p = 0.52$
	Set 2	87.13 ± 8.30	87.09 ± 9.85	89.23 ± 11.11	$\eta p^2 = 0.04$	$\eta p^2 = 0.13$	$\eta p^2 = 0.02$
	Set 3	86.07 ± 8.80	85.91 ± 7.64	89.73 ± 10.77			
SQ protocol MDF (%)	Set 1	89.31 ± 8.74	90.55 ± 9.63	91.40 ± 9.93	$F = 0.64$; $p = 0.54$	$F = 13.68$; $p < 0.001$*	$F = 1.23$; $p = 0.29$
	Set 2	87.06 ± 8.85	87.97 ± 9.83	90.19 ± 11.39	$\eta p^2 = 0.03$	$\eta p^2 = 0.44$	$\eta p^2 = 0.07$
	Set 3	86.01 ± 8.32	86.14 ± 8.48	90.73 ± 11.06			

Mean ± standard deviation. PLA, placebo; HD, high dose; LD, low dose; RMS, root mean square; MDF, median frequency; VL, vastus lateralis; VM, vastus medialis. Post, post-exercise measure; post-24, 24 h post-exercise measure. Isometric: values obtained from the isometric squat test; 60% 1RM load: values obtained from two full-squat (SQ) repetitions against the 60% 1RM load; SQ protocol: values obtained from SQ protocol, i.e., from three SQ sets of eight repetitions with 70% 1RM load. * Significant difference ($p \leq 0.05$).

was used to explore the effect of the interventions (LD, HD, and PLA) across time on the magnitude of each dependent variable and to address the presence of an order effect. A one-way repeated measures ANOVA was used to compare the total volume load. The Greenhouse–Geisser correction was applied when Mauchly's sphericity test was significant ($p \leq 0.05$). Statistical analyses were performed using the software package SPSS (IBM SPSS version 25.0, Chicago, IL, United States). Statistical significance was established at $p \leq 0.05$. To assess the magnitude of the differences, partial eta-squared values (ηp^2) were derived from ANOVA and were interpreted as low (<0.04), moderate (0.04–0.13), and large (>0.13). Bonferroni *post hoc* comparisons were used to evaluate pairwise differences. The effect size of *post hoc* comparisons was calculated by means of Cohen's d, which was interpreted as a low (<0.50), moderate (0.50–0.79), or large effect (>0.80) (Cohen, 1988).

Results

Electromyography

The descriptive values and statistical comparisons for EMG outcomes are presented in Table 1. Two-way repeated measures ANOVAs did not reveal any condition × time interaction (p range <0.26–0.69). However, a significant time effect for RMS at 60% 1RM, MDF at isometric SQ, MDF at 60% 1RM, and MDF during the SQ protocol (p range <0.001–0.006) was observed. The *post hoc* Bonferroni test for time only revealed significant differences at 60% load RMS for 1/2 ($p = 0.01$; d = 1.11) and 2/3 ($p = 0.006$; d = 1.19) set comparisons.

Countermovement jump test

The two-way repeated measures ANOVA revealed significant condition × time interactions for CMJ height (p <0.01) and a significant condition main effect (p <0.001) (Table 3). *Post hoc* Bonferroni tests showed that HD attained a higher CMJ height than PLA (p <0.001; d = 0.72) and LD (p <0.001; d = 0.37).

Dynamic and isometric squat tests

Regarding the SQ test with 60% 1RM, the two-way repeated measures ANOVA did not reveal significant condition × time interactions (p range = 0.74–0.95). However, a significant time effect (p <0.001) and condition effect (p = 0.03) for MPV were observed (Table 3). Moreover, the two-way repeated measures ANOVA did not reveal significant condition × time interactions (p range = 0.09–0.89) or significant differences for time (p-range =

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TABLE 2 Comparison of isometric mechanical responses to the three supplementation conditions using two-way repeated measures analysis of variance.

Variable	Time	Condition			ANOVA		
		PLA	LD	HD	Condition	Time	Condition × time
MIF (%)	Post	0.88 ± 0.13	0.90 ± 0.12	0.92 ± 0.13	F = 0.57; p = 0.57	F = 0.005; p = 0.95	F = 0.20; p = 0.81
	Post-24	0.93 ± 0.17	0.92 ± 0.19	0.95 ± 0.17	ηp^2 = 0.045	ηp^2 = 0.001	ηp^2 = 0.02
RFDmax (N·s^{-1})	Pre	4,562.8 ± 1,607.7	5,041.3 ± 1,676.7	4,363.2 ± 1,234.2	F = 0.03; p = 0.97	F = 0.30; p = 0.78	F = 1.46; p = 0.22
	Post	4,395.9 ± 1,689.3	3,935.8 ± 1,153.0	4,678.5 ± 1,658.9	ηp^2 = 0.07	ηp^2 = 0.12	ηp^2 = 0.06
	Post-24	4,894.2 ± 1,601.3	4,075.7 ± 1,337.7	4,752.2 ± 1,585.1			
RFD_{0-50} (N·s^{-1})	Pre	2,262.4 ± 1,189.2	2,765.4 ± 1,725.4	2,544.1 ± 1,150.3	F = 1.28; p = 0.29	F = 0.78; p = 0.46	F = 1.55; p = 0.19
	Post	2,467.7 ± 1,396.8	2,335.8 ± 1,408.5	2,375.3 ± 1,172.7	ηp^2 = 0.08	ηp^2 = 0.05	ηp^2 = 0.10
	Post-24	2,512.4 ± 1,332.5	2,006.3 ± 773.6	2,190.1 ± 1,249.6			
RFD_{0-100} (N·s^{-1})	Set 1	2,377.2 ± 1,325.0	2,921,4 ± 1,353.3	2,585.6 ± 1,998.9	F = 1.73; p = 0.19	F = 1.14; p = 0.33	F = 2.43; p = 0.10
	Set 2	2,039.6 ± 1,283.3	2,320.4 ± 1,229.5	1,968.5 ± 1,373.4	ηp^2 = 0.11	ηp^2 = 0.07	ηp^2 = 0.14
	Set 3	2,666.2 ± 1,432.5	2,129.3 ± 942.0	2,377.3 ± 1,196.7			
RFD_{0-150} (N·s^{-1})	Set 1	2,380.8 ± 1,655.6	2,958.4 ± 1,280.9	2,635.7 ± 1,075.2	F = 0.54; p = 0.58	F = 1.29; p = 0.28	F = 2.09; p = 0.09
	Set 2	2,670.8 ± 1,002.9	2,388.1 ± 1,184.6	2,129.5 ± 1,279.2	ηp^2 = 0.04	ηp^2 = 0.09	ηp^2 = 0.13
	Set 3	2,600.7 ± 1,330.5	2,260.5 ± 1,032.9	2,450.5 ± 1,275.4			
RFD_{0-200} (N·s^{-1})	Set 1	2,400.9 ± 1,322.2	2,697.3 ± 1,219.1	2,456.9 ± 1,052.1	F = 0.28; p = 0.76	F = 2.56; p = 0.10	F = 1.69; p = 0.16
	Set 2	2,384.1 ± 918.5	2,178.3 ± 1,101.1	1,973.3 ± 1,214.8	ηp^2 = 0.02	ηp^2 = 0.15	ηp^2 = 0.10
	Set 3	2,295.3 ± 1,430.7	2,067.3 ± 978.1	2,361.8 ± 1,214.6			
RFD_{0-400} (N·s^{-1})	Set 1	1,787.1 ± 974.8	1,764.9 ± 898.6	1,784.8 ± 809.4	F = 0.47; p = 0.62	F = 3.94; p = 0.06	F = 0.27; p = 0.89
	Set 2	1,652.5 ± 772.0	1,489.6 ± 733.9	1,521.3 ± 853,9	ηp^2 = 0.03	ηp^2 = 0.23	ηp^2 = 0.02
	Set 3	1,631.8 ± 1,045.1	1,610.3 ± 773.9	1,664.4 ± 912.0			

Mean ± standard deviation. PLA, placebo; HD, high dose; LD, low dose; MIF, maximal isometric force; RFDmax, maximal rate of force development; RFD_{0-50}, rate of force development from the onset of force production to 50 ms; RFD_{0-100}, rate of force development from the onset of force production to 100 ms; RFD_{0-150}, rate of force development from the onset of force production to 150 ms; RFD_{0-200}, rate of force development from the onset of force production to 200 ms; RFD_{0-400}, rate of force development from the onset of force production to 400 ms.

0.06–0.78) or condition effect (p range = 0.19–0.97) for any of the isometric outcomes (Table 2).

Full-squat protocol

One-way repeated measures ANOVA reported no significant differences between conditions for total volume load (F = 1.09; p = 0.35). The two-way repeated measures ANOVA did not reveal significant condition × time interactions for any variable (p range = 0.12–0.98). However, it revealed significant time effects (p range = 0.001–0.008) for all the analyzed outcomes. Moreover, a significant condition effect was observed for 60% load (p = 0.03), MPVmean (p = 0.02), and VLoss (p = 0.04) (Table 3). The Bonferroni *post hoc* test revealed no significant differences for conditions (p range = 0.06–0.07). The *post hoc* Bonferroni test for time analyses revealed significant differences between sets 1 and 2 for all outcomes (p range <0.001 to 0.007; d range = 0.47–0.73). However, Bonferroni *post hoc* time analyses reported significant differences between sets 1 and 3 for MPV (p <0.001; d = 0.80). For comparisons between sets 2 and 3, all movement velocity outcomes reported significant time differences (p range <0.001 to 0.04; d range = 0.21–0.78). The one-way ANOVA for intra-set analysis revealed significant differences in repetitions 13, 15, 16, 17, 23, and 24 (p range = 0.03–0.04). The *post hoc* Bonferroni test reported significant differences between HD and PLA in repetition 23 (p = 0.01; d = 0.65) and for HD and LD in repetitions 15 (p = 0.008; d = 0.50) and 16 (p = 0.004; d = 0.46) (Figure 2).

Discussion

The objective of this study was to explore, for the first time, the neuromuscular and mechanical responses to a capsaicinoid supplement in dynamic and isometric exercises. The main findings of this research indicate that a LD or HD of PC does not modulate the electrical signals of quadriceps muscles compared to a PLA. However, the HD condition attained higher velocity values during the main SQ test than LD and PLA. Furthermore, the HD condition also exhibited higher CMJ values across time compared to PLA and LD. Supporting our initial hypothesis, PC may produce an ergogenic effect on SQ performance and may improve mechanical

TABLE 3 Comparison of mechanical responses to the three supplementation conditions using two-way repeated measures analysis of variance and comparison of the descriptive total volume of the squat protocol between the three supplementation conditions using one-way repeated measures ANOVA.

Variable	Time	Condition			ANOVA		
		PLA	LD	HD	Condition	Time	Condition × time
Total volume load (kg)		2,089.1 ± 357.6	2,014.6 ± 380.9	2,101.7 ± 352.9	$F = 1.09$; $p = 0.35$		
60% load MPV ($m \cdot s^{-1}$)	Pre	0.91 ± 0.07	0.93 ± 0.06	0.93 ± 0.06	$F = 3.94$; $p = 0.03$*	$F = 50.89$; $p < 0.001$*	$F = 0.48$; $p = 0.74$
	Post	0.77 ± 0.09	0.80 ± 0.08	0.81 ± 0.12	$\eta p^2 = 0.19$	$\eta p^2 = 0.75$	$\eta p^2 = 0.03$
	Post-24	0.89 ± 0.07	0.93 ± 0.09	0.91 ± 0.08			
MPVbest ($m \cdot s^{-1}$)	Set 1	0.77 ± 0.04	0.78 ± 0.05	0.79 ± 0.06	$F = 1.40$; $p = 0.14$	$F = 65.53$; $p < 0.001$*	$F = 0.01$; $p = 0.90$
	Set 2	0.72 ± 0.04	0.72 ± 0.07	0.74 ± 0.07	$\eta p^2 = 0.09$	$\eta p^2 = 0.73$	$\eta p^2 = 0.013$
	Set 3	0.70 ± 0.05	0.71 ± 0.07	0.73 ± 0.05			
MPVmean ($m \cdot s^{-1}$)	Set 1	0.65 ± 0.04	0.66 ± 0.07	0.68 ± 0.07	$F = 4.14$; $p = 0.02$*	$F = 98.13$; $p < 0.001$*	$F = 0.50$; $p = 0.77$
	Set 2	0.60 ± 0.05	0.60 ± 0.07	0.64 ± 0.08	$\eta p^2 = 0.17$	$\eta p^2 = 0.83$	$\eta p^2 = 0.02$
	Set 3	0.57 ± 0.06	0.58 ± 0.08	0.61 ± 0.08			
VLoss (%)	Set 1	29.4 ± 8.5	29.7 ± 10.9	26.6 ± 9.4	$F = 3.73$; $p = 0.04$*	$F = 5.43$; $p = 0.008$*	$F = 0.11$; $p = 0.98$
	Set 2	29.9 ± 11.8	30.9 ± 10.2	26.6 ± 11.7	$\eta p^2 = 0.14$	$\eta p^2 = 0.21$	$\eta p^2 = 0.006$
	Set 3	34.2 ± 11.9	34.4 ± 12.2	29.8 ± 10.9			
CMJ height (cm)	Pre	39.94 ± 9.63	40.15 ± 9.61	40.18 ± 9.88	$F = 97.10$; $p < 0.001$*	$F = 2.82$; $p = 0.07$	$F = 3.48$; $p = 0.01$*
	Post	32.92 ± 8.24	33.13 ± 8.29	34.73 ± 9.08	$\eta p^2 = 0.80$	$\eta p^2 = 0.10$	$\eta p^2 = 0.13$
	Post-24	39.07 ± 9.12	40.02 ± 9.68	39.71 ± 9.71			

Mean ± standard deviation. PLA, placebo; HD, high dose; LD, low dose; MPV, mean propulsive velocity; VLoss, percentage of velocity loss during a set; MPVbest, the highest value of each set; MPVmean, the mean value of all repetitions conducted in each set; CMJ, countermovement jump. * Significant difference ($p \leq 0.05$).

recovery outcomes (e.g., CMJ height and post-test lifting velocity). On the other hand, the initial hypothesis on a nervous system modulator effect was rejected according to the collected electrical signals. Therefore, a HD of PC supplementation may attenuate mechanical fatigue after a submaximal high-intensity session. These effects on fatigue outcomes may not be mediated by the traditional neural mechanisms proposed in the aforementioned literature (Jiménez-Martínez et al., 2022).

In the present study, changes in electrical signals were not detected for RMS or MDF between PLA and LD or HD. However, the protocol was effective, inducing fatigue due to the reduction in the intensity of electrical signals across the SQ sets. In this regard, an increase in RMS may be explained by a higher degree of motor unit recruitment, higher firing rates, or the recruitment of higher-threshold motor units when greater electrical outputs are needed (Bigland-Ritchie et al., 1986; Hunter et al., 2004). By contrast, lower MDF values are highly correlated with a decline in the force production from the fresh state due to impairments in the neural conduction velocity and discharge rates of motor units (Allison and Fujiwara, 2002). Thus, during and after high-intensity exercise, EMG can reflect the changes in neural strategies of the muscle related to the increment in the metabolic activity, hydrogen ion accumulation, and other physiological events inducing fatigue (Mendez-Villanueva et al., 2008). Contrary to the present findings, the previous research has documented that topical capsaicin administration may alter Henneman's size principle (De Luca and Contessa, 2012), eliciting a greater number of motor units recruited in upper-limb isometric contractions (Evans et al., 2021). Nevertheless, the effects of an oral capsaicin supplement on electrical muscle signals have not been evaluated previously. In addition, although the mechanical performance was higher in the HD condition (i.e., lower velocity loss in all the sets), the null changes in electrical signals may be interpreted, bearing in mind that other physiological mechanisms may be presented in the ergogenic effect of PC supplementation (de Moura e Silva et al., 2021). Furthermore, if a direct neural mechanism would have appeared improving the neural output, HD may have attained higher RMS values during and in the post-squat window (Alix-Fages et al., 2022a), which suggests that PC does not provide an ergogenic effect on the acute recovery of the neural output. For this reason, PC may exert its main effects directly in the muscle junction upregulating the efflux of calcium channels and acetylcholine turnover without a direct effect on the neural raw output (de Moura e Silva et al., 2021).

Regarding mechanical outcomes, the present results have not displayed an ergogenic mechanical effect of PC on *post hoc* comparisons of velocity outcomes, although the CMJ height and intra-set lifting velocities were reported significant in the *post hoc* comparisons. However, as the velocity was higher in both HD and LD of PC, a plausible delaying effect on neuromuscular fatigue may have

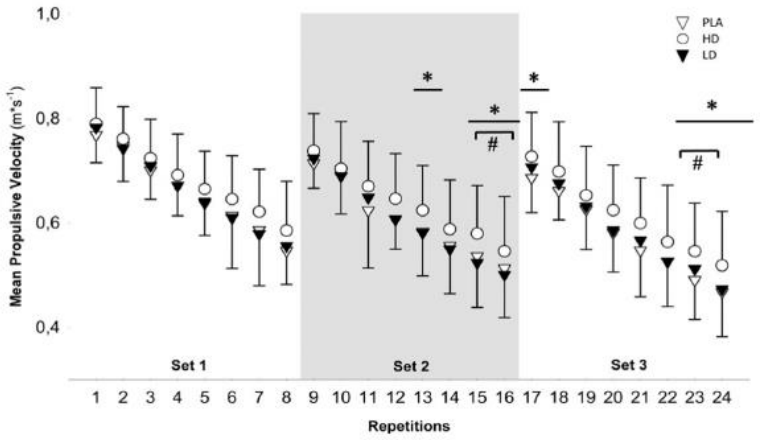

FIGURE 2
One-way repeated measures analysis of variance (ANOVA) of the intra-set comparisons of individual repetitions and repetitions across time for different supplementation conditions (PLA, HD, and LD). PLA, placebo; HD, high dose; LD, low dose (*$p_{ANOVA} \leq 0.05$; #$p_{Bonferroni} \leq 0.05$)

been presented (i.e., a lower velocity loss in all the sets and a higher CMJ height). Within this context, previous research has focused on the effects of capsaicin supplementation on strength endurance tasks performed until exhaustion (Jiménez-Martínez et al., 2022). For instance, a four sets at 70% 1RM until failure SQ protocol documented an ergogenic response to 12 mg of purified capsaicin in comparison to a placebo (de Freitas et al., 2018). In this study, the authors reported a higher number of repetitions until exhaustion and, consequently, a rise in the total mass lifted. However, the effects of capsaicin on mechanical fatigue were only approached by the inter-set analysis of the number of repetitions in the SQ exercise. Moreover, the effects of capsaicin on a non-exhaustive task after an SQ exercise have not been assessed previously (de Moura e Silva et al., 2021; Jiménez-Martínez et al., 2022). Accordingly, these aforementioned protocols may provide low applicability on the strength and conditioning or sports performance fields due to the high levels of fatigue induced (Pareja-Blanco et al., 2020b). Nevertheless, according to the current results and previous research, capsaicin may improve efforts performed close to muscular failure on account of the regulation of TRPV1 in the context of high degrees of metabolic fatigue during exercise (de Freitas et al., 2018; de Freitas et al., 2019), due to the responses of III and IV nerve afferent fibers (Taylor et al., 2016). For this reason, it could be hypothesized that the differences reported between the current study and the previous research may be explained by the fact that in the present study, the exercise volume was matched. This is corroborated by the growing gap reported between increasing MPVs in the intra-set analysis, where most of the significant differences were found in favor of the HD condition at the end of the last repetitions and mainly in the last set.

Concerning the influence of HD on mechanical recovery, the two dynamic indicators of fatigue (i.e., increasing MPV with 60% 1RM and CMJ height) exhibited higher values in the post-exercise window in favor of HD. According to the previous literature, SQ dynamic strength (Nuzzo et al., 2008) is highly correlated with the CMJ height (Bauer et al., 2019). Consequently, these effects on CMJ performance may have been mediated by the less mechanical fatigue accumulated according to the % of VLoss reported in the HD group. These findings are meaningful, as the reported improvements in the mechanical outcomes can be associated with a 2.5% enhancement of force production (Sánchez-Medina et al., 2017). Nonetheless, in both dynamic mechanical recovery variables, the post-24 h values returned close to the baseline, which suggests that this mechanical effect may be only considered during the first hours of the acute time course recovery window (Pareja-Blanco et al., 2019). Regarding isometric testing, as mentioned previously, the null effects of PC on MIF and RFD outcomes may be explained by the lower metabolic demands across the time required in isometric tasks (Wells et al., 2009). Accordingly, previous research did not report performance improvements from a low dose of capsaicin in knee-extension isokinetic exercises when the range of motion was restricted (Cross et al., 2020). Thus, PC's TRPV1 activity may enhance resistance training during dynamic exercises but not in short-duration isometric tasks. Moreover, this rationale could also be the cause of the contrary results observed between dynamic fatigue and isometric fatigue in the present study, given that afferent III and IV nerve fibers mainly detect metabolic discomfort during exercise (Collins et al., 2018; Alix-Fages et al., 2022a). Within this context, exercise "perceived pain" could be explained as a manifestation of these nerve fiber firing, which is a biochemical target of PC supplementation, and leads to a lesser extent of efficiency in the neuromuscular junction, reducing (e.g., regulation of calcium overload) the velocity of crossbridge cycling, a mechanism directly involved in dynamic contractions (Collins et al., 2018; de Moura e Silva et al., 2021; Jiménez-Martínez et al., 2022). Additionally, the lack of a dose–response effect in most of the variables studied (i.e., isometric and electrical) may be explained by the existence of a threshold in TRPV1 peripheral activation,

which is in line with the different doses of capsaicin employed in the previous literature (Jiménez-Martínez et al., 2022). In this sense, the current HD dose of PC may correspond to the most used evidenced ergogenic dose of capsaicin due to its near five-fold higher bioavailability (Turck et al., 2019; Framroze, 2022; Jiménez-Martínez et al., 2022; Jiménez-Martínez et al., 2023).

Collectively, an acute HD (i.e., 2.5 mg) of PC may reduce mechanical fatigue (i.e., higher CMJ height) after submaximal resistance exercise, as a consequence of a positive effect on the mechanical performance (i.e., higher MPV values and a lower % of VLoss) compared to LD and PLA. Therefore, PC may serve as a tool for reducing fatigue during high-volume resistance exercise workouts. Finally, a triple-blind, crossover, placebo-controlled design and the enrollment of trained subjects as described in previous research (de Freitas et al., 2018) were considered the important strengths of this current study. Nevertheless, some limitations may be remarkable. First, a nutritional follow-up of the subject's diet and supplementation was not directly registered. Second, this study was conducted on trained men under laboratory conditions; for this reason, the present finding may be cautiously interpreted in other populations and environments. Moreover, bipolar surface EMG might not detect the contractile properties of the muscle in isolation. Accordingly, future research studies may add more accurate measures, such as high-density EMG, for the assessment of the neural effects of PC. Finally, the rate of perceived exertion or perceived pain was not evaluated in this study, which may be useful in future studies for the understanding of the sensitivity mechanisms underlying supplementation with capsaicin. On the other hand, it would be valuable to determine the impact of PC on protocols where exercise volumes were not matched. In addition, sets may be performed until exhaustion, and a force platform and linear transducer may be used.

Conclusion

Acute PC ingestion may be considered an ergogenic aid (2.5 mg) for dynamic resistance exercise sessions when more than one exercise is performed. Consistently, mechanical fatigue after a submaximal exercise may be delayed and attenuated by PC. In addition, further research studies must deeply examine the existence of neural mechanisms underlying the capsaicinoid ergogenic impact.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Local Research Ethics Committee of Junta de Andalucía (Code: 0513-N-22). The patients/participants provided their written informed consent to participate in this study.

Author contributions

The study and methodology were conceived by PJ-M, FP-B, CA-F, and JC. JS-V, PC-D, CC-C, IA-I, and PJ-M were involved in data collection and treatment. The writing of the manuscript was carried out by PJ-M and CA-F. All authors contributed to the article and approved the submitted version.

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Conflict of interest

PJ-M and CA-F are scientific advisors of a sports supplement brand (Life Pro Nutrition).

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2023.1215644/full#supplementary-material

References

Alix-Fages, C., Del Vecchio, A., Baz-Valle, E., Santos-Concejero, J., and Balsalobre-Fernández, C. (2022a). The role of the neural stimulus in regulating skeletal muscle hypertrophy. *Eur. J. Appl. Physiol.* 122, 1111–1128. doi:10.1007/s00421-022-04906-6

Alix-Fages, C., Grgic, J., Jiménez-Martínez, P., Baz-Valle, E., and Balsalobre-Fernández, C. (2022b). Effects of mental fatigue on strength endurance: A systematic review and meta-analysis. *Mot. Control* 27, 442. doi:10.1123/mc.2022-0051

Allison, G. T., and Fujiwara, T. (2002). The relationship between EMG median frequency and low frequency band amplitude changes at different levels of muscle capacity. *Clin. Biomech.* 17, 464–469. doi:10.1016/S0268-0033(02)00033-5

Amann, M., Blain, G. M., Proctor, L. T., Sebranek, J. J., Pegelow, D. F., and Dempsey, J. A. (2011). Implications of group III and IV muscle afferents for high-intensity endurance exercise performance in humans. *J. Physiology* 59, 5299–5309. doi:10.1113/jphysiol.2011.213769

Arora, V., Campbell, J. N., and Chung, M.-K. (2021). Fight fire with fire: Neurobiology of capsaicin-induced analgesia for chronic pain. *Pharmacol. Ther.* 220, 107743. doi:10.1016/j.pharmthera.2020.107743

Baamonde, A., Lastra, A., Juarez, L., Hidalgo, A., and Menéndez, L. (2005). TRPV1 desensitisation and endogenous vanilloid involvement in the enhanced analgesia induced by capsaicin in inflamed tissues. *Brain Res. Bull.* 67, 476–481. doi:10.1016/j.brainresbull.2005.07.001

Bauer, P., Sansone, P., Mitter, B., Makivic, B., Seitz, L. B., and Tschan, H. (2019). Acute effects of back squats on countermovement jump performance across multiple sets of a contrast training protocol in resistance-trained men. *J. Strength Cond. Res.* 33, 995–1000. doi:10.1519/JSC.0000000000002422

Bigland-Ritchie, B. R., Dawson, N. J., Johansson, R. S., and Lippold, O. C. (1986). Reflex origin for the slowing of motoneurone firing rates in fatigue of human voluntary contractions. *J. Physiology* 379, 451–459. doi:10.1113/jphysiol.1986.sp016263

Blain, G. M., Mangum, T. S., Sidhu, S. K., Weavil, J. C., Hureau, T. J., Jessop, J. E., et al. (2016). Group III/IV muscle afferents limit the intramuscular metabolic perturbation during whole body exercise in humans. *J. Physiology* 594, 5303–5315. doi:10.1113/JP272283

Broxterman, R. M., Hureau, T. J., Layec, G., Morgan, D. E., Bledsoe, A. D., Jessop, J. E., et al. (2018). Influence of group III/IV muscle afferents on small muscle mass exercise performance: A bioenergetics perspective. *J. Physiology* 596, 2301–2314. doi:10.1113/JP275817

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences*. Hillsdale, N.J. Lawrence Erlbaum Associates.

Collins, B. W., Pearcey, G. E. P., Buckle, N. C. M., Power, K. E., and Button, D. C. (2018). Neuromuscular fatigue during repeated sprint exercise: Underlying physiology and methodological considerations. *Appl. Physiol. Nutr. Metab.* 43, 1166–1175. doi:10.1139/apnm-2018-0080

Cross, B. L., Parker, D., Langan, S. P., and Grosicki, G. J. (2020). Effect of a commercially available low-dose capsaicin supplement on knee extensor contractile function. *Int. J. Exerc. Sci.* 7, 312–318.

de Freitas, M. C., Cholewa, J. M., Freire, R. V., Carmo, B. A., Bottan, J., Bratfich, M., et al. (2018). Acute capsaicin supplementation improves resistance training performance in trained men. *J. Strength Cond. Res.* 32, 2227–2232. doi:10.1519/JSC.0000000000002109

de Freitas, M. C., Cholewa, J. M., Panissa, V. L. G., Toloi, G. G., Netto, H. C., Zanini de Freitas, C., et al. (2019). Acute capsaicin supplementation improved resistance exercise performance performed after a high-intensity intermittent running in resistance-trained men. J. Strength Cond. Res. *Publ.* 36, 130–134. Ahead of Print. doi:10.1519/JSC.0000000000003431

De Luca, C. J., and Contessa, P. (2012). Hierarchical control of motor units in voluntary contractions. *J. Neurophysiology* 107, 178–195. doi:10.1152/jn.00961.2010

de Moura e Silva, V. E. L., Cholewa, J. M., Billaut, F., Jäger, R., de Freitas, M. C., Lira, F. S., et al. (2021). Capsaicinoid and capsinoids as an ergogenic aid: A systematic review and the potential mechanisms involved. *Int. J. Sports Physiology Perform.* 16, 464–473. doi:10.1123/ijspp.2020-0677

Evans, V., Koh, R. G. L., Duarte, F. C. K., Linde, L., Amiri, M., and Kumbhare, D. (2021). A randomized double blinded placebo controlled study to evaluate motor unit abnormalities after experimentally induced sensitization using capsaicin. *Sci. Rep.* 11, 13793. doi:10.1038/s41598-021-93188-7

Fattori, V., Hohmann, M., Rossaneis, A., Pinho-Ribeiro, F., and Verri, W. (2016). Capsaicin: Current understanding of its mechanisms and therapy of pain and other pre-clinical and clinical uses. *Molecules* 21, 844. doi:10.3390/molecules21070844

Framroze, Z. (2022). Placebo controlled study to investigate the relationship between lowering serum zonulin levels and improved body weight composition using a daily oral dose of phenylcapsaicin. *Int. J. Clin. Ski.* 7, 1.

Glatthorn, J. F., Gouge, S., Nussbaumer, S., Stauffacher, S., Impellizzeri, F. M., and Maffiuletti, N. A. (2011). Validity and reliability of optojump photoelectric cells for estimating vertical jump height. *J. Strength Cond. Res.* 25, 556–560. doi:10.1519/JSC.0b013e3181ccb18d

González-Badillo, J. J., and Sánchez-Medina, L. (2010). Movement velocity as a measure of loading intensity in resistance training. *Int. J. Sports Med.* 31, 347–352. doi:10.1055/s-0030-1248333

González-Badillo, J. J., Yañez-García, J. M., Mora-Custodio, R., and Rodríguez-Rosell, D. (2017). Velocity loss as a variable for monitoring resistance exercise. *Int. J. Sports Med.* 38, 217–225. doi:10.1055/s-0042-120324

Hayman, M., and Kam, P. C. A. (2008). Capsaicin: A review of its pharmacology and clinical applications. *Curr. Anaesth. Crit. Care* 19, 338–343. doi:10.1016/j.cacc.2008.07.003

Hermens, H. J., Freriks, B., Disselhorst-Klug, C., and Rau, G. (2000). Development of recommendations for SEMG sensors and sensor placement procedures. *J. Electromyogr. Kinesiol.* 10, 361–374. doi:10.1016/S1050-6411(00)00027-4

Hunter, S. K., Duchateau, J., and Enoka, R. M. (2004). Muscle fatigue and the mechanisms of task failure. *Exerc. Sport Sci. Rev.* 32, 44–49. doi:10.1097/00003677-200404000-00002

Jiménez-Martínez, P., Alix-Fages, C., Janicijevic, D., Miras-Moreno, S., Chacón-Ventura, S., Martín-Olmedo, J. J., et al. (2023). Effects of phenylcapsaicin on aerobic capacity and physiological parameters in active young males: A randomized, triple-blinded, placebo-controlled, crossover trial. *Front. Physiol.* 14, 1190345. doi:10.3389/fphys.2023.1190345

Jiménez-Martínez, P., Ramírez-Campillo, R., Flandez, J., Alix-Fages, C., Baz-Valle, E., and Colado, J. C. (2022). Effects of oral capsaicinoids and capsinoids supplementation on resistance and high intensity interval training: A systematic review of randomized controlled trials. *J. Hum. Sport. Exer.* 18 (2), 375–389. doi:10.14198/jhse.2023.182.09

Kattla, S., and Lowery, M. M. (2010). Fatigue related changes in electromyographic coherence between synergistic hand muscles. *Exp. Brain Res.* 202, 89–99. doi:10.1007/s00221-009-2110-0

Martinopoulou, K., Donti, O., Sands, W. A., Terzis, G., and Bogdanis, G. C. (2022). Evaluation of the isometric and dynamic rates of force development in multi-joint muscle actions. *J. Hum. Kinet.* 81, 135–148. doi:10.2478/hukin-2021-0130

Mendez-Villanueva, A., Hamer, P., and Bishop, D. (2008). Fatigue in repeated-sprint exercise is related to muscle power factors and reduced neuromuscular activity. *Eur. J. Appl. Physiol.* 103, 411–419. doi:10.1007/s00421-008-0723-9

Nuzzo, J. L., McBride, J. M., Cormie, P., and McCaulley, G. O. (2008). Relationship between countermovement jump performance and multijoint isometric and dynamic tests of strength. *J. Strength Cond. Res.* 22, 699–707. doi:10.1519/JSC.0b013e31816d5eda

Okano, A. H., Fontes, E. B., Montenegro, R. A., Farinatti, P. de T. V., Cyrino, E. S., Li, L. M., et al. (2015). Brain stimulation modulates the autonomic nervous system, rating of perceived exertion and performance during maximal exercise. *Br. J. Sports Med.* 49, 1213–1218. doi:10.1136/bjsports-2012-091658

Ortega-Becerra, M., Sánchez-Moreno, M., and Pareja-Blanco, F. (2021). Effects of cluster set configuration on mechanical performance and neuromuscular activity. *J. Strength Cond. Res.* 35, 310–317. doi:10.1519/JSC.0000000000003907

Pareja-Blanco, F., Rodríguez-Rosell, D., Aagaard, P., Sánchez-Medina, L., Ribas-Serna, J., Mora-Custodio, R., et al. (2020b). Time course of recovery from resistance exercise with different set configurations. *J. Strength Cond. Res.* 34, 2867–2876. doi:10.1519/JSC.0000000000002756

Pareja-Blanco, F., Alcazar, J., Sánchez-Valdepeñas, J., Cornejo-Daza, P. J., Piqueras-Sanchiz, F., Mora-Vela, R., et al. (2020a). Velocity loss as a critical variable determining the adaptations to strength training. *Med. Sci. Sports Exerc.* 52, 1752–1762. doi:10.1249/MSS.0000000000002295

Pareja-Blanco, F., Rodríguez-Rosell, D., Sánchez-Medina, L., Gorostiaga, E., and González-Badillo, J. (2014). Effect of movement velocity during resistance training on neuromuscular performance. *Int. J. Sports Med.* 35, 916–924. doi:10.1055/s-0033-1363985

Pareja-Blanco, F., Villalba-Fernández, A., Cornejo-Daza, P., Sánchez-Valdepeñas, J., and González-Badillo, J. (2019). Time course of recovery following resistance exercise with different loading magnitudes and velocity loss in the set. *Sports* 7, 59. doi:10.3390/sports7030059

Piqueras-Sanchiz, F., Cornejo-Daza, P. J., Sánchez-Valdepeñas, J., Bachero-Mena, B., Sánchez-Moreno, M., Martín-Rodríguez, S., et al. (2021). Acute mechanical, neuromuscular, and metabolic responses to different set configurations in resistance training. J. Strength Cond. Res. *Publ.* 36, 2983–2991. Ahead of Print. doi:10.1519/JSC.0000000000004068

Poitras, I., Bielmann, M., Campeau-Lecours, A., Mercier, C., Bouyer, L. J., and Roy, J.-S. (2019). Validity of wearable sensors at the shoulder joint: Combining wireless electromyography sensors and inertial measurement units to perform physical workplace assessments. *Sensors* 19, 1885. doi:10.3390/s19081885

Sánchez-Medina, L., and González-Badillo, J. J. (2011). Velocity loss as an indicator of neuromuscular fatigue during resistance training. *Med. Sci. Sports Exerc.* 43, 1725–1734. doi:10.1249/MSS.0b013e318213f880

Sánchez-Medina, L., Pallarés, J., Pérez, C., Morán-Navarro, R., and González-Badillo, J. (2017). Estimation of relative load from bar velocity in the full back squat exercise. *Sports Med. Int. Open* 01, E80–E88. –E88. doi:10.1055/s-0043-102933

Sidhu, S. K., Weavil, J. C., Mangum, T. S., Jessop, J. E., Richardson, R. S., Morgan, D. E., et al. (2017). Group III/IV locomotor muscle afferents alter motor cortical and corticospinal excitability and promote central fatigue during cycling exercise. *Clin. Neurophysiol.* 128, 44–55. doi:10.1016/j.clinph.2016.10.008

Taylor, J. L., Amann, M., Duchateau, J., Meeusen, R., and Rice, C. L. (2016). Neural contributions to muscle fatigue: From the brain to the muscle and back again. *Med. Sci. Sports Exerc.* 48, 2294–2306. doi:10.1249/MSS.0000000000000923

Turck, D., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K. I., Kearney, J., Maciuk, A., et al. (2019). Safety of phenylcapsaicin as a novel food pursuant to Regulation (EU) 2015/2283. *EFS2* 17, 1. doi:10.2903/j.efsa.2019.5718

Wells, G. D., Selvadurai, H., and Tein, I. (2009). Bioenergetic provision of energy for muscular activity. *Paediatr. Respir. Rev.* 10, 83–90. doi:10.1016/j.prrv.2009.04.005

8.1.2 Article two

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Effects of different phenylcapsaicin doses on resistance training performance, muscle damage, protein breakdown, metabolic response, ratings of perceived exertion, and recovery: a randomized, triple-blinded, placebo-controlled, crossover trial

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ABSTRACT

Background: The aim of this study was to explore the effects of a low dose (LD) of 0.625 mg and a high dose (HD) of 2.5 mg of phenylcapsaicin (PC) on full squat (SQ) performance, active muscle (RPE-AM) and overall body (RPE-OB) ratings of perceived exertion, muscle damage, protein breakdown, metabolic response, and 24-h recovery in comparison to placebo (PLA).

Method: Twenty-five resistance-trained males (age = 21.00 ± 2.15 years, SQ 1-repetition maximum [1RM] normalized = 1.66 ± 0.22 kg) were enrolled in this randomized, triple-blinded, placebo-controlled, crossover trial. Participants completed 2 weekly sessions per condition (LD, HD, and PLA). The first session consisted of pre-blood testing of lactate, urea, and aspartate aminotransferases (AST) and 2 SQ repetitions with 60% 1RM followed by the resistance exercise protocol, which consisted of SQ sets of 3 × 8 × 70% 1RM monitoring lifting velocity. RPE-OB and RPE-AM were assessed after each set. After the first session, 2 SQ repetitions with 60% 1RM were performed, and blood lactate and urea posttests were collected. After 24 h, AST posttest and 1 × 2 × 60% 1RM were determined as biochemical and mechanical fatigue outcomes.

Results: HD reported significant differences for RPE-AM, AST, and SQ performance compared to LD and PLA. Post-hoc analyses revealed that HD attained faster velocities in SQ than LD (p = 0.008). HD induced a lower RPE-AM when compared with LD (p = 0.02) and PLA (p = 0.004). PLA resulted in higher AST concentrations

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at 24-h post than HD ($p = 0.02$). No significant differences were observed for the rest of the comparisons.
Conclusions: This study suggests that PC may favorably influence SQ performance, RPE-AM, and muscle damage compared to PLA. However, HD exhibited most of the biochemical and mechanical anti-fatigue effects instead of LD.

1. Introduction

Sports supplements are popular ergogenic aids among athletes aiming to improve their sport performance [1,2]. Recently, sports nutrition research has focused on the discovery of new active compounds which may be able to improve high-intensity training in different modalities [3,4]. Within this context, capsaicinoids have emerged as a plausible ergogenic aid for strength conditioning and high-intensity sports [5,6]. Capsaicinoids are a group of compounds naturally found in spicy chili peppers which are characterized by their vanilloid structure [7]. Capsaicin (8-methyl-N-vanillyl-trans-6-nonenamide), which is found in the placental tissue of *Capsicum* fruit, has emerged as the primary and most abundant capsaicinoid with therapeutic and physical performance relevance [6,8]. In this sense, capsaicin has exhibited improvements on several pathophysiological conditions, such as chronic musculoskeletal and neuropathic pain, gastrointestinal disruptions (e.g. gastroduodenal mucosal injury), and metabolic disorders (e.g. overweight) [9,10]. On the other hand, capsaicin seems to enhance sport performance by reducing ratings of perceived exertion and perceived discomfort while improving mechanical performance (e.g. total volume load) [5,11].

The aforementioned physiological effects of capsaicin have been associated with the transient receptor vanilloid 1 (TRPV1) [12]. TRPV1 content is especially high in afferents III and IV nerve fibers, which are linked to peripheral and central fatigue during high-intensity tasks [13–15]. The feedback from afferents III and IV could directly or indirectly reduce motoneuron firing and motor unit recruitment [14,15]. In this sense, the TRPV1 content of these nerve fibers (mainly in group IV afferents) has been proposed as a target for enhancing physical performance by using capsaicin [5,6,16]. Thus, as a TRPV1 agonist, capsaicin could improve muscle contraction by increasing the perceived heat analgesia and the release of calcium from the sarcoplasmic reticulum, which are linked to motor units function [5].

To date, capsaicinoid supplementation has mainly been tested on sports performance with encapsulated capsaicin formulations [5]. Purified acute (i.e. 45 min before exercise) oral capsaicin supplementation has demonstrated an ergogenic effect in upper- and lower-limb resistance training tasks [5,17]. For instance, de Freitas et al. [6] in a double-blind, randomized placebo-controlled trial found that acute 12 mg of capsaicin significantly increased the number of repetitions until failure, increased the total weight lifted, and reduced overall body RPE (RPE-OB) in squat with a not-volume-matched design. By contrast, a lower dose of purified capsaicin (i.e. 1.2 mg in gummy format) did not reach significant differences in summed torque or fatigue index of an isokinetic knee extension exercise in a randomized, double-blinded, controlled trial [18]. Within this context, although velocity-based training has been proposed as an objective approach to

monitoring resistance training load by reflecting the state of the neuromuscular system to produce force against a load [19,20], only one study has examined the effects of capsaicin on velocity variables in resistance training [17]. Furthermore, research approaching capsaicinoids effects on velocity-derived outcomes has only been reported for upper-limb exercises but not for any lower-limb activity (e.g. squat) [5,17].

Although capsaicin TRPV1 activity may be linked to its spiciness [8,9], encapsulated capsaicin has not gotten a direct pungent taste. However, previous research has reported intestinal discomfort after high-dose (HD) oral encapsulated capsaicin supplementation (25.8 mg) [21], which suggests that capsaicin may be irritating, in spite of the vehicle of administration used. Recently, a new synthetic analog called phenylcapsaicin (PC) has emerged as an alternative to traditional oral purified capsaicin supplementation [22]. PC is a microencapsulation of 98% of PC and 1–1.5% of cellulose and lipidic excipients as primary vehicles [22]. Through hepatic glucuronidation, PC is fast presented in tissues such as the small intestine, stomach, and liver after 0.5 h of ingestion [22]. Therefore, it is supposed that microencapsulation might entail a lower pungency power, less digestive system mucosa irritation, and potentially a higher bioavailability [10,22]. Therefore, given that purified capsaicin ergogenic dose oscillates around 12 mg [6], PC might exert positive ergogenic effects with lower doses (i.e. less than 2.5 mg). This reasoning may be aligned with the current European Food Safety Authority (EFSA) PC permitted limits for dietary supplements (i.e. 2.5 mg) [22].

On the other hand, acute muscle damage, protein breakdown, and recovery variables have not been assessed in response to capsaicin supplementation yet. As intense exercise may increase metabolic, biochemical, and neuromuscular fatigue in acute and short-term ways [11,23], exploring the biochemical, perceptual, and neuromuscular effects of capsaicin on these topics may also be relevant for researchers and practitioners.

Therefore, the aim of this study was to explore the effects of low dose (LD) and HD of PC on lower-limb performance in the full squat (SQ) exercise under velocity-based control, metabolic responses to exercise, acute biochemical muscle damage, protein breakdown, RPE, and perceived recovery in resistance-trained men. These assessments were approached under a randomized, triple-blinded, placebo-controlled, crossover design. It was hypothesized that PC may exert a positive impact in a dose–response way on velocity outcomes and RPE. However, a concomitant increment of perceived fatigue, protein breakdown, and muscle damage would have appeared after PC supplementation due to the increase in physical performance. It was expected that impairments in performance as well as muscle and protein damage were higher in the HD condition.

2. Materials and methods

2.1. Participants

Twenty-five healthy men (age = 21.0 ± 2.2 years, body mass = 76.5 ± 9.5 kg, height = 176.4 ± 7.5 cm, and SQ 1-repetition maximum [1RM] normalized to body mass = 1.66 ± 0.22) enrolled voluntarily to this study. Of the total sample, two participants dropped out of the study, one due to causes not related to the study, and the other after the placebo session. All participants were resistance-trained men with at least 2 years of experience

(experience = 3.61 ± 1.43 years). Exclusion criteria comprised cardiovascular, neurological, physical, and/or metabolic disorders that may disturb primary outcomes.

One week before the beginning of the study, SQ strength and anthropometric measurements (i.e. body mass and height) were tested for all participants. Participants were asked not to consume alcohol, caffeine, or other ergogenic aids. Besides, they could not to perform intense exercise or modify their macronutrients distribution, calorie intake, and food selection 24 and 48 h before each session. The experimental protocol was explained before the informed consent, and sample collection agreement was signed prior to the first experimental session. The study protocol adhered to and respected the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of Pablo of Olavide University (Code: 0513-N-22).

2.2. Experimental design

A randomized, triple-blinded, placebo-controlled crossover trial was used to explore the effects of PC on resistance training performance, muscle damage, and metabolic responses. Participants attended the laboratory twice per week for a total study duration of 3 weeks. Each week of the study consisted of a main experimental session and a follow-up session. For each condition, six capillary blood extractions, a warm-up, an SQ testing protocol, and a 24-h recovery and muscle damage follow-up session were performed.

The order of the interventions was randomized for all participants by an external researcher in a balanced way before the beginning of the study in order to reduce training bias risk. The Research Randomizer website (www.randomizer.org) was used. All procedures were completed at the same time of the day and under stable environmental

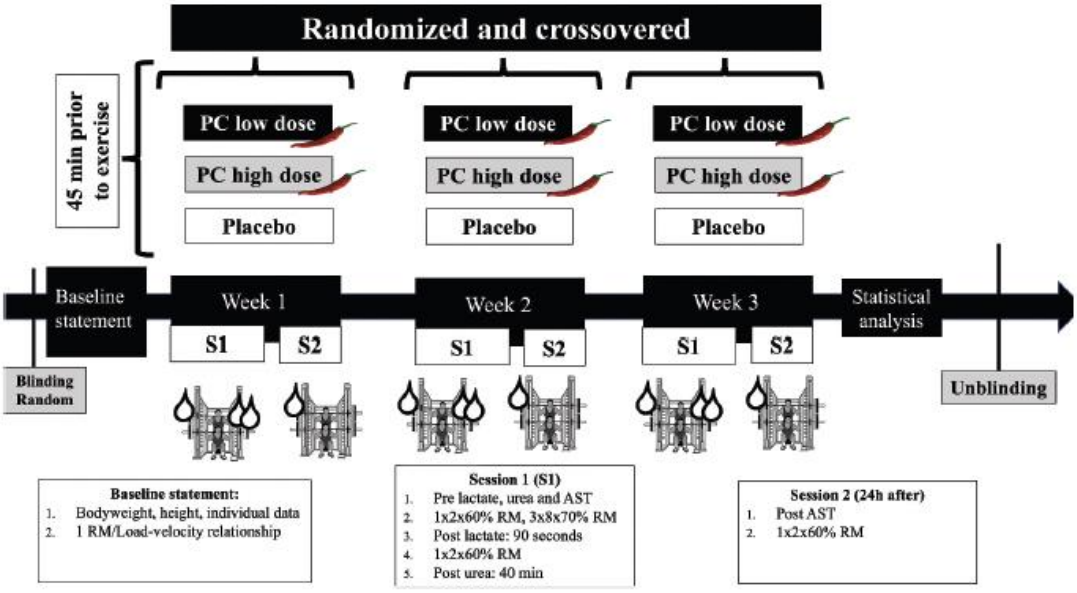

Figure 1. Overview of the experimental protocol and methodological aspects of the timeline. PC, phenylcapsaicin; S1/2, Session 1/2; 1RM, one-repetition maximum; AST aspartate aminotransferase.

conditions (22°C–24°C and 55% humidity) for each participant. The overall design of the study is depicted in Figure 1.

2.3. Supplementation procedures

To ensure blinding, supplements and placebo (Life Pro Nutrition industries, Madrid, Spain) were encapsulated and packaged with numbered labels by an independent researcher (i.e. not involved in the study). Packages and capsules were indistinguishable in appearance, smell, and taste, and their content was only revealed after an independent researcher performed the statistical analyses.

Oral ingestion of each condition was performed 45 min before the first physical testing session of each week, according to previous research [5,22]. Participants were encouraged to freely select one capsule of the assigned numbered condition under researcher supervision. Participants were only allowed to consume the selected capsule with water. Capsules contained a 0.625 mg LD of PC, a 2.5 mg HD of PC (Axivite, Malmö, Sweden), and a PLA composed of maltodextrin and excipients. According to the EFSA, both PC doses are considered in the safety range proposed by its expert panel judgment [22].

2.4. Blood testing

Blood samples were extracted and analyzed at four different times each week. Before the beginning of each first session, baseline capillary lactate, blood urea, and aspartate aminotransferase (AST) samples were collected from the index fingertip of each participant. The timeline for each biomarker was chosen due to suitability and reproducibility according to previous data [23,24]. Capillary blood extractions were conducted with a sterilized lancet after cleaning and drying the fingertip of participants before each attempt. Lactate Pro 2 LT-1730 (Arkray, Kyoto, Japan) was used for lactate measurements as it has been shown previously to be reliable throughout the physiological range of 1.0–18.00 $mmol \cdot l^{-1}$ [25]. Posttest lactate was approached 90 s after the last SQ set as a metabolic indicator of the exercise intensity [26]. Urea and AST were tested with 28.5–31.5 µl blood samples using automatic reflectance photometry (Reflotron, Roche, Boehringer Mannheim, Germany) [27,28] as whole-body protein breakdown and muscle damage measurements, respectively. For the determination of urea and AST, heparinized capillary tubes, pipettes, and the manufacturer's reagent strips were used immediately after each extraction [23]. According to previous research, posttests extractions and analyses were performed 40 min [23] after the last SQ set for urea as a purine cycle indicator [29] and before starting the 24-h follow-up session for AST [24] (Figure 1).

2.5. Resistance training protocol

2.5.1. Progressive loading test

Before the beginning of the study, each participant undertook an initial test with increasing loads for the individual determination of the 1RM and the load–velocity relationship in the SQ exercise [30]. For this purpose, a smith machine with no counterweight mechanism was used (Multipower Fitness Line, Peroga, Murcia, Spain). The mean propulsive velocity

(MPV) was directly measured for each repetition with a linear velocity transducer (T-Force System, Ergotech, Murcia, Spain) attached perpendicularly to the barbell [19].

Concerning the progressive loading test, the initial load was set at 30 kg, and it was progressively increased in 10 kg until the mean propulsive velocity (MPV) was 0.50 $m \cdot s^{-1}$. Then, the load was increased with smaller increments (2.5–5.0 kg) until the repetition could not be completed. Three repetitions were completed for light loads (≥1.00 $m \cdot s^{-1}$), two for medium loads (1.00–0.80 $m \cdot s^{-1}$), and one for the heaviest loads (≤0.80 $m \cdot s^{-1}$). Rest periods were 3 min for light and medium loads and 5 min for heavy loads. Only the best repetition (i.e. highest MPV) with each load was considered for load–velocity relationship calculation [31].

The SQ was performed with subjects starting from the upright position with the knees and hips fully extended, parallel feet and stance approximately shoulder-width apart, and the barbell resting across the back at the level of the acromion. Each subject descended in a continuous motion until the top of the thighs was below the horizontal plane (~35°–40° knee flexion), then immediately reversed motion, and raised back to the upright position. Unlike the eccentric phase that was performed at a controlled mean velocity (~0.50–0.65 $m \cdot s^{-1}$), participants were encouraged to always perform the concentric phase of the SQ at maximal intended velocity [32].

2.5.2. Warm-up

The standardized warm-up of the 2 weekly SQ sessions consisted of (I) 5 min of running at 9 $km \cdot h^{-1}$, (II) three sets of 10 repetitions of bodyweight squat, (III) three progressive countermovement jumps, and (IV) 3 sets of 2 SQ repetitions with the 40%, 50%, and 60% of 1RM resting 2 min between sets. These warm-up intensities were chosen based on previous research of the field [31] in order to progressively prepare subjects but not to fatigue them.

2.5.3. Full squat protocol

The execution technique has been described in the progressive loading test section. The SQ protocol consisted of three sets of eight repetitions with 70% 1RM with a 2-min rest period between sets. Therefore, training volume was matched for all sessions. Thus, velocity loss could be compared for the same training performed after different interventions.

According to warm-up sets velocities and the individual load–velocity relationship, 70% 1RM load was daily established for each participant. This load was chosen because this 1RM percentage may involve a submaximal non-extenuating (i.e. without reaching muscle failure) high effort for the selected repetitions [20]. Finally, as MPV and, consequently, the percentage of velocity loss (VL) are indicators of neuromuscular fatigue [20], two repetitions with 60% 1RM load were performed 3 min and 24 h after the last set of the 3 × 8 protocol. Velocity values were treated as the fastest, mean, and slowest obtained for the three sets. The percentage of mean and maximal VL was calculated in agreement with previous research [33].

2.6. Rating of perceived exertion and perceived recovery status assessment

Subjective fatigue and recovery assessment was conducted using the perceived recovery status (PRS), RPE-OB, and active muscle RPE (RPE-AM) scales. PRS, RPE-AM, and RPE-OB are subjective recovery and fatigue status pictographs where cutoff points ranged from 0 to 10. Although subjects were familiarized with both pictographs before the start of the study, both pictographs were presented during the previous session to the study start. In PRS, the perceived recovery is set between "very poorly recovered/extremely tired" (value 0) and "very well recovered/highly energetic" (value 10) [34,35]. PRS was explained again and evaluated before the start of the post-24-h follow-up session for each condition.

Fatigue was evaluated using RPE-AM and RPE-OB immediately after each 3 × 8 set [36]. RPE scales were explained after the 60% 1RM load and before the 3 × 8 protocol for each condition. The maximum perceived exertion was set on the value 10, which corresponds to reaching exhaustion (i.e. last set rating of exertion of the progressive loading test), and the basal intensity is represented with the value 0. RPE-AM was set up as the locally perceived exertion of the quadriceps and RPE-OB as the traditional general perceived exertion of the whole body [36]. Both validated scales were printed, and participants were able to visualize each one when they required them.

2.7. Sample size calculation

Sample size calculation was performed using the G* POWER software (Heinrich-Heine-Universität Düsseldorf, Germany) with an alpha of 0.05, a statistical power of 0.80, and an effect size of 0.60 based on previous resistance training performance (i.e. total mass lifted) in the previous literature [6]. Accordingly, at least 21 participants were required for this study. Plausible drop-out rate was set on 15%, and 25 subjects were recruited [37,38].

2.8. Statistical analysis

Data are presented as means and standard deviations (Mean ± SD). The normal distribution of the variables (Shapiro–Wilk test) and the homogeneity of the variances (Levene's test) were tested for each variable ($p > 0.05$). A two-way repeated measures analysis of variance (ANOVA) (condition × time) was used to explore the effect of the interventions (LD, HD, and PLA) along the time on the magnitude of each dependent biochemical and perceptual variable. Bonferroni post-hoc comparison was performed when ANOVA significance was reached. A one-way repeated measures ANOVA was used to compare velocity analyses. The Greenhouse–Geisser correction was applied when Mauchly's sphericity test was significant ($p \leq 0.05$). The Cohen's *d* effect size (ES) with 95% confidence intervals was calculated to evaluate the magnitude of the differences using the following scale: negligible (<0.20), small (0.20–0.49), moderate (0.50–0.79), and large (≥0.80) [39]. If non-parametric data were examined, Friedman and post-hoc Wilcoxon were used instead. Statistical analyses were performed using the software package SPSS (IBM SPSS version 25.0, Chicago, IL, USA). Statistical significance was set at $p \leq 0.05$.

Table 1. Metabolic response to the different conditions of phenylcapsaicin supplementation.

		Condition			ANOVA		
Variable	Time	PLA	LD	HD	Condition	Time	Condition × time
Lactate (mmol/L)	Pre	1.9 ± 0.6	1.7 ± 0.4	1.6 ± 0.4	F = 1.49	F = 269.62	F = 0.94
	Post	12.4 ± 3.5	12.7 ± 4.5	11.5 ± 2.8	$p = 0.23$	$p < 0.001$*	$p = 0.34$
Urea (mg/dl)	Pre	32.0 ± 11.6	29.8 ± 10.7	30.3 ± 10.4	F = 0.11	F = 32.44	F = 0.94
	Post	38.9 ± 17.4	40.8 ± 14.2	37.5 ± 18.2	$p = 0.89$	$p < 0.001$*	$p = 0.39$
AST (U/L)	Pre	18.6 ± 9.9	17.4 ± 11.3	16.4 ± 11.3	Pre; $p^{\#} = 0.77$		
	Post	29.6 ± 8.6	27.1 ± 14.7	22.4 ± 10.9	Post; $p^{\#} = 0.03$*		

Mean ± standard deviation. AST, Aspartate aminotransferase; PLA, Placebo; HD, High dose; LD, Low dose. Pre; measurements; Taken each week before the first session. Post; Taken 90 s after squat testing for lactate, 40 min for urea, and 24 h for AST. * Significant difference ($p \leq 0.05$). # non-parametric outcome.

3. Results

3.1. Blood testing

Two-way repeated measures ANOVAs reported significant differences of lactate and urea concentrations for time (pre-post comparisons) ($F = 269.62$, $p < 0.001$) but not for condition ($F = 1.49$, $p = 0.23$) or condition × time interaction ($F = 0.94$, $p = 0.34$) (Table 1). For AST, Friedman's test revealed no significant differences between conditions in pre-values ($p = 0.77$), showing similar baseline levels. However, significant differences between the conditions were found for AST post-values ($p = 0.03$, Table 1). Post-hoc Wilcoxon test revealed significantly higher post-levels of AST for PLA compared to HD ($p = 0.02$).

3.2. Resistance training protocol

One-way repeated measures ANOVAs of movement velocity outcomes revealed significant effects for mean velocity and maximal VL (p range ≤ 0.001 to 0.05, Table 2). Bonferroni post-hoc comparisons revealed significant differences between HD and LD for maximal VL ($p = 0.008$), and it was almost achieved between HD and PLA for the mean velocity variable ($p = 0.06$). The magnitude of the differences between the different conditions ranged from negligible to large (Table 3).

Table 2. Mechanical characteristics of the training session carried out under different conditions of phenylcapsaicin supplementation.

	Condition			
Variable	PLA	LD	HD	ANOVA
Repetitions (n)	23.8 ± 0.6	24.0 ± 0.0	24.0 ± 0.0	F =#, p=#
Fastest-V ($m \cdot s^{-1}$)	0.78 ± 0.04	0.78 ± 0.03	0.80 ± 0.06	F = 2.45; $p = 0.09$
Mean-V_t ($m \cdot s^{-1}$)	0.59 ± 0.05	0.61 ± 0.07	0.64 ± 0.07	F = 4.14, $p = 0.02$*
Slowest-V_t ($m \cdot s^{-1}$)	0.43 ± 0.10	0.45 ± 0.09	0.49 ± 0.09	F = 2.69, $p = 0.08$
MeanLoss-V (%)	31.2 ± 9.9	31.7 ± 9.3	27.7 ± 8.8	F = 2.79, $p = 0.07$
MaxLoss-V (%)	36.9 ± 10.8	38.0 ± 10.6	32.8 ± 8.3	F = 0.33, $p = 0.05$*

Mean ± standard deviation. Repetitions, Repetitions performed in the protocol; fastest-V, the highest velocity measured in the 3 sets; mean-V, mean velocity of all repetitions during the 3 sets; Slowest-V, Slowest velocity measured in the 3 sets; MeanLoss-V, mean percent loss in velocity from the fastest to the slowest repetition over the 3 sets; MaxLoss-V, maximum percent loss in velocity from the fastest to the slowest repetition over the 3 sets; PLA, Placebo; LD, Low dose; HD, High dose; * Significant difference ($p \leq 0.05$); # value not defined.

Table 3. Cohen's d effect size (ES) with 95% confidence intervals (CI) comparing mechanical outcomes between conditions.

	LD *vs.* PLA	HD *vs.* PLA	LD *vs.* HD
Fastest-V ($m \cdot s^{-1}$)	2.22 (1.76, 2.67)	0.41 (0.00, 0.82)	1.17 (0.65, 1.17)
Mean-V_t ($m \cdot s^{-1}$)	0.08 (–0.36, 0.51)	0.53 (0.08, 0.99)	–0.37 (–0.65, –0.08)
Slowest-V_t ($m \cdot s^{-1}$)	0.16 (–0.36, 0.069)	0.53 (–0.06, 1.13)	–0.37 (–0.65, –0.08)
MeanLoss-V ($m \cdot s^{-1}$)	0.09 (–0.26, 0.44)	–0.33 (–0.80, 0.14)	0.38 (0.06, 0.69)
MaxLoss-V ($m \cdot s^{-1}$)	–0.15 (–0.21, 0.52)	–0.28 (–0.73, 0.17)	0.45 (0.15, 0.75)

Mean ± standard deviation. Repetitions, Repetitions performed in the protocol; 60% fastest-V, highest velocity measured in the 60% sets; fastest-V, highest velocity measured in the 3 sets; mean-V, mean velocity of all repetitions during the 3 sets; Slowest-V, Slowest velocity measured in the 3 sets; MeanLoss-V, mean percent loss in velocity from the fastest to the slowest repetition over the 3 sets; MaxLoss-V, maximum percent loss in velocity from the fastest to the slowest repetition over the 3 sets; PLA, Placebo; LD, Low dose; HD, High dose. A positive ES indicates a higher value for HD compared to PLA, LD compared to PLA, and LD compared to HD.

3.3. Rating of perceived exertion and perceived recovery status assessments

Two-way repeated measures ANOVAs for RPE-OB reported significant differences for time ($F = 49.00$, $p < 0.001$) but not for condition ($F = 2.77$, $p = 0.07$) or condition × time interaction ($F = 1.339$, $p = 0.26$) (Figure 2A). However, Bonferroni post-hoc analyses revealed no significant differences. For RPE-AM, both condition ($F = 9.19$, $p < 0.001$) and time ($F = 36.154$, $p < 0.001$) reached significant differences but not condition × time interaction ($F = 0.553$, $p = 0.70$). Bonferroni post-hoc analyses showed significant differences for all time comparisons (p range ≤ 0.001 to 0.002) and for comparisons between PLA and HD ($p = 0.004$) and between HD and LD ($p = 0.02$) (Figure 2B). On the other hand, one-way repeated measures ANOVAs found no significant differences between conditions for PRS ($F = 0.698$, $p = 0.46$, Figure 2C).

4. Discussion

This is the first study where the impact of a capsaicinoid has been evaluated on several topics, such as muscle damage, protein breakdown, recovery, peripheral perceived exertion, and velocity-performance variables with an acute 24-h design in strength-trained men. The main finding of this study was that although HD reduced RPE-AM and enhanced mechanical performance, it also exhibited lower muscle damage in comparison to PLA and LD. The main HD effects were documented through reductions in maximal VL and eliciting a positive trend in the velocity of the slowest repetitions compared to LD and PLA. Collectively, contrary to the initial hypothesis, the ergogenic effects of PC on performance variables were only verified for HD. In addition, a significant dose–response relationship for LD and HD was not fulfilled for any of the particular outcomes. On the other hand, contrary to our initial hypothesis, HD was effective in reducing muscle damage. Therefore, the results of the present study confirm a plausible ergogenic effect of PC on strength training and performance, but an HD seems to be necessary.

Regarding metabolic blood testing, previous literature has described that the involvement of major muscle mass areas (e.g. lower-limb exercise) and the execution time are key factors in lactate response [40]. In this sense, a higher velocity loss is linearly correlated with an increase in lactate levels after SQ exercise [20]. Accordingly, resistance training stimulus applied in the present study was effective inducing a lactate response between pre- and post-exercise states. These results agree with

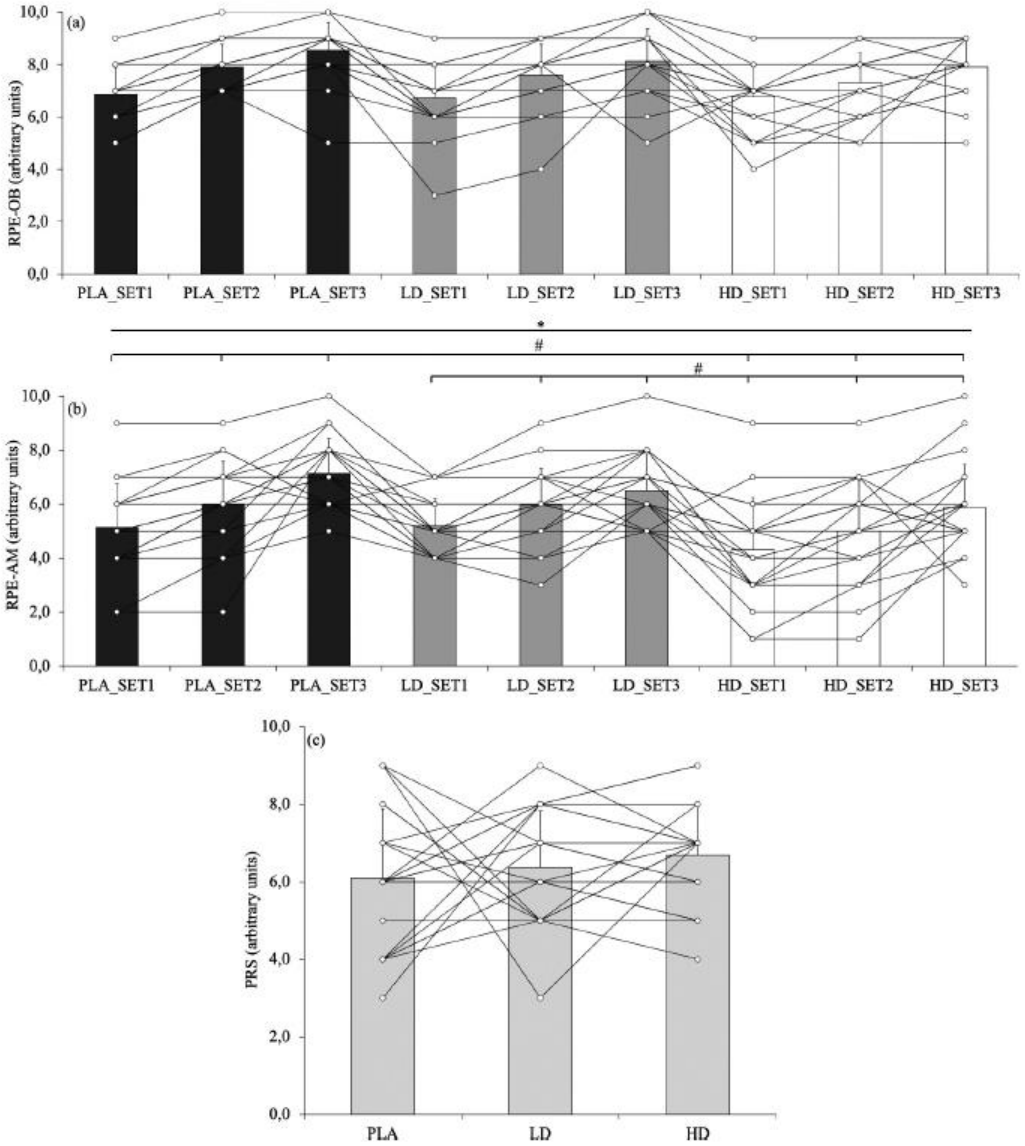

Figure 2. Individual (points) and mean (bars) values of: (a) RPE-OB, overall body rating of perceived exertion; (b) RPE-AM, active muscle rating of perceived exertion; (c) PRS, perceived recovery status for the different supplementation conditions (PLA, HD, and LD). PLA, Placebo; HD, High dose; LD, Low dose. (* $p_{ANOVA} \leq 0.05$; # $p_{Bonferroni} \leq 0.05$).

the only previous study where lactate was assessed after capsaicin supplementation [6]. Within this context, in our study, post-exercise lactate levels tended to be lower in HD conditions compared to PLA and LD. Preclinical data have shown that lactate is a potent endogenous inhibitor of TRPV1 activity [41]. For this reason, PC may have modulated sarcoplasmic calcium efflux channels lowering lactate levels [41]. This finding agrees with a previous study [6], in which although subjects significantly performed more repetitions after capsaicin ingestion compared to placebo, non-significant lower lactate levels were reported for capsaicin condition. This reduction

on lactate kinetics after oral capsaicin administration has also been reported in other high-intensity exercise modalities, such as running time trial [42].

Concerning protein breakdown, previous literature has observed an accelerated catabolic effect on purine nucleotides in skeletal muscle after strenuous exercise [29,43]. As a waste product of ammonium, urea levels are linked to exercise intensity (i.e. closeness to muscle failure), which can be assessed through velocity loss [20,29]. Previous research has fixed purine breakdown threshold on 35% of VL in the SQ exercise [20]. In the present study, all protocols induced moderate increases in urea levels following exercise. However, none of the protocols achieved this cutoff threshold. Nevertheless, although significant differences were not reported between conditions, the absolute increase tended to be lower in HD, which reported a significantly lower maximal VL. In agreement with protein breakdown, strenuous exercise also raises inflammation and oxidative stress, which can be observed in different muscle damage biomarkers [44,45]. AST is a liver and musculoskeletal enzyme which reacts 24 h after demanding exercise [24,45,46]. In this regard, HD exhibited lower levels of AST than PLA after 24 h. This finding may be linked to the lower mechanical stress suffered during the HD conditions since HD obtained higher velocities and lower VL values. This mechanical hypothesis may be more intuitive instead of a direct "antioxidant" or recovery effect of PC due to its synthetic composition [22].

Related to the SQ protocol, the present results agree with the previous literature where capsaicin enhanced performance when it was acutely ingested [5,6]. In the previous research, capsaicin (i.e. 12 mg) raised the total number of repetitions performed until failure in a 4 × 70% 1RM protocol [6]. Within this context, as MPV is a reliable predictor of the number of repetitions [47], the observed trend of higher MPV values in the last repetitions (i.e. the slowest repetitions) of HD might have implied an increase in the total repetitions until exhaustion if the volume had not been matched. Furthermore, as previous research elicited further benefits of capsaicin in the last set of higher-volume training protocols, plausible greater effects would have been reported by increasing the total number of sets [3,6]. Regarding the potential ergogenic mechanisms underlying capsaicin effects on SQ performance, according to previous research, they may be linked to an increase of calcium released by the sarcoplasmic reticulum, higher acetylcholine levels, and its analgesic effect [5,48]. As afferents III and IV fibers seem to contribute to the development of central fatigue at spinal and supraspinal levels of the central nervous system [13,49], this "desensitizer" agent may provide a higher tolerance to firing rate reductions during strenuous exercise. Thus, as muscle force is critically affected by the motor unit activity [50], and MPV is a mechanical manifestation of muscle force [20], the analgesic effect of PC may have played a retardant effect on neuromuscular fatigue improving MPV, especially at the last repetitions. Consequently, the ergogenic effect of PC on mechanical outcomes might be explained by a plausible reduction in neural fatigue [5,51].

Concerning perceived effort variables, previous research has reported RPE-OB reductions after 12 mg of acute capsaicin supplementation [3,6]. Although a positive trend was observed, our results did not support these RPE-OB reductions when PC, in HD or LD, was ingested in comparison to PLA. In this sense, most of the previous research protocols were performed until muscle failure where the plausible analgesic effect of capsaicinoids may be higher [6,48]. On the other hand, in the present study, local quadriceps RPE-AM was highly affected by HD in

comparison to LD and PLA. Considering that the RPE can be modulated by changes in the neuronal circuitry of the brain [52,53] and afferent feedback from III and IV afferents may raise exercise-related discomfort [11] and RPE [16], this effect of PC on RPE-AM could be mediated by TRPV1 interaction. In addition, since HD but not LD produced significant effects on RPE-AM, peripheral analgesic effects of PC [54] during exercise could be dose-dependent. This conjectu[re] agrees with the only previous study where a LD of a regularly bioavailable capsaicin supplement was consumed before a resistance training protocol [18]. In this study, fatigue index and isometric knee torque were not affected after 1.2 mg of capsaicin [18]. On the other hand, although PC in HD exerted a positive effect on muscle damage and RPE-AM, PC effects did not produce any improvements in PRS. Hence, the possible analgesic effect of PC may only appear acutely. Intriguingly, as previous research [5] hypothesized that capsaicin may increase injury risk due to its analgesic effect, in this study, only two participants dropped out, one for personal reason and the other during the placebo session.

Collectively, our findings suggest that a 2.5 mg dose of PC provides a plausible ergogenic effect on strength performance, muscle damage, and peripheral perceived exertion in comparison to PLA and a lower dose of 0.625 mg. This novel information is valuable because never before a capsaicinoid has been evaluated concerning muscle damage, protein breakdown, and peripheral fatigue. Finally, this study presents important strengths such as a triple-blinded, placebo-controlled crossover design, trained participant enrollment, and the use of velocity measures as performance indicators. However, some limitations should be addressed. First, electromyographical assessment may have completed the internal reliability of the peripheral effects of PC. Another possible limitation is that the study was only carried out in male athletes, for this reason, these results may not be extrapolated to other populations such as female athletes or untrained subjects. Furthermore, these results should be cautiously interpreted for other exercises and tasks not performed in a laboratory environment. Finally, as in this study only three sets of SQ in a single training session were performed, further research may evaluate the effects of phenylcapsaicin during longer workouts and chronic trials to verify whether or not these benefits are replicated in these conditions.

5. Conclusions

The results of the present study suggest that an HD (2.5 mg) of PC supplementation ingested 45 min before exercise may increase SQ performance and reduce muscle damage, as well as peripheral quadriceps perceived exertion in strength-trained subjects in comparison to an LD (0.625 mg) and PLA. Therefore, the ergogenic effect of PC may appear after a "dose" threshold is reached.

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Data availability statement

Datasets used in this study are available from the corresponding author under reasonable request.

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Authors´ contributions

The study and methodology were conceived using JCC, PJM, FPB, and CAF. PCD, JSV, IAI, CCC, PJM were involved in data collection and data treatment, writing was conducted by CAF and PJM, all authors reviewed critically the manuscript under JCC and FPB supervision.

References

1. MaughanRJ, BurkeLM, DvorakJ, et al. IOC consensus statement: dietary supplements and the high-performance athlete. Int J Sport Nutr Exerc Metab. 2018;28(2):22.
2. Cano-GonzálezH, Jiménez-MartínezP, Baz-ValleE, et al. Nutritional and supplementation strategies of Spanish natural elite bodybuilders in pre-contest. Gazzetta Medica Italiana. 2022;181.
3. de FreitasMC, CholewaJM, PanissaVLG, et al. Acute capsaicin supplementation improved resistance exercise performance performed after a high-intensity intermittent running in resistance-trained men. J Strength Cond Res [Internet]. 2019 Sep 16;36(1):130–134. cited2021Publish Ahead of Print. Available from. DOI:10.1519/JSC.0000000000003431
4. WalterAA, HerdaTJ, RyanED, et al. Acute effects of a thermogenic nutritional supplement on cycling time to exhaustion and muscular strength in college-aged men. J Int Soc Sports Nutr. 2009;6(1):15. DOI:10.1186/1550-2783-6-15
5. Jiménez-MartínezP, Ramírez-CampilloR, FlandezJ, et al. Effects of oral capsaicinoids and capsinoids supplementation on resistance and high intensity interval training: a systematic

review of randomized controlled trials. J HumSportexer. 2022;18(2). DOI:10.14198/jhse.2023.182.09
6. de FreitasMC, CholewaJM, FreireRV, et al. Acute capsaicin supplementation improves resistance training performance in trained men. J Strength Cond Conditioning Res. 2018;32(8):2227–2232. DOI:10.1519/JSC.0000000000002109
7. LangY, KisakaH, SugiyamaR, et al. Functional loss of pAMT results in biosynthesis of capsinoids, capsaicinoid analogs, in *capsicum annuum* cv. CH-19 sweet. Plant J. 2009;59(6):953–961. DOI:10.1111/j.1365-313X.2009.03921.x
8. SharmaSK, VijAS, SharmaM. Mechanisms and clinical uses of capsaicin. Eur J Pharmacol. 2013;720:55–62.
9. BasithS, CuiM, HongS, et al. Harnessing the therapeutic potential of capsaicin and its analogues in pain and other diseases. Molecules. 2016;21(8):966.
10. FramrozeZ. Placebo controlled study to investigate the relationship between lowering serum zonulin levels and improved body weight composition using a daily oral dose of phenylcapsaicin. Int J Clin Ski. 2022;16(7):251.
11. TaylorJL, AmannM, DuchateauJ, et al. Neural contributions to muscle fatigue: from the brain to the muscle and back again. Med Sci Sports Exerc. 2016;48(11):2294–2306.
12. de Mourae E Silva Viton E L, Cholewa Jason M, Francois Billaut, Ralf, Jäger, Marcelo C de, Freitas, Fabio S, Lira, Fabrício E, Rossi. Capsaicinoid and capsinoids as an ergogenic aid: a systematic review and the potential mechanisms involved. Int J Sports Physiol Perform. 2021;16:464–473.
13. CollinsBW, PearceyGEP, BuckleNCM, et al. Neuromuscular fatigue during repeated sprint exercise: underlying physiology and methodological considerations. Appl Physiol Nutr Metab. 2018;43(11):1166–1175.
14. Alix-FagesC, Del VecchioA, Baz-ValleE, et al. The role of the neural stimulus in regulating skeletal muscle hypertrophy. 2022. [[cited 2022 Mar 17]; Available from]. Internet Eur J Appl Physiol. 122(5):1111–1128. 10.1007/s00421-022-04906-6.
15. Alix-FagesC, GrgicJ, Jiménez-MartínezP, et al. Effects of mental fatigue on strength endurance: a systematic review and meta-analysis. Motor Control. 2022;27(2):442–461.
16. AmannM, BlainGM, ProctorLT, et al. Group III and IV muscle afferents contribute to ventilatory and cardiovascular response to rhythmic exercise in humans. J Appl Physiol. 2010;109(4):966–976.
17. da SilvaBVC, MotaGR, MarocoloM, et al. Acute supplementation with capsaicin enhances upper-limb performance in male jiu-jitsu athletes. Sports. 2022;10(8):120.
18. CrossBL, ParkerD, LanganSP, et al. Effect of a commercially available low-dose capsaicin supplement on knee extensor contractile function. Int J Exer Sci 2020;7: 312.
19. González-BadilloJJ, Sánchez-MedinaL. Movement velocity as a measure of loading intensity in resistance training. Int J Sports Med. 2010;31(05):347–352.
20. Sánchez-MedinaL, González-BadilloJJ. Velocity loss as an indicator of neuromuscular fatigue during resistance training. Med Sci Sports Exerc. 2011;43(9):1725–1734.
21. OpheimMN, RankinJW. Effect of capsaicin supplementation on repeated sprinting performance. J Strength Cond Conditioning Res. 2012;26(2):319–326.
22. EFSA Panel on Nutrition, Novel Foods and Food Allergens (EFSA NDA Panel), TurckD, CastenmillerJ, Safety of phenylcapsaicin as a novel food pursuant to regulation (EU) 2015/2283.EFSA J.InternetAvailable from2019:[cited 2022 Sep 14]17.10.2903/j.efsa.2019.57186.
23. IzquierdoM, Lez-IzalMG, Navarro-Amezquetal, et al. Effects of strength training on muscle fatigue mapping from surface EMG and blood metabolites. Appl Sci. 2010;43(2):303–311. DOI:10.1249/MSS.0b013e3181edfa96
24. PalS, ChakiB, ChattopadhyayS, et al. High-intensity exercise induced oxidative stress and skeletal muscle damage in postpubertal boys and girls: a comparative study. J Strength Cond Conditioning Res. 2018;32(4):1045–1052.
25. PyneDB, BostonT, MartinDT, et al. Evaluation of the lactate pro blood lactate analyser. Eur J Appl Physiol. 2000;82(1–2):112–116.

26. Mora-CustodioR, Rodríguez-RoselID, Yáñez-GarcíaJM, et al. Effect of different inter-repetition rest intervals across four load intensities on velocity loss and blood lactate concentration during full squat exercise. J Sports Sci. 2018;36(24):10.
27. CattozzoG, FranziniC, HubbuchA, et al. Evaluation of determination of uric acid in serum and whole blood with the reflotron. Clin Chem. 1988;34(2):414–416.
28. PearsonJR, DusenburyLJ, Bakes-MartinR, et al. Evaluation of a simple method for measuring blood cholesterol levels using non-laboratory observers. Am J Med. 1988;85(3):369–374.
29. WarburtonDER, WelshRC, HaykowskyMJ. Biochemical changes as a result of prolonged strenuous exercise. Br J Sports Med. 2002;36(4):301–303.
30. González-BadilloJJ, Yañez-GarcíaJM, Mora-CustodioR, et al. Velocity loss as a variable for monitoring resistance exercise. Int J Sports Med. 2017;38(03):217–225.
31. Piqueras-SanchizF, Cornejo-DazaPJ, Sánchez-ValdepeñasJ, et al. Acute Mechanical, Neuromuscular, and Metabolic Responses to Different Set Configurations in Resistance Training. J Strength Cond Conditioning Res. 2021;36(11):2983–2991. Internet. [cited 2022 Sep 18];Publish Ahead of Print. Available from. https://journals.lww.com/10.1519/JSC.0000000000004068
32. Pareja-BlancoF, AlcazarJ, Sánchez-ValdepeñasJ, et al. Velocity loss as a critical variable determining the adaptations to strength training. Med Sci Sports Exerc. 2020;52(8):1752–1762. DOI:10.1249/MSS.0000000000002295
33. González-BadilloJ, Rodríguez-RoselID, Sánchez-MedinaL, et al. Short-term recovery following resistance exercise leading or not to failure. Int J Sports Med. 2015;37(04):295–304. DOI:10.1055/s-0035-1564254
34. LaurentCM, GreenJM, BishopPA, et al. A practical approach to monitoring recovery: development of a perceived recovery status scale. Journal of Strength and Conditioning Research. 2010;25(3):620–628.
35. ColadoJC, PedrosaFM, JuesasA, et al. Concurrent validation of the OMNI-Resistance exercise scale of perceived exertion with elastic bands in the elderly. Exp Gerontol. 2018;103:11–16.
36. SwankA, RobertsonRJ. Effect of induced alkalosis on perception of exertion during intermittent exercise. J Appl Physiol. 1989;67(5):6.
37. de MortonNA. The PEDro scale is a valid measure of the methodological quality of clinical trials: a demographic study. Aust J Physiother. 2009;55(2):129–133.
38. Sánchez-MorenoM, Cornejo-DazaPJ, González-BadilloJJ, et al. Effects of velocity loss during body mass prone-grip pull-up training on strength and endurance performance. J Strength Cond Conditioning Res. 2020;34(4):911–917.
39. CohenJ. Statistical power analysis for the behavioral sciences. 2nd ed. Hillsdale, N.J: Lawrence Erlbaum Associates; 1988.
40. WirtzN, WahlP, KleinöderH, et al. Lactate kinetics during multiple set resistance exercise. Journal of sports science & medicine. 2014;13(1):73–77.
41. de la RocheJ, WaltherI, LeonowW, et al. Lactate is a potent inhibitor of the capsaicin receptor TRPV1. Sci Rep. 2016;6(1):36740. DOI:10.1038/srep36740
42. de FreitasMC, CholewaJM, GobboLA, et al. Acute capsaicin supplementation improves 1,500-m running time-trial performance and rate of perceived exertion in physically active adults. J Strength Cond Conditioning Res. 2018;32(2):572–577.
43. SuttonJR, ToewsCJ, WardGR, et al. Purine metabolism during strenuous muscular exercise in man. Metabolism. 1980;29(3):254–260.
44. BairdMF, GrahamSM, BakerJS, et al. Creatine-kinase- and exercise-related muscle damage implications for muscle performance and recovery. J Nutr Metab. 2012;2012:1–13.
45. BessaAL, OliveiraVN, AgostiniGG, et al. Exercise intensity and recovery biomarkers of injury. Inflamm Oxidative Stress. 2016;30(2):311–319. DOI:10.1519/JSC.0b013e31828f1ee9
46. Villavicencio KimJ, WuGY. Body building and aminotransferase elevations: a review. J Clin Transl Hepatol. 2020;8(2):161–167.
47. Morán-NavarroR, Martínez-CavaA, Sánchez-MedinaL, et al. Movement velocity as a measure of level of effort during resistance exercise. J Strength Cond Conditioning Res. 2019;33(6):1496–1504.

48. de MouraE, SilvaVEL, CholewaJM, et al. Chronic capsiate supplementation increases fat-free mass and upper body strength but not the inflammatory response to resistance exercise in young untrained men: a randomized, placebo-controlled and double-blind study. J Int Soc Sports Nutr. 2021;18(1):50. DOI:10.1186/s12970-021-00446-0
49. KaufmanMP, IwamotoGA, LonghurstJC, et al. Effects of capsaicin and bradykinin on afferent fibers with ending in skeletal muscle. Circ Res. 1982;50(1):133–139.
50. Del VecchioA, CasoloA, NegroF, et al. The increase in muscle force after 4 weeks of strength training is mediated by adaptations in motor unit recruitment and rate coding. J Physiol. 2019;597(7):1873–1887. DOI:10.1113/JP277250
51. HeckmanCJ, EnokaRM.Physiology of the motor neuron and the motor unit.Handb Clin Neurophysiol.InternetAvailable fromElsevier.2004:cited 2021 Nov 17119–147https://linkinghub.elsevier.com/retrieve/pii/S1567423104040067 .
52. Alix-FagesC, García-RamosA, Calderón-NadalG, et al. Anodal transcranial direct current stimulation enhances strength training volume but not the force–velocity profile. Eur J Appl Physiol. 2020;120(8):1881–1891. DOI:10.1007/s00421-020-04417-2
53. OkanoAH, FontesEB, MontenegroRA, et al. Brain stimulation modulates the autonomic nervous system, rating of perceived exertion and performance during maximal exercise. Br J Sports Med. 2015;49(18):1213–1218. DOI:10.1136/bjsports-2012-091658
54. AroraV, CampbellJN, ChungM-K. Fight fire with fire: neurobiology of capsaicin-induced analgesia for chronic pain. Pharmacol Ther. 2021;220:107743.

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Effects of phenylcapsaicin on aerobic capacity and physiological parameters in active young males: a randomized, triple-blinded, placebo-controlled, crossover trial

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Objective: Phenylcapsaicin (PC) is a new capsaicin analog which has exhibited a higher bioavailability. This sudy assessed the effects of a low dose (LD) of 0.625 mg and a high dose (HD) of 2.5 mg of PC on aerobic capacity, substrate oxidation, energy metabolism and exercise physiological variables in young males.

Materials and methods: Seventeen active males (age = 24.7 ± 6.0 years) enrolled to this randomized, triple-blinded, placebo-controlled, crossover trial. Participants attended the laboratory on 4 sessions separated by 72–96 h. A submaximal exercise test [to determine maximal fat oxidation (MFO) and the intensity at MFO (FATmax)] followed by a maximal incremental test (to determine $VO2_{max}$) were performed in a preliminary session. The subsequent sessions only differed in the supplement ingested [LD, HD or placebo (PLA)] and consisted of a steady-state test (60 min at FATmax) followed by a maximal incremental test. Energy metabolism, substrate oxidation, heart rate, general (gRPE) and quadriceps (RPEquad) rate of perceived exertion, skin temperature and thermal perception were tested.

Results: Clavicle thermal perception was lower in HD compared to PLA and LD (p = 0.04) across time. HD reduced maximum heart rate in comparison to PLA and LD (p = 0.03). LD reported higher general RPE (RPEg) values during the steady-state test compared to PLA and HD across time (p = 0.02). HD and LD elicited higher peak of fat oxidation during the steady-state test compared with PLA (p =

0.05). Intra-test analyses revealed significant differences for fat oxidation (FATox) in favor of HD and LD compared to PLA (p = 0.002 and 0.002, respectively), and for carbohydrate oxidation (CHOox) (p = 0.05) and respiratory exchange ratio (RER) (p = 0.03) for PLA. In the incremental test, only general RPE at 60% of the maximal intensity (W) differed favoring HD ($p \leq 0.05$).

Conclusion: Therefore, PC may contribute to increase aerobic capacity through the improvement of fat oxidation, maximum heart rate and perceptual responses during exercise.

KEYWORDS

substrate oxidation, ergogenic aid, phenylcapsaicin, exercise metabolism, exercise capacity

1 Introduction

Capsaicinoids are a group of substances found in spicy fruits such as chili peppers (González-Cano et al., 2022; Jiménez-Martínez et al., 2022). Among the variety of substances included in this category, capsaicin has emerged as the most used ergogenic compound (de Moura e Silva et al., 2021). Capsaicin vanillyl moiety is responsible for its pungent properties, which are associated with its ergogenic effects (Jiménez-Martínez et al., 2022). However, although capsaicin spiciness has been proposed as an athletic enhancer mechanism, new non-pungent analogs have emerged as a plausible alternative (Luo et al., 2011). Capsinoids are a group of capsaicin non-pungent analogs (e.g., capsiate) found in sweet peppers (Lang et al., 2009). Although capsaicinoids and capsinoids have a similar chemical structure, these substances present differences in their functional properties and their metabolism. Accordingly, capsinoids are rapidly metabolized and conjugated after their ingestion (EFSA, 2012), resulting in non-detectable circulating levels in the bloodstream (EFSA, 2012). By contrast, after their ingestion, different capsaicin formulations have a fast effect on different tissues, such as the small intestine, liver, and stomach (Turck et al., 2019). Recent advances in this field have discovered a new capsaicin analog called phenylcapsaicin (PC), which has shown a higher bioavailability (Turck et al., 2019) in comparison to purified capsaicin. PC formulations are composed of 98% PC and 1.0%–1.5% of cellulose and lipidic excipients as vehicles. As a consequence, PC might reduce the ergogenic dose of capsaicin (Turck et al., 2019) and consequently the spicy perception and gastrointestinal discomfort after its ingestion.

The ergogenic effects of capsaicinoids and capsinoids are mediated by their interaction with the transient receptor vanilloid 1 (TRPV1) (Jiménez-Martínez et al., 2022). TRPV1 receptors are found in afferent III and IV nerve fibers, which are key for the development of central fatigue during exercise (Collins et al., 2018) as the increased firing in these afferents could inhibit motor neurons firing directly and through the presynaptic inhibition of Ia afferents reducing or affecting supraspinal levels to reduce the descending drive to the motor units (Taylor et al., 2016; Alix-Fages et al., 2022a). Besides, ingesting these substances enhance muscle contraction as a consequence of the improvement in motoneurons recruitment, calcium release, and perceived analgesia (de Moura e Silva et al., 2021). Additionally, other potential ergogenic mechanisms related to TRPV1 stimulation concern metabolic effects such as an increase in fatty acid oxidation (more FFAs available for beta-oxidation), an increase in glycogen sparing, and a positive effect in the acetylcholine turnover (de Moura e Silva et al., 2021). However, current human evidence regarding the positive metabolic impact of these substances is scarce, albeit the plausible mechanisms aforementioned are well documented in preclinical models (Basith et al., 2016; Jiménez-Martínez et al., 2022).

In the present study, only active males were recruited as previous research has exhibited that the effects of capsaicin on substrate oxidation are unaffected by the sex of the subjects (Lejeune et al., 2003). To date, only two studies have reported the metabolic effects of a capsaicinoid on substrate oxidation (Lim et al., 1997; Rossi et al., 2022). In the first study, participants exhibited equal RER values after 12 mg of capsiate supplementation during exercise performed at 70% of the maximal aerobic speed (Rossi et al., 2022). In the second study, a meal with 10 g of hot peppers 2.5 h prior to 1 h of aerobic exercise at 60% $VO2_{peak}$ showed an increase in RER compared to placebo (Lim et al., 1997). An important limitation in current research is that human substrate oxidation is not usually reported in previous studies (Santos et al., 2022). For instance, 10 mg/kg of capsiate supplementation 60 min prior to aerobic exercise decreased RER in mice males (Santos et al., 2022). On the other hand, 12 mg of dihydrocapsiate supplementation has not exhibited significant differences in fat oxidation (FATox), non-esterified fatty acids, energy expenditure (EE), and skin temperature in sedentary overweight men during aerobic exercise (Osuna-Prieto et al., 2022). Furthermore, most current studies do not standardize maximal fat oxidation (MFO) measurement in the pre-test, which may bias the metabolic endpoints measured (Santos et al., 2022). Concerning the metabolic effects of these substances on high-intensity training (i.e., resistance training and high-intensity interval training), 12 mg of capsaicin supplementation has exhibited lower lactate levels compared to placebo after four sets until failure in the squat exercise (de Freitas et al., 2018a). This reduction in lactate values has also been reported in other high-intensity tasks, such as repeated sprints after 12 mg of capsaicin supplementation (de Freitas et al., 2018b). Additionally, previous research has documented a negligible effect of capsaicin supplementation on heart rate (Giuriato et al., 2022). Overall, evidence regarding the metabolic impact of capsaicinoids and capsinoids during exercise is scarce and inconclusive. Furthermore, the effects of PC on physiological exercise variables and sport performance have not been tested yet. For this reason, if new capsaicin formulations, which are supposed to be more bioavailable (e.g., PC), produce an

TABLE 1 Characteristics of the study participants (n = 17).

	Mean ± standard deviation
Age (years)	24.7 ± 6.0
Height (cm)	175.9 ± 7.6
Body mass (kg)	77.2 ± 11.7
Muscle mass (%)	41.2 ± 6.2
Fat mass (%)	6.9 ± 5.3
FATmax (W)	68.8 ± 37.7
MFO (g/min)	0.27 ± 0.09

FATmax, intensity linked to maximal fat oxidation; MFO, maximal fat oxidation.

ergogenic effect on substrate oxidation, energy metabolism and aerobic capacity and if a dose threshold exists have to be investigated. Note that PC intake should reduce spicy perception and gastrointestinal discomfort, as the ergogenic dose might be lower compared to capsaicin (Turck et al., 2019).

Therefore, this study aimed to assess the effects of a low dose (LD) of 0.625 mg and a high dose (HD) of 2.5 mg of PC on aerobic capacity, energy metabolism, substrate oxidation and other physiological variables such as circulating lactate levels, energy expenditure, body temperature response, ratings of perceived exertion (RPE) and perceived temperature compared to placebo in active males. We hypothesized that PC supplementation will increase FATox, skin body temperature, mechanical performance and energy expenditure while will decrease RPE in a dose-dependent manner.

2 Materials and methods

2.1 Participants

Sample size calculation was performed using the G* POWER software (Heinrich-Heine-Universität Düsseldorf, Germany) with an alpha of 0.05, an effect size of 0.4 and a statistical power of 0.80. Based on previous studies (Josse et al., 2010; Osuna-Prieto et al., 2022), 12 participants were required to establish statistical differences between conditions. To ensure the detection of differences 17 physically active males were enrolled in the study (Table 1). Participants enrolled in the study through a poster that was shared on social media. None of the participants reported any physical limitation or health condition that could compromise cycling performance. Participants were instructed not to perform any intense physical exercise during the 2 days preceding each visit to the laboratory and from consuming stimulant beverages or any dietary supplement within 24 h preceding each testing session. Before being included in the study, all potential participants were comprehensively informed about the study purpose, procedures and the benefits, risks, and discomforts that might result from participation. Each participant provided informed consent and was free to withdraw from the study at any time. The study protocol adhered to the tenets of the last revised Declaration of Helsinki and was approved by the Institutional Review Board (blinded for peer review).

2.2 Experimental design

A randomized, triple-blinded, placebo-controlled crossover trial was used to analyze the effects of PC on energy expenditure and substrate oxidation, skin body temperature, heart rate and perceptual responses to submaximal steady-state and maximal effort cycling tests (Figure 1). Participants attended the laboratory four times, separated by 72–96 h to ensure a complete recovery from central and peripheral fatigue (Carroll et al., 2017; Alix-fages et al., 2023). Before the preliminary session, participants anthropometrical [(i.e., height, body mass, % muscle, % body fat (Tanita BC 418 segmental, Tokyo, Japan; Seca 202 Stadiometer, Seca Ltd., Hamburg, Germany)] and sociodemographic characteristics were obtained (see Table 1). Then, two tests, a submaximal incremental exercise test followed by a maximal effort incremental test, were conducted in the preliminary session. The submaximal exercise test was used to determine the MFO and the cycling power values (W) at MFO (FATmax intensity). The maximal effort test assessed maximal oxygen consumption ($VO2_{max}$) and the maximal cycling power achieved during the test. The three experimental sessions were identical, only differing in the supplement (PLA, LD of PC, and HD of PC), which was only administered 45 min before the first cycling task. In each experimental session, participants performed a steady-state test (60 min at FATmax) followed by a maximal incremental effort test (25 W increments every min until volitional exhaustion). Each participant was constantly tested at the same time of the day and under similar environmental conditions (22ºC–24°C and 55% humidity).

2.3 Supplementation procedures

Capsules were ingested with water. The contents of the capsules were as follows: a low dose (LD) of 0.625 mg of PC, a high dose (HD) of 2.5 mg HD of PC (Axivite, Malmö, Sweden), and a PLA composed of maltodextrin and excipients. According to EFSA, both PC doses are considered in the safety range proposed by its expert panel judgment (Turck et al., 2019). Supplements and placebo were encapsulated and packaged with alphanumeric labels to ensure blinding. Accordingly, an independent technician (i.e., not involved in the study) prepared the capsules in the original producer's industry (Life Pro Nutrition industries, Madrid, Spain). Participants selected one capsule of the daily randomized assigned condition under the supervision of at least one researcher. To reduce possible bias, an independent researcher assigned participants to each condition with the Research Randomizer online software (www.randomizer.org). Packages and capsules were indistinguishable in appearance, smell and taste and their content was only revealed after an independent researcher performed the statistical analyses to prevent evaluators' bias. Adverse effects were not reported for any of the conditions.

2.4 Dietary intake standardization

To ensure intra and inter-individual reliability in metabolic variables (e.g., FATox), dietary intake was standardized at least

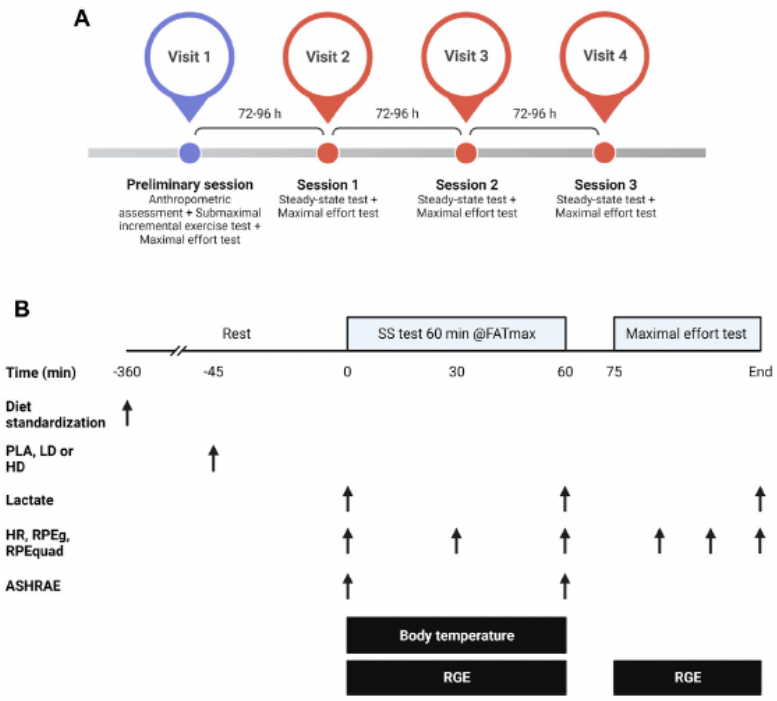

FIGURE 1
General overview of the study **(A)** and design of the experimental sessions **(B)** aiming to analyze the effects of two doses of phenylcapsaicin on metabolic, thermal, perceptual, and mechanical responses to steady-state and maximal cycling tests. HD, high dose; LD, low dose; PLA, placebo; HR, heart rate; RGE, respiratory gas exchange; RPEg, general ratings of perceived exertion; RPEquad, ratings of perceived exertion of quadriceps; SS, steady-state. Created with BioRender.com.

6 h previous to exercise testing (Amaro-Gahete et al., 2018; Rothschild et al., 2022). For this purpose, participants performed each test with at least 6 h of fasting prior to the start of each session. The last meal before the fasting period was standardized with 45 g of maltodextrin powder and 30 g of protein powder for all participants (Life Pro Nutrition industries, Madrid, Spain).

2.5 Exercise procedures

The three tests were performed employing an electronically braked cycle ergometer (Excalibur Sport; Lode, Groningen, Netherlands). Respiratory gas exchange was monitored during all tests with a gas analyzer (Ergocard CPX, Medisoft, Belgium). The testing procedures and variables collected in each test are described below:

2.5.1 Submaximal incremental exercise test

This test was used to determine MFO and FATmax in the preliminary session. The exercise protocol started with a 3 min stage at 25 W, and the intensity was increased in steps of 25 W every 3 min until the respiratory exchange ratio (RER) was ≥1 for at least 30 s (Sanchez-Delgado et al., 2018). During the submaximal exercise test, VO2 and carbon dioxide production (VCO2) data were averaged over the last 60 s of each 3 min stage. Then, FATox was calculated from the aforementioned values. FATox values (g/min) from the different stages of the submaximal exercise test were plotted against the relative exercise intensity (W). Third-degree polynomial regression was built to determine the absolute MFO (g/min) (Midgley et al., 2007; Sanchez-Delgado et al., 2018). EE was estimated with Weir's abbreviated equations (Weir, 1949) and FATox and CHOox with Frayn's stoichiometric equations assuming a negligible urinary nitrogen excretion (Frayn, 2016; Osuna-Prieto et al., 2022). Subsequently, RER was calculated from the CHOox and FATox obtained data. This procedure was further used for the next sessions. The equations used are presented hereunder:

$$EE\,(kcal/min) = (1.106 * VCO_2) + (3.941 * VO_2)$$
$$RER = (VCO_2/VO_2)$$
$$CHOox\,(g/min) = (4.55 * VCO_2) - (3.21 * VO_2)$$
$$FATox\,(g/min) = (1.67 * VCO_2) - (1.67 * VO_2)$$

2.5.2 Maximal effort test

This test was performed in the preliminary and the three experimental sessions, 15 min after the first test of each session. The exercise protocol started with a 1 min stage at 25 W, and the

intensity was increased in steps of 25 W every min until i) volitional exhaustion was reached, or ii) participants had to stop because of peripheral fatigue. The dependent variables considered in this test were: i) final stage completed in the protocol, ii) $VO2_{max}$, iii) circulating lactate concentration recorded 90 s after test cessation with a portable lactate analyzer (Lactate PRO2, Arkray, Kyoto, Japan) (Crotty et al., 2021), iv) general (RPEg) and quadriceps (RPEquad) ratings of perceived exertion (RPE 0-10) and heart rate (Polar RS800, Polar Electro Inc., Woodbury, NY, United States) (Hernando et al., 2018) at an intensity representing the 30%, 60%, and 90% of the maximal power attained at the final stage completed in the preliminary session. Lactate blood was extracted with a sterilized lancet after cleaning and drying the fingertip of participants before each attempt. VO2 was monitored using a galvanic fuel cell, and VCO2 was evaluated using a non-dispersive infrared sensor. The gas analyzer was calibrated following standard gas concentrations as suggested by the manufacturer.

2.5.3 Steady-state test

Participants cycled for 60 min at the intensity of FATmax determined in the preliminary session. Circulating lactate, RPEg, RPEquad, and heart rate were recorded pre-exercise, in the middle of the test (30 min), and at the end (60 min). The skin body temperature was also recorded throughout the test with a set of 8 DS-1922 L iButtonTM wireless thermometers (Thermochron, Dallas, TX, United States) (van Marken Lichtenbelt et al., 2006; Smith et al., 2010) attached to the participant's skin in different places: i) forehead, ii) right scapula, iii) left chest, iv) right% deltoid), v) left elbow, vi) left hand, vii) right thigh and viii) right gastrocnemius. Data were processed as mean blocks of 5 min. Consequently, a total of 12 temperature stages were recorded and averaged for the final analysis.

The equation used to assess the effects of PC and PLA on body temperature is described hereunder:

Overall mean skin temperature = (Forehead*0.07)
+ (Right Scapula*0.175)
+ (Left Chest*0.175)
+ (Right Deltoid*0.07)
+ (Left Elbow*0.07)
+ (Left Hand*0.05)
+ (Right Thigh*0.19)
+ (Right Gastrocnemius*0.2)

Martinez-Tellez et al. (2017) the mean and maximum heart rates recorded throughout the test were also compared between the experimental conditions. The substrate oxidation (fat and carbohydrates), energy expenditure, and RER during the 60 min steady-state test were also estimated and compared between the conditions. FATox, CHOox and EE were also expressed as the area under the curve (AUC) using the trapezoidal rule. For metabolic variables, the highest value achieved during the test (i.e., peak) and the individual analysis of each stage (i.e., intra-test analysis) were performed. Finally, the American Society of Heating, Refrigerating, and Air Conditioning Engineers (ASHRAE) scale was used to record thermal perception before and after the test. The scale was recorded for the following body areas: i) clavicle, ii) abdominal, iii) arms, iv) hands, v) legs, vi) feet, and vii) overall body. Each item is scored with the following values: cold (−3), cool (−2), slightly cool (−1), neutral (0), slightly warm (1), warm (2), to hot (3).

2.6 Statistical analysis

Data are presented as means and standard deviations (Mean ± SD). The normal distribution of all the variables presented was tested with the Shapiro-Wilk test, and the homogeneity of the variances with the Levene's test ($p > 0.05$). A two-way repeated measures analysis of variance (ANOVA) (condition × time) was used to analyze the effect of the supplementation (LD, HD, PLA) across the time on each dependent metabolic, performance, and perceptual variable. F value was retrieved from each ANOVA calculation. A Bonferroni *post hoc* comparison was performed when ANOVA significance was reached. A one-way repeated measures ANOVA was used to compare intra-test effects for each stage and for the maximal metabolic values of the steady-state test. The Greenhouse-Geisser correction was applied when Mauchly's sphericity test was significant ($p \leq 0.05$). For non-parametric data, Friedman's test and *post hoc* Wilcoxon corrections were used instead. Statistical analyses were performed using the software package SPSS (IBM SPSS version 25.0, Chicago, IL, United States). Statistical significance was set at $p \leq 0.05$. The magnitude of the differences was assessed with partial eta squared values (ηp^2) derived from ANOVAs and were interpreted as low (<0.04), moderate (0.04–0.13) and large (>0.13) for all the parametric outcomes. The effect size of the *post hoc* comparisons was calculated by means of Cohen's d, which was interpreted as a low (<0.50), moderate (0.50–0.80), or large effect (>0.80) (Cohen, 1988).

3 Results

3.1 Circulating lactate levels, RPE and heart rate during steady-state test

Two-way repeated measures ANOVAs did not reveal significant differences for condition in circulating lactate levels, general and local RPE, nor heart rate (p range = 0.08–0.56) (Table 2). Significant differences were reported in time for heart rate, RPEg, and RPEquad ($p \leq 0.001$) (Table 2). Bonferroni *post hoc* analyses revealed significant differences for all time comparisons (pre, "30" and "60") in heart rate, RPEg, and RPEquad (p range <0.001–0.004). Significant condition × time interaction was only found for RPEg due to higher values in LD compared to PLA (d = 27) and HD (d = 0.27) (Table 2).

3.2 *Skin body temperature*, maximal metabolic respiratory variables and mean and maximum heart rate during steady-state test

One-way repeated measures ANOVAs did not reveal significant differences in skin body temperature (p = 0.27) or mean heart rate (p = 0.24) (Table 3). Maximal carbohydrate oxidation, energy expenditure and RER did not differ between

TABLE 2 Two-way repeated measures analysis of variance (ANOVA) comparing circulating lactate levels, general and local RPE, and heart rate between the different experimental conditions during the steady-state test.

Variable	Time	Condition			ANOVA		
		PLA	LD	HD	Condition	Time	Condition × time
Lactate (mmol/L)	Pre	1.54 ± 0.38	2.08 ± 2.21	2.43 ± 3.49	F = 0.58, p = 0.56 ηp^2 = 0.04	F = 0.03, p = 0.86 ηp^2 = 0.02	F = 2.68, p = 0.08 ηp^2 = 0.16
	Post	3.37 ± 4.54	1.36 ± 0.43	1.63 ± 0.51			
RPEg (a.u.)	Pre	0.19 ± 0.40	0.06 ± 0.25	0.00 ± 0.00	F = 1.08, p = 0.33 ηp^2 = 0.06	F = 47.40, p < 0.001* ηp^2 = 0.76	F = 3.15, p = 0.02* ηp^2 = 0.17
	30′	1.94 ± 1.12	2.06 ± 1.53	2.06 ± 1.34			
	60′	2.75 ± 1.48	3.63 ± 2.31	2.94 ± 1.88			
RPEquad (a.u.)	Pre	0.25 ± 0.58	0.25 ± 0.45	0.06 ± 0.25	F = 2.65, p = 0.08 ηp^2 = 0.15	F = 44.30, p < 0.001* ηp^2 = 0.74	F = 1.62, p = 0.20 ηp^2 = 0.10
	30′	1.94 ± 1.29	2.56 ± 1.71	2.19 ± 1.56			
	60′	3.06 ± 1.61	3.69 ± 2.18	3.19 ± 1.80			
Heart rate (b/min)	Pre	70.4 ± 10.2	66.6 ± 8.6	67.9 ± 9.6	F = 2.28, p = 0.11 ηp^2 = 0.13	F = 64.86, p < 0.001* ηp^2 = 0.81	F = 0.85, p = 0.45 ηp^2 = 0.05
	30′	105.5 ± 15.9	103.1 ± 16.59	102.2 ± 17.8			
	60′	109.3 ± 19.6	107.4 ± 18.1	103.9 ± 17.4			

Mean ± standard deviation. PLA, Placebo; HD, high dose; LD, low dose; Pre; Before the start of the session; 30′, at minute 30 of steady-state test; 60′, at minute 60 of steady test; RPEg, general ratings of perceived effort; RPEquad, ratings of perceived effort in quadriceps; a.u., arbitrary units. * Significant difference: $p \leq 0.05$.

TABLE 3 One-way repeated measures analysis of variance (ANOVA) comparing skin body temperature, mean and maximum heart rate, and maximal metabolic respiratory variables between the three experimental conditions during the steady-state test.

Variable	Conditions			ANOVA
	PLA	LD	HD	
Skin body temperature (°C)	30.27 ± 1.02	30.48 ± 1.22	30.02 ± 1.22	F = 1.32, p = 0.27, ηp^2 = 0.09
Mean heart rate (bpm)	100.73 ± 14.96	99.97 ± 14.18	97.66 ± 15.22	F = 1.47, p = 0.24, ηp^2 = 0.09
Maximum heart rate (bpm)	114.13 ± 18.24	112.31 ± 18.53	108.75 ± 16.83	F = 3.23, p = 0.03*, ηp^2 = 0.19
FOpeak (g/min)	0.28 ± 0.09	0.32 ± 0.11	0.33 ± 0.11	F = 3.20, p = 0.05*, ηp^2 = 0.16
CHOOXpeak (g/min)	1.12 ± 0.60	1.03 ± 0.50	1.07 ± 0.50	F = 3.05, p = 0.10, ηp^2 = 0.15
MEEpeak (kcal/min)	5.99 ± 1.90	6.03 ± 1.85	6.10 ± 1.92	F = 0.20, p = 0.77, ηp^2 = 0.01
MRERpeak (g/min)	0.92 ± 0.05	0.91 ± 0.05	0.91 ± 0.06	F = 0.80, p = 0.45, ηp^2 = 0.05

Mean ± standard deviation. PLA, Placebo; HD, high dose; LD, low dose; FOpeak, Peak of fat oxidation; CHOOXpeak, Peak of carbohydrate oxidation; MEEpeak, Peak of energy expenditure; MRERpeak, Peak of respiratory exchange ratio. * Significant difference: $p \leq 0.05$.

conditions (p ranged from 0.10 to 0.77). However, significant differences were found for maximum heart rate (p = 0.03) and MFO (p = 0.05) (Table 3), where PLA reached the maximum and HD the lowest values. However, Bonferroni *post hoc* did not revealed differences between conditions in any of the outcomes (p range = 0.09 to 0.99; d range = 0.20–0.31).

3.3 Thermal perception during steady-state test

Significant differences for condition were not reported for any of the ASHRAE outcomes measured (p range = 0.17 to 0.78; ηp^2 range = 0.01–0.12). However, significant differences were reported in time for all the variables ($p \leq 0.001$; ηp^2 range = 0.52–0.73). A significant condition × time interaction was found for the clavicle (p = 0.04; ηp^2 = 0.16) area due to the lower value of HD in comparison to LD an PLA. The other areas did not exhibit condition × time interactions (p range = 0.12 to 0.60; ηp^2 range = 0.04–0.12) (Figure 2).

3.4 Energy expenditure and substrate oxidation during steady-state test

No significant differences were found for AUC EE, AUC FATox, and AUC CHOox (p range = 0.09 to 0.54; ηp^2 range = 0.04–0.18). Two-way repeated measures ANOVAs exhibited non-significant differences for condition in FAToxidation (p = 0.06; ηp^2 = 0.14), CHOox (p = 0.19; ηp^2 = 0.10), EE (p = 0.54; ηp^2 = 0.008), and RER (p = 0.21; ηp^2 = 0.10). However, two-way repeated measures

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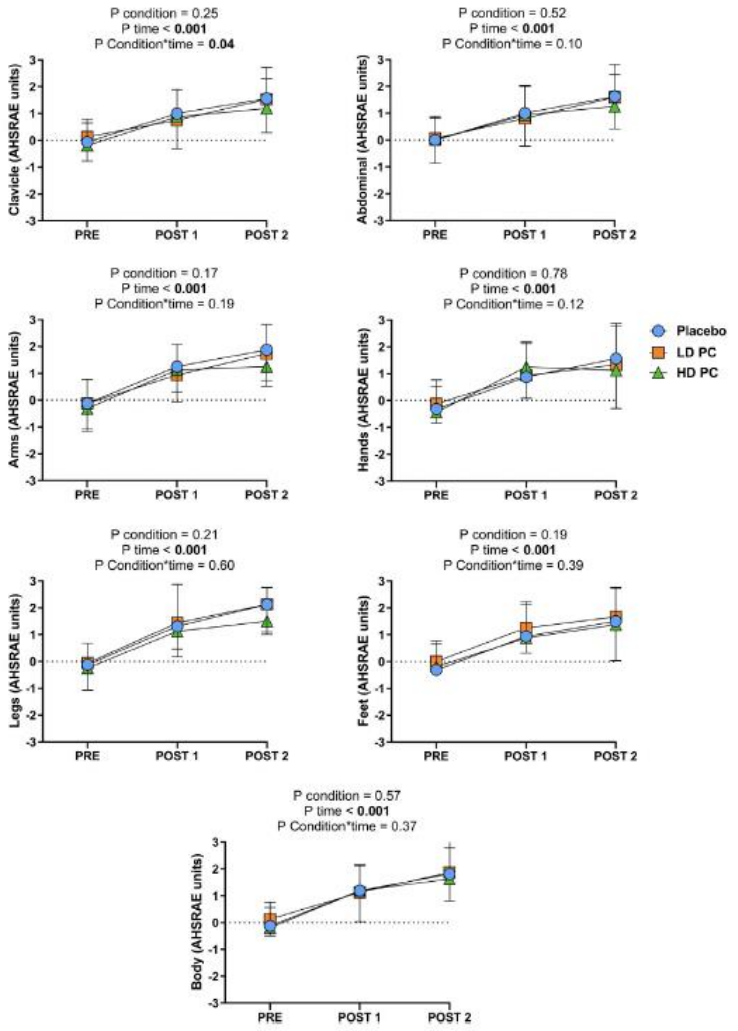

FIGURE 2
Two-way repeated measures analysis of variance (ANOVA) comparing the effects of consuming different dosis of phenylcapsaicin (HD and LD) or placebo (PLA) on thermal perception (ASHRAE scale) at different time points. PLA, Placebo; HD, High dose; LD, Low dose; PC, Phenylcapsaicin. PRE: Before the start of the session; Post 1: Between the steady state test and maximal effort test; Post 2: After the maximal effort test.

ANOVA revealed significant differences for time in all the aforementioned variables ($p \leq 0.001$; ηp^2 range = 0.54–0.79) but not for condition × time interaction (p range = 0.17 to 0.96; ηp^2 range = 0.003–0.06). Intra-test one-way repeated measures ANOVA only exhibited a significant effect on FATox at min 5 ($p = 0.005$; $\eta p^2 = 0.28$), 10 ($p \leq 0.001$; $\eta p^2 = 0.29$), and 55 ($p = 0.04$; $\eta p^2 = 0.24$) for HD and LD and for CHOox at min 5 ($p = 0.05$; $\eta p^2 = 0.25$) and RER ($p = 0.003$; $\eta p^2 = 0.25$) at min 5 in favor of PLA but not for any variable in any other stage. Post-hoc Bonferroni reported significant differences in favor of HD and LD between PLA/HD at 5 min ($p = 0.002$; d = 0.92), PLA/LD at 5 min ($p = 0.002$; d = 0.74), PLA/HD ($p = 0.002$; d = 0.66) and PLA/LD at 10 min ($p = 0.002$; d = 0.56) for FATox (Figure 3).

3.5 Maximal effort test

None of the variables (i.e., heart rate, lactate, RPEg at 30% and 90%, and RPEquad) recorded during the maximal effort test differed between the experimental conditions (p ranged from 0.011 to 0.915) except RPEg at 60% due to the lower values of HD compared to LD and PLA ($p = 0.05$) (Table 4).

4 Discussion

This study aimed to evaluate for the first time the effects of two doses of PC on several metabolic and perceptual responses during a

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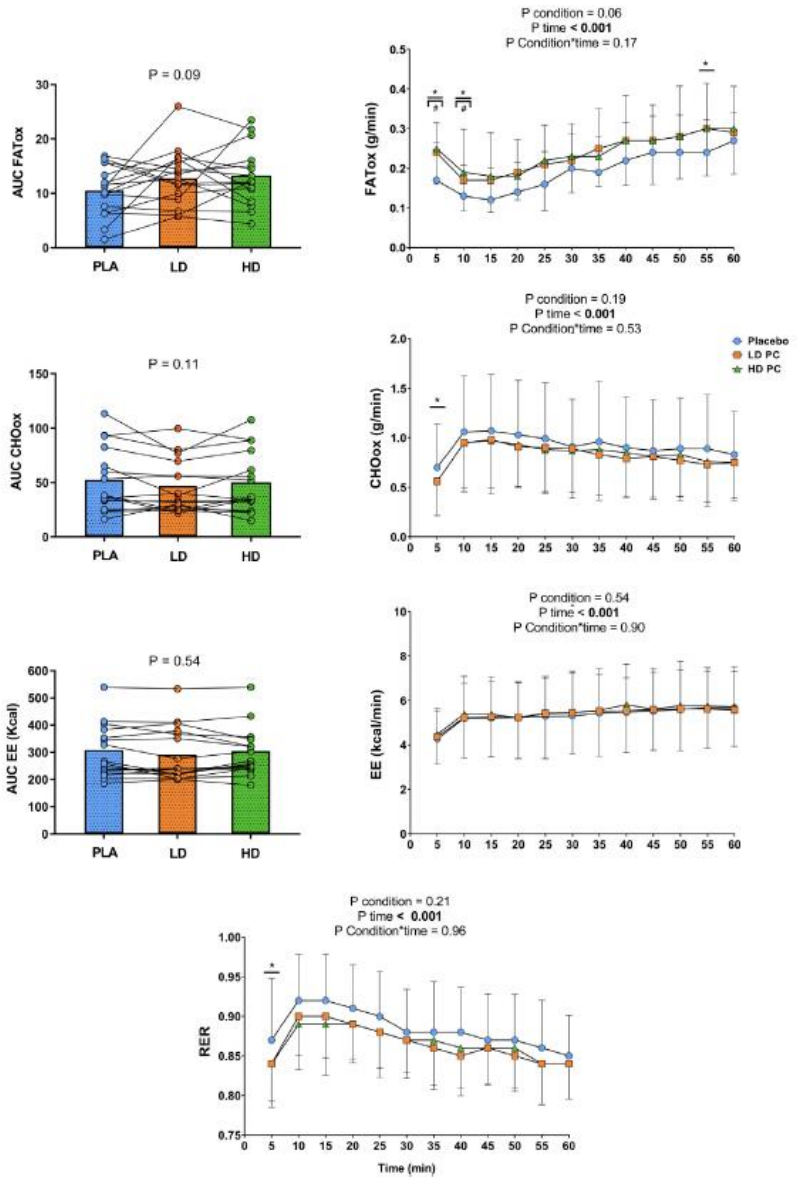

FIGURE 3
Two-way repeated measures analysis of variance (ANOVA) comparing the effects of phenylcapsaicin and placebo (PLA, HD, LD) across time on substrate oxidation, energy expenditure and respiratory exchange ratio during the 60 min steady-state test. AUC, Area Under the curve; FATox, Fat oxidation; CHOox, Carbohydrates oxidation; EE, Energy expenditure; RER, Respiratory exchange ratio; PLA, Placebo; HD, High dose; LD, Low dose; PC, Phenylcapsaicin. Intra-test analysis (one-way repeated measures ANOVA): *$p_{ANOVA} \leq 0.05$; #$p_{Bonferroni} \leq 0.05$.

steady-state and a maximal incremental test. The main finding of this study was that LD and HD of PC increase fat oxidation during different stages of the steady-state test and the peak of fat oxidation (FOpeak) in comparison to PLA. Furthermore, both PC doses, exhibited a reduction in CHOox and RER during the first stage of the steady-state test which suggest a shift on substrate oxidation. HD of PC also reduced maximum heart rate during the steady-state test. Contrary to our hypothesis, LD elicited higher RPEg values compared to PLA and HD during the steady-state test. Intriguingly, although skin body temperature was not affected by PC, the thermal perception was significantly lower in the supraclavicular area for HD. Nevertheless, only RPEg at 60% of maximal intensity was

TABLE 4 One-way repeated measures analysis of variance (ANOVA) or Friedman's test comparing different variables obtained during the incremental test.

Variable	Conditions			ANOVA or Friedman's
	PLA	LD	HD	
Final stage (W)	296.4 ± 43.7	291.1 ± 43.4	292.9 ± 37.2	$F = 0.48$, $p = 0.62$, $\eta p^2 = 0.05$
$VO2_{max}$ (mL/min/kg)	30.9 ± 7.3	30.5 ± 7.4	30.9 ± 5.8	$F = 0.08$, $p = 0.91$, $\eta p^2 = 0.01$
Lactate (mmol/L)	9.0 ± 3.7	8.0 ± 2.8	8.4 ± 3.3	$F = 0.51$, $p = 0.60$, $\eta p^2 = 0.04$
RPEg 30% (a.u.)	1.4 ± 1.3	1.7 ± 1.2	1.4 ± 0.8	$\chi = 1.08$, $p = 0.58$
RPEg 60% (a.u.)	3.8 ± 1.8	3.9 ± 2.0	3.4 ± 1.9	$\chi = 5.84$, $p = 0.05$*
RPEg 90% (a.u.)	7.1 ± 1.6	7.0 ± 1.7	6.9 ± 1.7	$\chi = 0.62$, $p = 0.73$
RPEquad 30% (a.u.)	1.8 ± 1.4	2.1 ± 1.4	1.8 ± 1.3	$\chi = 0.97$, $p = 0.61$
RPEquad 60% (a.u.)	4.8 ± 1.5	4.9 ± 1.5	4.4 ± 1.5	$\chi = 3.73$, $p = 0.15$
RPEquad 90% (a.u.)	7.9 ± 0.9	7.9 ± 0.9	8.1 ± 1.2	$\chi = 2.60$, $p = 0.27$
Heart rate 30% (bpm)	106.5 ± 11.1	107.7 ± 9.4	105.8 ± 12.5	$F = 0.44$, $p = 0.64$, $\eta p^2 = 0.03$
Heart rate 60% (bpm)	140.2 ± 15.6	139.9 ± 15.0	135.9 ± 13.8	$F = 2.40$, $p = 0.11$, $\eta p^2 = 0.15$
Heart rate 90% (bpm)	169.9 ± 15.0	171.0 ± 13.7	170.1 ± 14.9	$F = 0.48$, $p = 0.49$, $\eta p^2 = 0.03$

Mean ± standard deviation. ANOVA, analysis of variance; PLA, Placebo; LD, low dose; HD, high dose; $VO2_{max}$, maximal oxygen consumption; RPEg, general rate of perceived exertion; RPEquad, quadriceps rate of perceived exertion; a.u., arbitrary units. * Significant difference: $p \leq 0.05$.

lowered with the HD of PC supplementation during the incremental test. Therefore, the present results suggest that PC enhances fat oxidation during aerobic exercise and modulate the perceptual responses to exercise and the maximum heart rate. However, these perceptual and heart rate effects are only reported when the HD is ingested.

In the present study, the FOpeak during the steady-state test and the rate of fat oxidation during different stages of this test were higher for HD and LD in comparison to PLA (see Figure 3). In addition, both PC doses also exhibited lower carbohydrates oxidation and RER values during the first stage of the steady-state test, which may suggest a shift on substrate oxidation induced by PC (de Moura e Silva et al., 2021). These results are reported for the first time with the use of PC. Furthermore, only two studies have previously documented the metabolic effects of a capsaicinoid on substrate oxidation, reporting contradictory findings between them (Lim et al., 1997; Rossi et al., 2022). The increase of fat oxidation reported in the current study might be explained by the exercise protocol employed and the type of supplement ingested (EFSA, 2012; Turck et al., 2019). Additionally, contrary to previous research, in the present study MFO and FATmax were calculated for each participant which matched the metabolic and mechanical training intensities of the subjects. This fact is essential due to different factors such as the exercise intensity, metabolic cart use, or the ergometer employed, influence the metabolic responses during exercise (Amaro-Gahete et al., 2018; Amaro-Gahete et al., 2019). Concerning the type of supplement used, previous studies revealed that capsinoids supplementation does not modulate the metabolic responses during aerobic exercise (Osuna-Prieto et al., 2022; Rossi et al., 2022). Although a MFO protocol was used by Osuna-Prieto et al. (2022), previous research has reported that capsinoids are hydrolyzed in the gastrointestinal tract, and their metabolites are excreted rapidly after their ingestion (Bernard et al., 2008). Capsiate circulating levels are significantly much lower in comparison to capsaicin levels, and their final linkage with TRPV1 is approximately 1/10 compared to capsaicinoids such as PC (Sasahara et al., 2010). Accordingly, PC might be able to activate in a higher way TRPV1 receptors leading to a greater increase in fatty acid oxidation and increasing glycogen sparing. This finding may be important due to the relevant link between muscle glycogen and performance in aerobic sports (Murray and Rosenbloom, 2018).

Although the TRPV1 temperature threshold is set at 43°C on dorsal root ganglia cells, capsaicin seems to decrease the activation threshold to 36.8°C (Szolcsányi, 2015). In humans, topical capsaicin has been demonstrated to increase heat loss by increasing peripheral vasodilation thereby improving the skin's vasoconstrictive tone, increasing heat dissipation during exercise and increasing thermal perception and heat stress (Botonis et al., 2019). However, oral capsiate supplementation has not exhibited any of these effects on overweight participants (Osuna-Prieto et al., 2022). As our study presents an encapsulated formulation of PC, the null effect on skin body temperature may be related to the nature of the vehicle used. Accordingly, if a non-encapsulated powder formulation might have been ingested, a thermoregulatory response could be expected due to the direct contact of PC with the esophagus and gastrointestinal tissues (Szolcsányi, 2015). However, in the present study, the thermal perception was reduced in the clavicle area for the HD condition. Because PC was encapsulated and a burning reflux effect was not reported by any of the participants, this finding contrasts most literature where capsaicinoids spiciness is discussed (Naves et al., 2019). A plausible explanation for this issue may be that the digestion of the capsules did not produce an irritant effect, albeit they may have increase the internal temperature (i.e., not directly measured with skin temperature assessment) (Patcharatrakul et al., 2020). As a

consequence, subjects may have experienced a counterregulatory response to PC at the end of the test on the aforementioned area (see Figure 2). Moreover, LD seems to be an insufficient dose to alter the termal perception. Besides, as heat perception contributes to the development of fatigue during exercise, this finding may be helpful to counteract fatigue in aerobic sports (Willmott et al., 2020). Overall, future research should assess if this finding depends on the ingestion of PC instead of traditional capsaicin formulations, the effect of the application vehicle on capsaicin's pungency perception and the plausible existence of a counterregulatory mechanism of PC on thermal perception.

The effects of capsaicin and red peppers on cardiovascular responses are currently controversial due to the vast heterogeneity between protocols and doses used in previous literature (Shirani et al., 2021). However, recent research has shown that supplementation with purified capsaicin does not alter heart rate during an incremental exercise test in a cycloergometer until exhaustion (Giuriato et al., 2022). These studies employed intensities above 80% of $VO2_{max}$ (Grgic et al., 2022), which therefore are not extrapolable to lower intensities. In the present study, although the medium heart rate did not differ between conditions, HD exhibited a significantly lower maximum heart rate during the steady-state test. As in the present study the intensity was matched in the steady-state test, it is possible that under HD supplementation, subjects experienced a higher mechanical efficiency, which may have reduced the relative workload, resulting in lower cardiovascular demands during the test (Mikus et al., 2009). In addition, circulating lactate response across time trended to be lower with both PC doses in the steady-state test. Previous preclinical research has shown that lactate is a potent endogenous inhibitor of TRPV1 activity (de la Roche et al., 2016). According to this possible mechanism, PC may have modulated sarcoplasmic calcium efflux channels lowering lactate levels during both tests (de la Roche et al., 2016). This finding agrees with previous research in resistance training and high-intensity running (de Freitas et al., 2018b; de Freitas et al., 2018a). Furthermore, this lactate lowering effect of PC may also be partially explained by the shift on susbtrate oxidation produced by this substance and provides novel information about the physiological independence between this effect and the intensity of the exercise used. However, contrary to previous research where capsaicin supplementation led to lower RPE values, in our data, participants showed higher RPE scores under the LD condition, and improvements were not reported with HD compared to PLA (de Freitas et al., 2018a). The low intensity demanded in this task may be insufficient to report an ergogenic effect of PC on perceptual variables (Jiménez Martínez et al., 2023). However, LD of PC reported for the first time a worsening effect of a capsaicinoid on RPE. Therefore, if PC at LD in low intensity tasks produces a counterregulatory effect on perceptual performance or if the sensitivity of RPE under these novel conditions is altered, should be corroborate in further studies.

Concerning the incremental test, the HD of PC reduced RPEg at 60% of the maximal intensity achieved. Additionally, the reduction in RPE values during high-intensity exercise after capsaicin supplementation is well documented (Jiménez-Martínez et al., 2022). During high-intensity exercise capsaicinoids reduce perceived exertion due to TRPV1 activation. TRPV1 are linked to afferent III and IV nerve fibers (Collins et al., 2018). These fibers influence central fatigue during exercise, which finally reduce motorneuron firing during high-intensity tasks (Alix-Fages et al., 2022b; Jiménez Martínez et al., 2023). For this reason, HD might have produce a "desensitizer" effect leading to a lower percepetion of exertion in the maximal incremental test. This threshold may not be achieved by the LD. Additionally, these findings align with previous research that has reported that capsaicin supplementation does not increase $VO2_{max}$ or any other metabolic outcome during high-intensity exercise, although the time to exhaustion in interval training is increased (de Freitas et al., 2019).

5 Strengths and limitations

This study presents essential strengths such as the control of the fasting conditions and the meal before each session, and the randomized, triple-blinded, crossover design. Nonetheless, some limitations should be addressed. First, the study only included active males, and these results may not be extrapolated to other populations. Secondly, these results can not be extrapolated to other exercise modalities or intensities. Finally, the chronic effects of PC on metabolic responses during exercise should not be extrapolated from this acute study.

6 Conclusion

The results of the present study suggest that LD and HD of PC modulate the metabolic response (FATox, CHOox and RER) to exercise and HD of PC reduces maximum heart rate values during aerobic exercise. However, PC only improves the perceptual responses (i.e., RPEg and clavicle thermal perception) to exercise when it is consumed in HD.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Portal de Ética de la Investigación Biomédica de Andalucía. The patients/participants provided their written informed consent to participate in this study.

Author contributions

PJ-M, AG-R, CA-F, and JC conceived the study and methodology. JM-O, JD, LJ-F, SM-M, SC-V, JO-P, FA-G, and DJ were involved in data collection and treatment; writing was conducted by PJ-M, DJ, AG-R, and CA-F. All authors reviewed and approved the final version of the manuscript. All authors listed

have made a substantial, direct, and intellectual contribution to the work and approved it for publication. All authors contributed to the article and approved the submitted version.

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Conflict of interest

PJ-M, DJ, JM-O, and CA-F are scientific advisors of a sports supplement brand (Life Pro Nutrition).

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

Publisher's note

References

Alix-Fages, C., Del Vecchio, A., Baz-Valle, E., Santos-Concejero, J., and Balsalobre-Fernández, C. (2022a). The role of the neural stimulus in regulating skeletal muscle hypertrophy. *Eur. J. Appl. Physiol.* 122, 1111–1128. doi:10.1007/s00421-022-04906-6

Alix-Fages, C., Grgic, J., Jiménez-Martínez, P., Baz-Valle, E., and Balsalobre-Fernández, C. (2022b). Effects of mental fatigue on strength endurance: A systematic review and meta-analysis. *Mot. Control* 27, 442–461. doi:10.1123/mc.2022-0051

Alix-fages, C., Baz-valle, E., González-cano, H., Jiménez-martínez, P., and Balsalobre-fernández, C. (2023). Mental Fatigue From Smartphone Use or Stroop Task Does Not Affect Bench Press Force – velocity Pro fi le, One-Repetition Maximum, or Vertical Jump Performance. *Mot. Control*, 1–14. doi:10.1123/mc.2022-0133

Amaro-Gahete, F. J., Sanchez-Delgado, G., and Ruiz, J. R. (2018). Commentary: Contextualising maximal fat oxidation during exercise: Determinants and normative values. *Front. Physiol.* 9, 1460. doi:10.3389/fphys.2018.01460

Amaro-Gahete, F. J., Sanchez-Delgado, G., Jurado-Fasoli, L., De-la-O, A., Castillo, M. J., Helge, J. W., et al. (2019). Assessment of maximal fat oxidation during exercise: A systematic review. *Scand. J. Med. Sci. Sports* 29, 910–921. doi:10.1111/sms.13424

Basith, S., Cui, M., Hong, S., and Choi, S. (2016). Harnessing the therapeutic potential of capsaicin and its analogues in pain and other diseases. *Molecules* 21, 966. doi:10.3390/molecules21080966

Bernard, B. K., Watanabe, E., Kodama, T., Tsubuku, S., Otabe, A., Nakajima, M., et al. (2008). Studies of the toxicological potential of capsinoids: V. Genotoxicity studies of dihydrocapsiate. *Int. J. Toxicol.* 27, 59–72. doi:10.1080/10915810802513536

Botonis, P. G., Miliotis, P. G., Kounalakis, S. N., Koskolou, M. D., and Geladas, N. D. (2019). Thermoregulatory and cardiovascular effects of capsaicin application on human skin during dynamic exercise to temperate and warm conditions. *Physiol. Rep.* 7, e14325. doi:10.14814/phy2.14325

Carroll, T. J., Taylor, J. L., and Gandevia, S. C. (2017). Recovery of central and peripheral neuromuscular fatigue after exercise. *J. Appl Physiol.* 122, 1068–1076. doi:10.1152/japplphysiol.00775.2016

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences*. 2nd ed. Hillsdale, N.J.: Lawrence Erlbaum Associates. doi:10.1234/12345678

Collins, B. W. C., Pearcey, G., Buckle, N. C. M., Power, K. E., and Button, D. C. (2018). Neuromuscular fatigue during repeated sprint exercise: Underlying physiology and methodological considerations. *Appl. Physiol. Nutr. Metab.* 43, 1166–1175. doi:10.1139/apnm-2018-0080

Crotty, N. M., Boland, M., Mahony, N., Donne, B., and Fleming, N. (2021). Reliability and validity of the lactate Pro 2 analyzer. *Meas. Phys. Educ. Exerc. Sci.* 25, 202–211. doi:10.1080/1091367X.2020.1865966

de Freitas, M. C., Cholewa, J. M., Freire, R. V., Carmo, B. A., Bottan, J., Bratfich, M., et al. (2018a). Acute capsaicin supplementation improves resistance training performance in trained men. *J. Strength Cond. Res.* 32, 2227–2232. doi:10.1519/jsc.0000000000002109

de Freitas, M. C., Cholewa, J. M., Gobbo, L. A., de Oliveira, J. V. N. S., Lira, F. S., and Rossi, F. E. F. E. (2018b). Acute capsaicin supplementation improves 1,500-M running time-trial performance and rate of perceived exertion in physically active adults. *J. strength Cond. Res.* 32, 572–577. doi:10.1519/JSC.0000000000002329

de Freitas, M. C., Billaut, F., Panissa, V. L. G., Rossi, F. E., Figueiredo, C., Caperuto, E. C., et al. (2019). Capsaicin supplementation increases time to exhaustion in high-intensity intermittent exercise without modifying metabolic responses in physically active men. *Eur. J. Appl. Physiol.* 119, 971–979. doi:10.1007/s00421-019-04086-w

de la Roche, J., Walther, I., Leonow, W., Hage, A., Eberhardt, M., Fischer, M., et al. (2016). Lactate is a potent inhibitor of the capsaicin receptor TRPV1. *Sci. Rep.* 6, 36740. doi:10.1038/srep36740

de Moura e Silva, V. E. L., Cholewa, J. M., Billaut, F., Jäger, R., de Freitas, M. C., Lira, F. S., et al. (2021). Capsaicinoid and capsinoids as an ergogenic aid: A systematic review and the potential mechanisms involved. *Int. J. Sports Physiol. Perform.* 16, 464–473. doi:10.1123/IJSPP.2020-0677

EFSA (2012). Scientific opinion on dihydrocapsiate. *EFSA J.* 10, 1–28. doi:10.2903/j.efsa.2012.2812

Frayn, K. N. (2016). Calculation of substrate oxidation rates *in vivo* from gaseous exchange. *J. Appl. Physiol.* 121, 628–634. doi:10.1152/jappl.1983.55.2.628

Giuriato, G., Venturelli, M., Matias, A., Soares, E. M. K. V. K., Gaetgens, J., Frederick, K. A., et al. (2022). Capsaicin and its effect on exercise performance, fatigue and inflammation after exercise. *Nutrients* 14, 232. doi:10.3390/nu14020232

González-Cano, H., Jiménez-Martínez, P., Baz-Valle, E., Contreras-Fernández, C., Colado, J. C., and Alix-Fages, C. (2022). Nutritional and supplementation strategies of Spanish natural elite bodybuilders in pre-contest. *Gazz. Med. Ital.* 132. doi:10.23736/S0393-3660.22.04877-X

Grgic, J., Memon, A. R., Chen, S., Ramirez-Campillo, R., Barreto, G., Haugen, M. E., et al. (2022). Effects of capsaicin and capsiate on endurance performance: A meta-analysis. *Nutrients* 14, 4531. doi:10.3390/nu14214531

Hernando, D., Garatachea, N., Almeida, R., Casajús, J. A., and Bailón, R. (2018). Validation of heart rate monitor polar RS800 for heart rate variability analysis during exercise. *J. strength Cond. Res.* 32, 716–725. doi:10.1519/JSC.0000000000001662

Jiménez Martínez, P., Cornejo Daza, P. J., Sánchez-Valdepeñas, J., Asín-Izquierdo, I., Cano-Castillo, C., Alix-Fages, C., et al. (2023). Effects of different phenylcapsaicin doses on resistance training performance, muscle damage, protein breakdown, metabolic response, ratings of perceived exertion, and recovery: A randomized, triple-blinded, placebo-controlled, crossover trial. *J. Int. Soc. Sports Nutr.* 20, 2204083. doi:10.1080/15502783.2023.2204083

Jiménez-Martínez, P., Ramirez-Campillo, R., Handez, J., Alix-Fages, C., Baz-Valle, E., and Colado, J. C. (2022). Effects of oral capsaicinoids and capsinoids supplementation on resistance and high intensity interval training: A systematic review of randomized controlled trials. *J. Hum. Sport Exerc.* 18, 75–91. doi:10.14198/jhse.2023.182.09

Josse, A. R., Sherriffs, S. S., Holwerda, A. M., Andrews, R., Staples, A. W., and Phillips, S. M. (2010). Effects of capsinoid ingestion on energy expenditure and lipid oxidation at rest and during exercise. *Nutr. Metab. (Lond).* 7, 65. doi:10.1186/1743-7075-7-65

Lang, Y., Kisaka, H., Sugiyama, R., Nomura, K., Morita, A., Watanabe, T., et al. (2009). Functional loss of pAMT results in biosynthesis of capsinoids, capsaicinoid analogs, in capsicum annuum cv. CH-19 sweet. *Plant J.* 59, 953–961. doi:10.1111/j.1365-313X.2009.03921.x

Lejeune, M. P. G. M., Kovacs, E. M. R., and Westerterp-Plantenga, M. S. (2003). Effect of capsaicin on substrate oxidation and weight maintenance after modest body-weight loss in human subjects. *Br. J. Nutr.* 90, 651–659. doi:10.1079/bjn2003938

Lim, K., Yoshioka, M., Kikuza, S., Kiyonaga, A., Tanaka, H., Shindo, M., et al. (1997). Dietary red pepper ingestion increases carbohydrate oxidation at rest and during exercise in runners. *Med. Sci. Sport. Exerc.* 29, 355–361. doi:10.1097/00005768-199703000-00010

Luo, X. J., Peng, J., and Li, Y. J. (2011). Recent advances in the study on capsaicinoids and capsinoids. *Eur. J. Pharmacol.* 650, 1–7. doi:10.1016/j.ejphar.2010.09.074

Martinez-Tellez, B., Sanchez-Delgado, G., Acosta, F. M., Alcantara, J. M. A., Boon, M. R., Rensen, P. C. N., et al. (2017). Differences between the most used equations in BAT-human studies to estimate parameters of skin temperature in young lean men. *Sci. Rep.* 7, 10530. doi:10.1038/s41598-017-10444-5

Midgley, A. W., McNaughton, L. R., Polman, R., and Marchant, D. (2007). Criteria for determination of maximal oxygen uptake: A brief critique and recommendations for future research. *Sports Med.* 37, 1019–1028. doi:10.2165/00007256-200737120-00002

Mikus, C. R., Earnest, C. P., Blair, S. N., and Church, T. S. (2009). Heart rate and exercise intensity during training: Observations from the DREW Study. *Br. J. Sports Med.* 43, 750–755. doi:10.1136/bjsm.2008.046342

Murray, B., and Rosenbloom, C. (2018). Fundamentals of glycogen metabolism for coaches and athletes. *Nutr. Rev.* 76, 243–259. doi:10.1093/NUTRIT/NUY001

Naves, E. R., de Ávila Silva, L., Sulpice, R., Araújo, W. L., Nunes-Nesi, A., Peres, L. E. P., et al. (2019). Capsaicinoids: Pungency beyond capsicum. *Trends Plant Sci.* 24, 109–120. doi:10.1016/j.tplants.2018.11.001

Osuna-Prieto, F. J., Acosta, F. M., Perez, U. A., Le, D. A., Riquelme-gallego, B., Merchan-ramirez, E., et al. (2022). Dihydrocapsiate does not increase energy expenditure nor fat oxidation during aerobic exercise in men with overweight/obesity: A randomized, triple-blinded, placebo-controlled, crossover trial. *J. Int. Soc. Sports Nutr.* 19, 417–436. doi:10.1080/15502783.2022.2099757

Patcharatrakul, T., Kriengkirakul, C., Chaiwatanarat, T., and Gonlachanvit, S. (2020). Acute effects of red chili, a natural capsaicin receptor agonist, on gastric accommodation and upper gastrointestinal symptoms in healthy volunteers and gastroesophageal reflux disease patients. *Nutrients* 12, 3740. doi:10.3390/nu12123740

Rossi, P. A. Q., Lira, F. S., Bezerra, V. R., Clark, N. W., Fukuda, D. H., and Panissa, V. L. G. (2022). Acute response to capsiate supplementation at rest and during exercise on energy intake, appetite, metabolism, and autonomic function: A randomized trial. *J. Am. Nutr. Assoc.* 41, 541–550. doi:10.1080/07315724.2021.1938294

Rothschild, J. A., Kilding, A. E., Stewart, T., and Plews, D. J. (2022). Factors influencing substrate oxidation during submaximal cycling: A modelling analysis. *Sport. Med.* 52, 2775–2795. doi:10.1007/s40279-022-01727-7

Sanchez-Delgado, G., Alcantara, J. M. A., Ortiz-Alvarez, L., Xu, H., Martinez-Tellez, B., Labayen, I., et al. (2018). Reliability of resting metabolic rate measurements in young adults: Impact of methods for data analysis. *Clin. Nutr.* 37, 1618–1624. doi:10.1016/j.clnu.2017.07.026

Santos, F., Eduardo, F., Caetano, D., Clark, N., and Panissa, G. (2022). Can capsaicinoids and their analogs affect energy expenditure and substrate oxidation during exercise ? A systematic review with acute studies. *Hum. Nutr. Metab.* 29, 200156. doi:10.1016/j.hnm.2022.200156

Sasahara, I., Furuhata, Y., Iwasaki, Y., Inoue, N., Sato, H., Watanabe, T., et al. (2010). Assessment of the biological similarity of three capsaicin analogs (Capsinoids) found in non-pungent chili pepper (CH-19 Sweet) fruits. *Biosci. Biotechnol. Biochem.* 74, 274–278. doi:10.1271/bbb.90570

Shirani, F., Foshati, S., Tavassoly, M., Clark, C. C. T., and Rouhani, M. H. (2021). The effect of red pepper/capsaicin on blood pressure and heart rate: A systematic review and meta-analysis of clinical trials. *Phytother. Res.* 35, 6080–6088. doi:10.1002/ptr.7217

Smith, A. D. H., Crabtree, D. R., Bilzon, J. L. J., and Walsh, N. P. (2010). The validity of wireless iButtons and thermistors for human skin temperature measurement. *Physiol. Meas.* 31, 95–114. doi:10.1088/0967-3334/31/1/007

Szolcsányi, J. (2015). Effect of capsaicin on thermoregulation: An update with new aspects. *Temp. (Austin, Tex.)* 2, 277–296. doi:10.1080/23328940.2015.1048928

Taylor, J. L., Amann, M., Duchateau, J., Meeusen, R., and Rice, C. L. (2016). Neural contributions to muscle fatigue: From the brain to the muscle and back again. *Med. Sci. Sport. Exerc.* 48, 2294–2306. doi:10.1249/mss.0000000000000923

Turck, D., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K. I., Kearney, J., Maciuk, A., et al. (2019). Safety of phenylcapsaicin as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA J.* 17, e05718. doi:10.2903/j.efsa.2019.5718

van Marken Lichtenbelt, W. D., Daanen, H. A. M., Wouters, L., Fronczek, R., Raymann, R. J. E. M., Severens, N. M. W., et al. (2006). Evaluation of wireless determination of skin temperature using iButtons. *Physiol. Behav.* 88, 489–497. doi:10.1016/j.physbeh.2006.04.026

Weir, J. D. (1949). New methods for calculating metabolic rate with special reference to protein metabolism. *J. Physiol.* 109, 1–9. doi:10.1113/jphysiol.1949.sp004363

Willmott, A. G. B., Hayes, M., James, C. A., Gibson, O. R., and Maxwell, N. S. (2020). Heat acclimation attenuates the increased sensations of fatigue reported during acute exercise-heat stress. *Temperature* 7, 178–190. doi:10.1080/23328940.2019.1664370

8.2 Articles published related to the compendium topic but not included

González-Cano, H., **Jiménez-Martínez, P.**, Baz-Valle, E., Contreras, C., Colado, JC., Alix-Fages, C. (2023). Nutritional and supplementation strategies of Spanish natural elite bodybuilders in precontest. *Gazz Med Ital - Arch Sci Med*; 181:000-000. DOI: 10.23736/S0393-3660.22.04877-X)

Jiménez-Martínez, P., Ramirez-Campillo, R., Flandez, J., Alix-Fages, C., Baz-Valle, E., & Colado, J.C. (2022). Effects of oral capsaicinoids and capsinoids supplementation on resistance and high intensity interval training: A systematic review of randomized controlled trials. *Journal of Human Sport and Exercise, in press.* https://doi.org/10.14198/jhse.2023.182.09

8.3 Other scientific contributions authored by the doctoral candidate

8.3.1 Articles published not related to the compendium topic

Alix-Fages, C., Baz-Valle, E., González-Cano, H., **Jiménez-Martínez, P.**, & Balsalobre-Fernández, C. (2023). Mental fatigue from smartphone use or stroop task does not affect bench press force-velocity profile, one-repetition maximum, or vertical jump performance. Motor control, 1–14. Advance online publication. https://doi.org/10.1123/mc.2022-0133

Alix-Fages, C., Grgic, J., **Jiménez-Martínez, P.**, Baz-Valle, E., & Balsalobre Fernández, C. (2022). Effects of mental fatigue on strength endurance: A systematic review and meta-analysis. Motor control, 27(2), 442–461. https://doi.org/10.1123/mc.2022-005

Alix-Fages, C., **Jiménez-Martínez, P.**, de Oliveira, D. S., Möck, S., Balsalobre-Fernández, C., & Del Vecchio, A. (2023). Mental fatigue impairs physical performance but not the neural drive to the muscle: a preliminary analysis. European journal of applied physiology, 10.1007/s00421-023-05189-1. Advance online publication. https://doi.org/10.1007/s00421-023-05189-1

Colado, J. C., Gené-Morales, J., **Jiménez-Martínez, P.**, Flandez, J., Ferri-Caruana, A. M., & Babiloni-Lopez, C. (2023). Rating of Perceived Exertion in the First Repetition is Related to the Total Repetitions Performed in Elastic Bands Training. *Motor control*, *27*(4), 830–843. https://doi.org/10.1123/mc.2023-0017

Gene-Morales, J., Saez-Berlanga, A., Babiloni-Lopez, C., **Jiménez-Martínez, P.**, Ferri-Carruana, A. M., Martin-Rivera, F., & Colado, J. C. (2023). Effects of an elastomeric technology garment on different external and internal load variables: A pilot study. Scientific Journal of Sport and Performance, 2(2), 165–176. https://doi.org/10.55860/BXNK5984

Jiménez-Martínez, P., Ramirez-Campillo, R., Alix-Fages, C., Gene-Morales, J., García-Ramos, A., & Colado, J. C. (2023). Chronic resistance training effects on serum adipokines in type 2 diabetes mellitus: A systematic review. Healthcare (Basel, Switzerland), 11(4), 594. https://doi.org/10.3390/healthcare11040594

Juesas, A., Gargallo, P., Gene-Morales, J., Babiloni-López, C., Saez-Berlanga, A., **Jiménez-Martínez, P.**, Casaña, J., Benitez-Martinez, J. C., Ramirez-Campillo, R., Chulvi-Medrano, I., & Colado, J. C. (2023). Effects of microfiltered seawater intake and variable resistance training on strength, bone health, body composition, and quality of life in older women: A 32-week randomized, double-blinded, placebo-controlled trial. International journal of environmental research and public health, 20(6), 4700. https://doi.org/10.3390/ijerph20064700

Kramer-Ramos, A., Pujol-Calafat, A., **Jiménez-Martínez, P.**, & Alix-Fages, C. (2022). Effects of high-intensity interval training on patients with type 2 diabetes mellitus: A narrative review. Scientific Journal of Sport and Performance, 2(1), 36-43. https://doi.org/10.55860/STBC2316

8.3.2 Manuscripts under review

Babiloni-López, C., **Jiménez-Martínez, P.**, Alix-Fages, C., Saez-Berlanga, A., Juesas, A., Gargallo, P., Gene-Morales, J. & Colado, J.C., (2023). Normative values of physical fitness tests stratified by sex and age in healthy older adults and their association with isokinetic lower limb strength: A 550 participants cross-sectional study.

Ben-Ayed, K., Hammami, R., Gene-Morales, J, Ajailia, A., Werfelli, A., Rebai, H., **Jiménez-Martínez, P.**, Flandez, J. & Colado, J. C. (2023). Effects of two different plyometric jump protocols performed following the warm-up on sprint and change-of-direction performance in youth elite volleyball players. *Manuscript Submitted for Publication*.

Bouafif, N., Hammami, R., Mahmoudi, A., **Jiménez-Martínez, P.**, Alix-Fages, C., Garcia-Ramos, A., Gene-Morales, J., Gaied-Chortane, S. & Colado, J.C. (2023). Reliability of single-leg maximal dynamic strength performance and inter-limb asymmetries in pre-pubertal soccer players. *Manuscript Submitted for Publication*.

Colado, J.C., Gene-Morales, J., **Jiménez-Martínez, P.**, Saez-Berlanga, A., Ferri-Caruana, A.M. & Babiloni-Lopez, C., (2023). Validity and reliability of the resistance intensity scale for exercise (RISE) with elastic bands for being applied according to the velocity-based training approach. *Manuscript Submitted for Publication*.

González-Cano, H., Martín-Olmedo, J.J., Baz-Valle, E., Contreras, C., Schoenfeld, B.J., García-Ramos, A., **Jiménez-Martínez, P.**, & Alix-Fages, C. (2023). Exploring the boundaries

of natural muscle mass development: A cross-sectional anthropometric study of elite natural bodybuilders on competition day. *Manuscript Submitted for Publication*.

Hammami, R., Gene-Morales, J., **Jiménez-Martínez, P.**, R., Mahmoudi, A., Rebai, H. & Colado, J.C. (2023). Effect of low, medium and high volume of nordic hamstring curl training on linear sprint and change of direction performance in youth soccer players. *Manuscript Submitted for Publication.*

Janicijevic, D., Miras-Moreno, S., Morenas-Aguilar M.D., **Jiménez-Martínez, P.**, Alix-Fages, C., & García-Ramos, A. (2023). Relationship between perceptual and mechanical markers of fatigue during bench press and bench pull exercises: Impact of inter-set rest period length. *Manuscript Submitted for Publication*.

Jiménez-Martínez, P., Alix-Fages, C., Helms, E.R., Espinar, S., González-Cano, H., Baz-Valle, E., García-Ramos, A., Colado, J.C. (2023). Dietary supplementation habits in international natural bodybuilders during pre-competition. *Manuscript Submitted for Publication*.

Juesas, A., Gargallo, P., Gene-Morales, J., Babiloni-López, C., Saez-Berlanga, A., **Jiménez-Martínez, P.**, Casaña, J., Benitez-Martinez, J.C. & Colado, J. C. (2023). Could deep-sea water supplementation and variable resistance training improve blood pressure, hepatic, and, renal biomarkers in older women? A 32-weeks, double-blinded, randomized, placebo-controlled trial. *Manuscript Submitted for Publication*.

Juesas, A., Gargallo, P., Gene-Morales, J., Tamayo, E., **Jiménez-Martínez, P.**, Casaña, J., Benitez-Martinez, J. C., & Colado, J. C. (2023). Multicomponent and power training improves metabolic and inflammatory parameters, body composition, and physical function in older women with metabolic syndrome. *Manuscript Submitted for Publication*.

Miras-Moreno, S., Morenas-Aguilar M.D., Martín-Olmedo, J. J., Janicijevic, D., Cwiklinska, M., Alix-Fages, C., **Jiménez-Martínez, P.** & García-Ramos, A. (2023). Effects of intra-training Cluster Dextrin supplementation on resistance training performance and physiological parameters. *Manuscript Submitted for Publication*.

Serrano-Jiménez, M., Kramer-Ramos K., **Jiménez-Martínez, P.**, Serrano-Jiménez, M., García-Ramos A. & Alix-Fages, C. (2023). Effects of eccentric and concentric home-based resistance training on subacromial impingement syndrome compared to traditional treatment in telematic and face-to-face interventions. *Manuscript Submitted for Publication*.

8.3.3 Congress communications and scientific speeches

POLITÉCNICA INEF Departamento de Salud y Rendimiento Humano SDF SIMPOSIO DE FUERZA

XIV SIMPOSIO INTERNACIONAL DE ACTUALIZACIONES EN ENTRENAMIENTO DE LA FUERZA

Se certifica que

Gene-Morales J., Jiménez-Martínez P., Saez-Berlanga A., Babiloni-Lopez C., Juesas A., Tellols S., Gené-Sampedro A., Colado J.C. ha presentado el **Póster** titulado **"Análisis de presión intraocular en el ejercicio de sentadilla: Comparación entre bandas elásticas y discos"** en el XIV Simposio Internacional de Actualizaciones en Entrenamiento de la Fuerza realizado en la **Facultad de Ciencias de la Actividad Física y del Deporte (INEF)** de la **Universidad Politécnica de Madrid**, entre los días 16 y 17 de diciembre de 2022.

Madrid, 18 de diciembre de 2022

Id. 65. Number of certificates: 8

Pedro J. Benito Peinado
Presidente del Comité Organizador

Ana B. Peinado Lozano
Presidenta del Comité Científico

MATRIX ENFAF HARBIZ NIEMS PRO

XIV SIMPOSIO INTERNACIONAL DE ACTUALIZACIONES EN ENTRENAMIENTO DE LA FUERZA

Se certifica que

Babiloni, C., Jiménez-Martínez, P., Mollá G., Saez-Berlanga A., Gene-Morales J., Juesas A., Alix-Fages C., Colado J.C. ha presentado el **Póster** titulado **"Activación electromiográfica del pectoral mayor durante ejercicios de fuerza de empuje horizontal"** en el XIV Simposio Internacional de Actualizaciones en Entrenamiento de la Fuerza realizado en la **Facultad de Ciencias de la Actividad Física y del Deporte (INEF)** de la **Universidad Politécnica de Madrid,** entre los días 16 y 17 de diciembre de 2022.

Madrid, 18 de diciembre de 2022

Pedro J. Benito Peinado
Presidente del Comité Organizador

Ana B. Peinado Lozano
Presidenta del Comité Científico

Id: 11 Number of certificates: 6

XIV SIMPOSIO INTERNACIONAL DE ACTUALIZACIONES EN ENTRENAMIENTO DE LA FUERZA

Se certifica que

Saez-Berlanga, A., Gene-Morales, J., Jiménez-Martínez, P., Babiloni, C., Juesas, A., Medina, J., Gené-Sampedro, A., Colado, J. C. ha presentado el **Póster** titulado **"Efectos de resistencias variables y continuas sobre presión intraocular y presión arterial en entrenamiento de potencia""** en el XIV Simposio Internacional de Actualizaciones en Entrenamiento de la Fuerza realizado en la **Facultad de Ciencias de la Actividad Física y del Deporte (INEF)** de la **Universidad Politécnica de Madrid**, entre los días 16 y 17 de diciembre de 2022.

Madrid, 18 de diciembre de 2022

Pedro J. Benito Peinado
Presidente del Comité Organizador

Ana B. Peinado Lozano
Presidenta del Comité Científico

XII Congreso Internacional de la Asociación Española de Ciencias del Deporte

Certificado de Comunicación

El Comité Organizador certifica que la comunicación con título:

Efectos sobre la activación neuromuscular de una novedosa prenda deportiva con tecnología elastomérica

ha sido presentada en la categoría **ORAL** por los siguientes autores:

Angel Saez-Berlanga, Carlos Babiloni-López, Pablo Jiménez-Martínez, Alejandro Silvestre-Herrero, Javier Gene-Morales, Josep Romero-Sánchez, Luis Alvarenga, Fernando Martin, Juan C Colado,

en el **XII Congreso Internacional de la Asociación Española de Ciencias del Deporte**

celebrado en Madrid el 21, 22 y 23 de junio de 2023, con sede en la Universidad Autónoma de Madrid.

Madrid, 23 de junio de 2023

Dr. Ricardo de la Vega
Comité organizador

Dr. Víctor Cuadrado
Comité organizador

Dr. Tomás García
Presidente AECD

Asociación Española de Ciencias del Deporte

Universidad Autónoma de Madrid

XII Congreso Internacional
de la Asociación Española
de Ciencias del Deporte

Certificado Mesa de Expertos

El Comité Organizador certifica que

D. Pablo Jiménez

ha participado en la Mesa de Expertos en Nuevas Tecnologías y E-Sports titulada:

Avances en fisiología y biomecánica aplicados a la ganancia de masa muscular

en el **XII Congreso Internacional de la Asociación Española de Ciencias del Deporte**

celebrado en Madrid el 21, 22 y 23 de junio de 2023, con sede en la Universidad Autónoma de Madrid.

Madrid, 23 de junio de 2023

Dr. Ricardo de la Vega
Comité organizador

Dr. Víctor Cuadrado
Comité organizador

Dr. Tomás García
Presidente AECD

Universidad Autónoma de Madrid

XII Congreso Internacional de la Asociación Española de Ciencias del Deporte

Certificado de Comunicación

El Comité Organizador certifica que

Pablo Jiménez Martínez

ha presentado la **PONENCIA** titulada:

Beneficios de los programas de entrenamiento concéntricos o excéntricos en la tendinopatía del manguito de los rotadores en comparación a la terapéutica tradicional

en el **XII Congreso Internacional de la Asociación Española de Ciencias del Deporte**

celebrado en Madrid el 21, 22 y 23 de junio de 2023, con sede en el Universidad Autónoma de Madrid.

Madrid, 23 de junio de 2023

Dr. Ricardo de la Vega
Comité organizador

Dr. Víctor Cuadrado
Comité organizador

Dr. Tomás García
Presidente AECD

Universidad Autónoma de Madrid

XII Congreso Internacional de la Asociación Española de Ciencias del Deporte

Certificado de Comunicación

El Comité Organizador certifica que la comunicación con título:

Beneficios de los programas de entrenamiento concéntricos o excéntricos en la tendinopatía del manguito de los rotadores en comparación a la terapéutica tradicional

ha sido presentada en la categoría **PÓSTER** por el siguiente autor:

Pablo Jiménez Martínez

en el **XII Congreso Internacional de la Asociación Española de Ciencias del Deporte**

celebrado en Madrid el 21, 22 y 23 de junio de 2023, con sede en el Universidad Autónoma de Madrid.

Madrid, 23 de junio de 2023

Dr. Ricardo de la Vega
Comité organizador

Dr. Víctor Cuadrado
Comité organizador

Dr. Tomás García
Presidente AECD

Asociación Española de Ciencias del Deporte

UAM Universidad Autónoma de Madrid

XII Congreso Internacional
de la Asociación Española
de Ciencias del Deporte

Certificado de Comunicación

El Comité Organizador certifica que la comunicación con título:

Repeticiones y velocidad media propulsiva en el press de hombro con prendas con tecnología elastomérica versus placebo

ha sido presentada en la categoría **ORAL** por los siguientes autores:

Javier Gene-Morales, Angel Saez-Berlanga, Carlos Babiloni-López, Pablo Jiménez-Martínez, Alejandro Silvestre-Herrero, Javier Martínez-Puente, Ana María Ferri-Caruana, Dani Gil-Fetes, Juan C Colado,

en el **XII Congreso Internacional de la Asociación Española de Ciencias del Deporte**

celebrado en Madrid el 21, 22 y 23 de junio de 2023, con sede en la Universidad Autónoma de Madrid.

Madrid, 23 de junio de 2023

Dr. Ricardo de la Vega
Comité organizador

Dr. Víctor Cuadrado
Comité organizador

Dr. Tomás García
Presidente AECD

Asociación Española de Ciencias del Deporte

UAM Universidad Autónoma de Madrid

Facultad de Ciencias del Deporte I Universidad de Granada, Granada I España

A
Universidad de Valencia

Departamento de Educación Física y Deportiva
Prof. Dr. Amador García Ramos

Ctra de Alfacar, S/N 18071 Granada
E-Mail: amagr@ugr.es

Granada, 25/01/2023

Presentación, análisis y debate de proyectos de investigación en curso, por parte del doctorando Pablo Jiménez Martínez

Con este certificado con validez académica se certifica que el doctorando Pablo Jiménez Martínez ha realizad presentaciones y análisis referentes a los proyectos de investigación desarrollados acorde a su tesis doctoral. Esta presentaciones han sido realizadas entre Diciembre de 2022 y Enero de 2023, con una duración total de 20 hora bajo la organización del foro de debate del grupo Análisis del movimiento humano (CTS-362).

Las presentaciones expuestas versan con los siguientes títulos:

- Efectos de la fenilcapsaicina sobre el rendimiento neuromuscular y metabólico en el entrenamiento de la fuerza.
- Influencia de los cambios estructurales entre nuevos capsaicinoides y capsinoides en comparación a sus homólogos tradicionales en el rendimiento físico deportivo.
-Efectos de la suplementación con fenilcapsaicina sobre la oxidación de ácidos grasos, el gasto energético, la respuesta metabólica y la temperatura corporal durante el ejercicio.

Prof. Dr. Amador García Ramos

Firma:

Amador García Ramos
Firmado digitalmente por Amador García Ramos
Fecha: 2023.01.25 18:22:26 +01'00'

8.3.4 Patent applications

patent varemerke design
Patentstyret

Pablo Jiménez Martinez
Calle [redacted]
Spania

Oslo, 2023.03.15

Application no: 20230263
Applicant: Axichem AB

Notification for the attention of the inventor

Considering your interests as an inventor the Norwegian Industrial Property Office will hereby notify you of being indicated as inventor for the following patent application.

Details concerning the application:

Application no: 20230263
Applicant: Axichem AB
Application date: 2023.03.13

The following inventor(s) is~~(are)~~ listed:

Torsten Helsing, Kleppevegen 39, 5300 KLEPPESTØ, Norge
Lucas Altepost, Odden 18, 1397 NESØYA, Norge
Carlos Alix Fages, Plaza Sandoval, 5, 2C, MURCIA, Spania
Pablo Jiménez Martinez, Calle Espoz y Mina 191º Derecha, ALBACETE, Spania

Sincerely,

Hild Danielsen
Telephone: +47 22 38 74 41

8.4 Research stays

The doctoral candidate has conducted two research stays that are presented hereunder:

FACULTAD DE CIENCIAS DEL DEPORTE
Universidad de Granada

UNIVERSIDAD DE GRANADA

Facultad de Ciencias del Deporte I Universidad de Granada, Granada I España

A
Universidad de Valencia

Departamento de Educación Física y Deportiva
Prof. Dr. Amador García Ramos

Ctra de Alfacar, S/N 18071 Granada
E-Mail: amagr@ugr.es

Granada, 31/01/23

Desarrollo de estancias y proyectos de investigación

Con este certificado con validez académica se certifica que el doctorando Pablo Jiménez Martínez ha realizado trabajos de investigación en relación a su tesis doctoral en la Universidad de Granada en las fechas compendiadas entre el 4 de Noviembre y el 29 de Noviembre de 2022 y el 9 de Enero y el 22 de Enero de 2023. La validez de esta actividad corresponde a 200 horas y ha sido dirigida por el Dr. Amador García Ramos.

Los trabajos desarrollados han incluido:

- Aprendizaje del uso de analizador de gases con el objetivo de determinar y cuantificar la oxidación de sustratos energéticos y el gasto energético durante el ejercicio.

-Desarrollo experimental del proyecto: "Efectos metabólicos de la suplementación con fenilcapsaicina en un test de máxima oxidación de ácidos grasos y en un test incremental: Un diseño aleatorizado, cruzado, triple ciego, comparado con placebo".

-Planteamiento y diseño de un nuevo protocolo experimental para determinar el impacto de la suplementación con ciclodextrina en el rendimiento neuromuscular en el entrenamiento de fuerza bajo el control de la velocidad.

Prof. Dr. Amador García Ramos

Firma:

Amador García Ramos
Firmado digitalmente por Amador García Ramos
Fecha: 2023.01.31 14:46:30 +01'00'

Friedrich-Alexander-Universität
Technische Fakultät

FAU Erlangen-Nürnberg I Henkestr. 91, 91052 Erlangen

To
University of Valencia

Department Artificial Intelligence in Biomedical Engineering

Professur für Neuromuscular Physiology and Neural Interfacing

Prof. Dr. Alessandro Del Vecchio

Henkestr. 91, 91052 Erlangen
Telefon: +49 9131 85-70940
E-Mail: alessandro.del.vecchio@fau.de
www.techfak.fau.de

Erlangen, den 03.07.2023

Stay at Friedrich-Alexander-University Nürnberg-Erlangen for researching purpose of PhD student Pablo Jiménez Martínez. Verification document:

The purpose of this document is to verify the stay conducted by the doctoral student Pablo Jiménez Martínez. The rationale of this stay was to acquire some corticospinal signals as well as dynamic and isometric forces with the use of quattrocento devices from the tibialis anterior and quadriceps muscles. For this reason, the doctoral student has successfully learned to use high-density electromyography with the quattrocento software and hardware. This stay was conducted between the **20/03/23** and **03/07/23**

Of note, research about the impact of mental fatigue on connectivity signals and motor units' behavior during a fatiguing physical exercise was also performed. Furthermore, the doctoral student was imbedded in an international project in which the effects of different joint angles in the knee and the ankle were evaluated on different neurophysiological outcomes, such as the recruitment threshold and the discharge rate.

As a result of this stay, the doctoral student has participated in the following scientific items:
Articles pending to be published from the conducted research during the stay:

- "Resting time influences the rate of force development and maximal isometric force in young subjects: a randomized trial".
- "Effects of different contraction configurations on electrophysiological outcomes in young active subjects".
- "Mental fatigue impairs physical performance but not the neural drive to the muscle"
- "Foot and hip positions influence the neural drive to the quadriceps muscles in Olympic athletes".

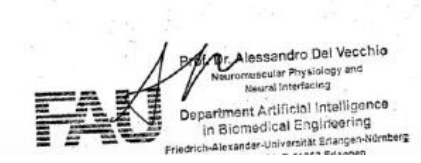
Poster and oral presentations:

- "Effects of mental fatigue on motor units' behavior during a fatiguing task". XII Congreso Internacional de la Asociación Española de Ciencias del Deporte, Madrid (UAM) 2023.

- Tutor in Spain: Dr. Juan Carlos Colado Sánchez
- Tutor in Germany and lab-head: Prof. Dr. Alessandro Del Vecchio

Yours sincerely,

Prof. Dr. Alessandro Del Vecchio



8.5 Funding

The doctoral candidate has obtained private financial support, as well as, he has won a grant to conduct his international research stay:

The studies one, two and three (chapters 2, 3 and 4) of this doctoral thesis have been supported with research material provided by Axichem AB (Malmö, Sweden). However, the company was not involved in study design, data collection, or data entry, and there were no restrictions on analysis, writing, or publication**.**

The travel grant won is presented hereunder:

VNIVERSITAT DE VALÈNCIA

Servei de Relacions Internacionals
Universitat de València
Palau de Cerveró
Pl. Cisneros, 4
46003 València
http://www.uv.es/relint

CARLOS POMER MONFERRER,

Jefe del Servicio de Relaciones Internacionales y Cooperación

INFORMA

que **PABLO JIMENEZ MARTINEZ** , estudiante del Programa de Doctorado 3172 - Programa de Doctorado en Actividad Física y Deporte y con Documento Nacional de Identidad [redacted] ha resultado beneficiario/a de una beca de movilidad internacional para estudiantes de doctorado de la Universitat de València para el año 2023 .

Esta ayuda ha facilitado una estancia de investigación realizada en Friedrich-Alexander-Universität Erlangen-Nürnberg (Alemania).

Valencia, 04/07/2023

8.6 Ethics committee approval

Junta de Andalucía
Consejería de Salud y Familias

Informe Dictamen Favorable
Proyecto Investigación Biomédica
C.P. phenyilcapsaicin and resistance training - C.I. 0513-N-22
16 de mayo de 2022

CEI de los Hospitales Universitarios Virgen Macarena y Virgen del Rocío

D. Carlos García Pérez
Secretario del CEI de los Hospitales Universitarios Virgen Macarena y Virgen del Rocío

CERTIFICA

Que el CEI de los Hospitales Universitarios Virgen Macarena y Virgen del Rocío en su reunión del día 28/04/2022, acta CEI_04/2022 ha evaluado la propuesta del promotor referida al estudio:

Título: Efectos de la suplementación con Fenilcapsaicina sobre el rendimiento neuromuscular y metabólico en sentadilla: Un estudio controlado aleatorizado

Código Promotor: phenyilcapsaicin and resistance training **Código Interno:** 0513-N-22
Promotor: Investigador
Monitor/CRO: Investigador
Versión Protocolo Evaluada: v.2-29/03/2022
Versión Hoja Información al Paciente Evaluada: HIP/CI / v.2-29/03/2022

1º. Considera que

- El estudio se plantea siguiendo los requisitos de la Ley 14/2007, de 3 de julio, de Investigación Biomédica y su realización es pertinente.
- Se cumplen los requisitos necesarios de idoneidad del protocolo en relación con los objetivos del estudio y están justificados los riesgos y molestias previsibles para el sujeto.
- Son adecuados tanto el procedimiento para obtener el consentimiento informado como la compensación prevista para los sujetos por daños que pudieran derivarse de su participación en el estudio.
- El alcance de las compensaciones económicas previstas no interfiere con el respeto a los postulados éticos.
- La capacidad de los Investigadores y los medios disponibles son apropiados para llevar a cabo el estudio.

2º. Por lo que este CEI emite un **DICTAMEN FAVORABLE.**

UCAM
UNIVERSIDAD CATÓLICA SAN ANTONIO

COMITÉ DE ÉTICA DE LA UCAM

DATOS DEL PROYECTO

Título:	"Hábitos nutricionales y de entrenamiento, y variables antropométricas de culturistas naturales a nivel nacional"	
Investigador Principal	**Nombre**	**Correo-e**
Dr.	Carlos Javier Contreras Fernández	cjcontreras@ucam.edu

INFORME DEL COMITÉ

Fecha	**27/01/2022**	**Código**	**CE012209**

Tipo de Experimentación

Investigación experimental clínica con seres humanos	
Investigación experimental no clínica con seres humanos	
Utilización de tejidos humanos procedentes de pacientes, personas sanas, tejidos embrionarios o fetales	
Utilización de tejidos humanos, tejidos embrionarios o fetales procedentes de bancos de muestras o tejidos	
Investigación observacional, psicológica o comportamental en humanos	X
Uso de datos personales, información genética, etc.	X
Experimentación animal	
Utilización de agentes biológicos de riesgo para la salud humana, animal o las plantas	
Uso de organismos modificados genéticamente (OMGs)	

Comentarios Respecto al Tipo de Experimentación
Nada Obsta

Comentarios Respecto a la Metodología de Experimentación
Nada Obsta

UCAM
UNIVERSIDAD CATÓLICA SAN ANTONIO

COMITÉ DE ÉTICA DE LA UCAM

Sugerencias al Investigador

A la vista de la solicitud de informe adjunto por el Investigador y de las recomendaciones anteriormente expuestas el dictamen del Comité es:

Emitir Informe Favorable	X
Emitir Informe Desfavorable	
Emitir Informe Favorable condicionado a Subsanación	

MOTIVACIÓN
Incrementará conocimientos en su área

Vº Bº El Presidente,

Fdo.: José Alberto Cánovas Sánchez

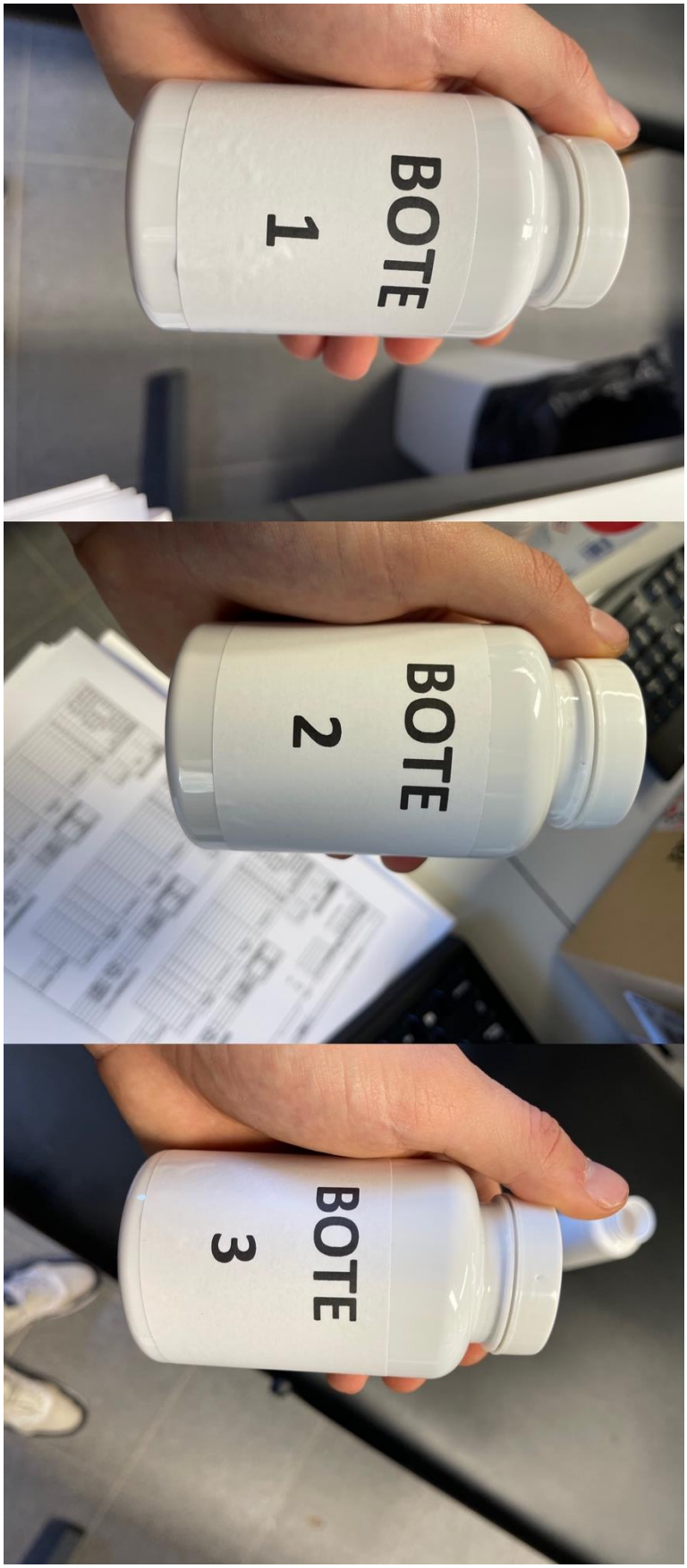
El Secretario,

Fdo.: José Alarcón Teruel

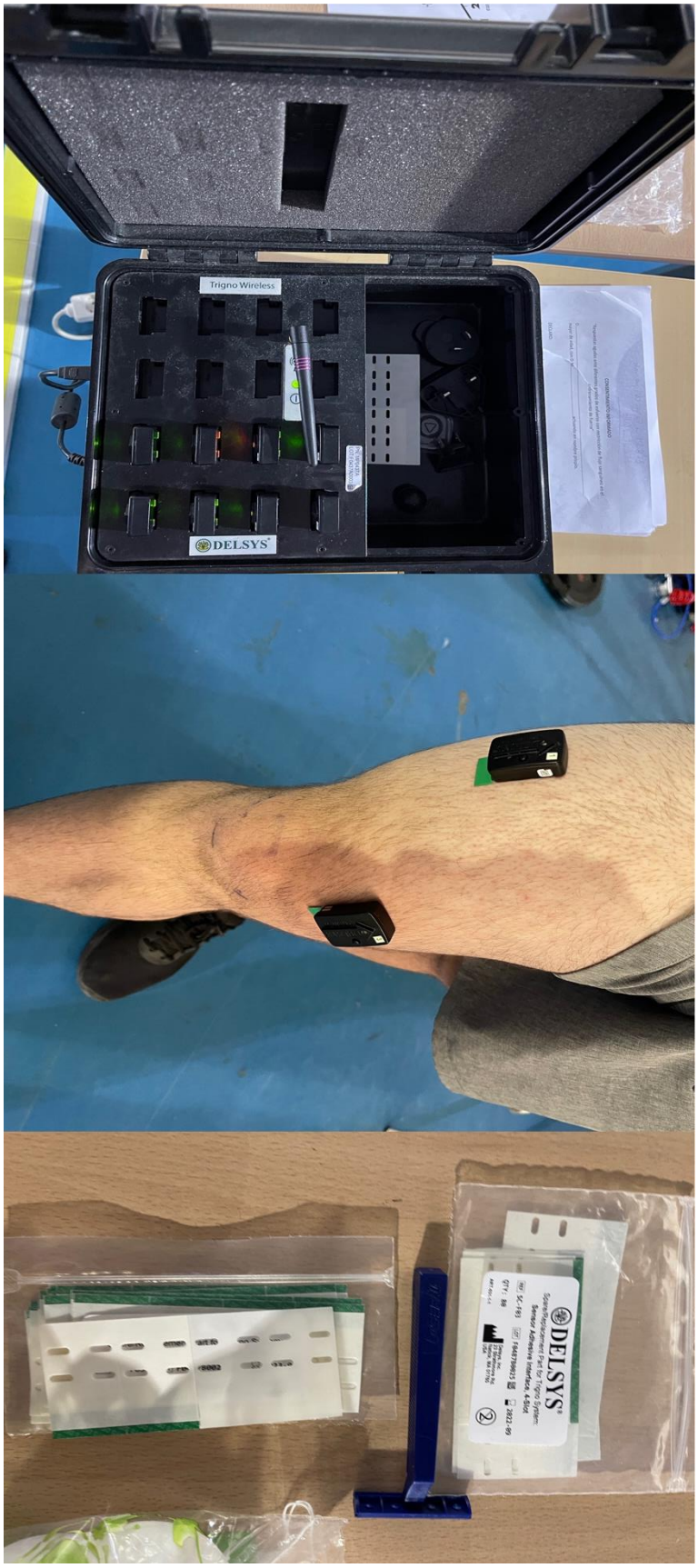
8.7 Tools and procedures

Overview of the laboratory and devices employed in the resistance training studies

Blinded packages of supplements used in all the experimental studies

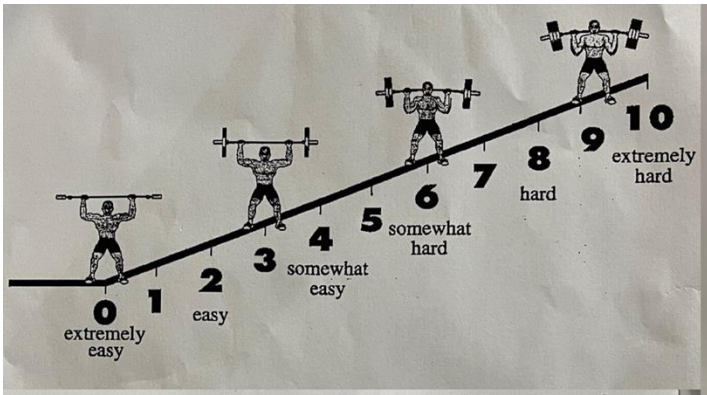


Electromyography equipment used during the first study

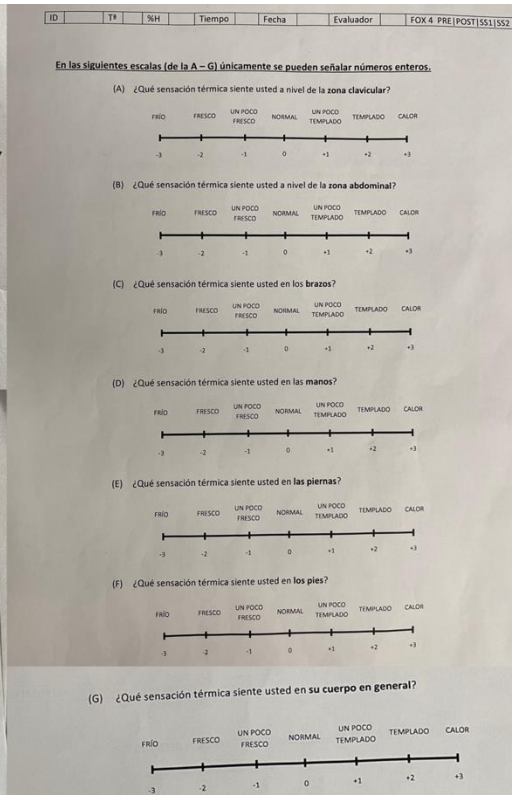


Equipment used in the assessment of blood biochemical variables during the second study

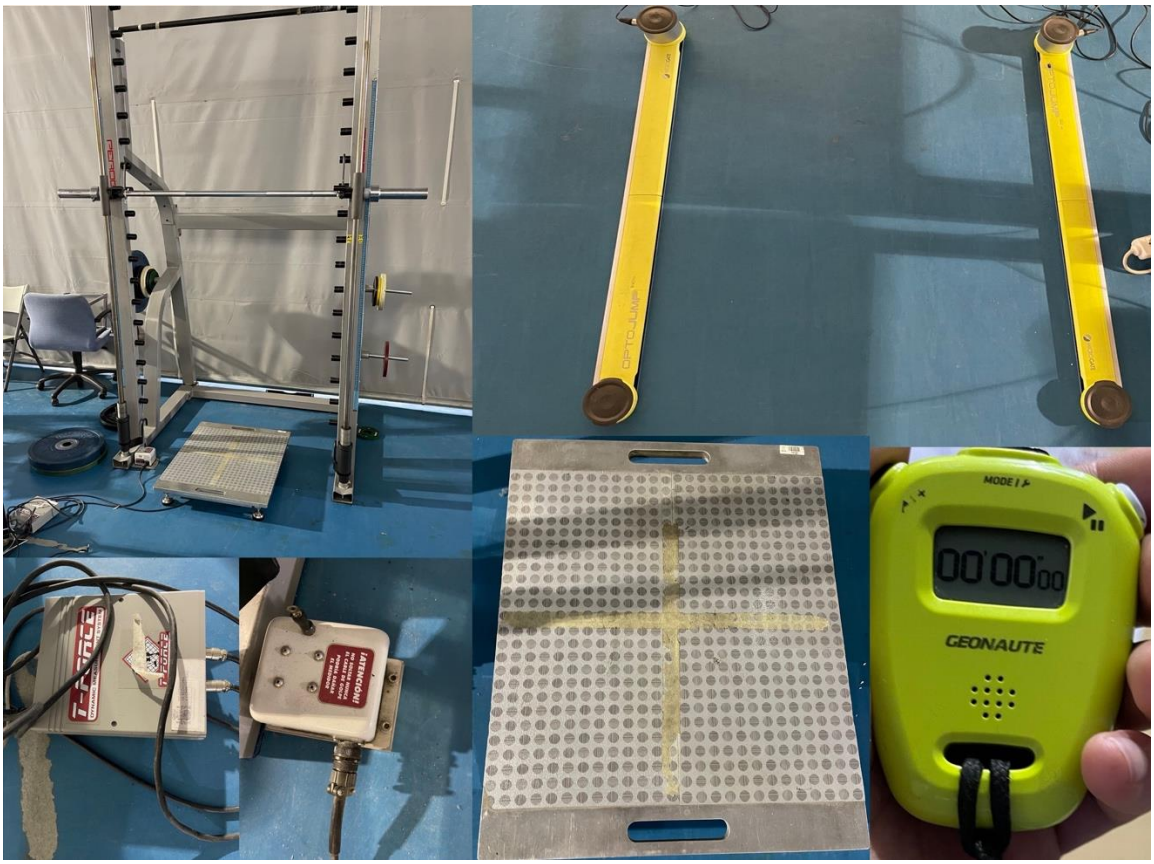
Perceived exertion and thermal scales used in the second and third studies



10 Very well recovered / Highly energetic
9
8 Well recovered / Somewhat energetic
7
6 Moderately recovered
5 Adequately recovered
4 Somewhat recovered
3
2 Not well recovered / Somewhat tired
1
0 Very poorly recovered / Extremely tired



Devices used to evaluate mechanical variables during the first and second studies



Procedures of capillary blood extraction and analysis

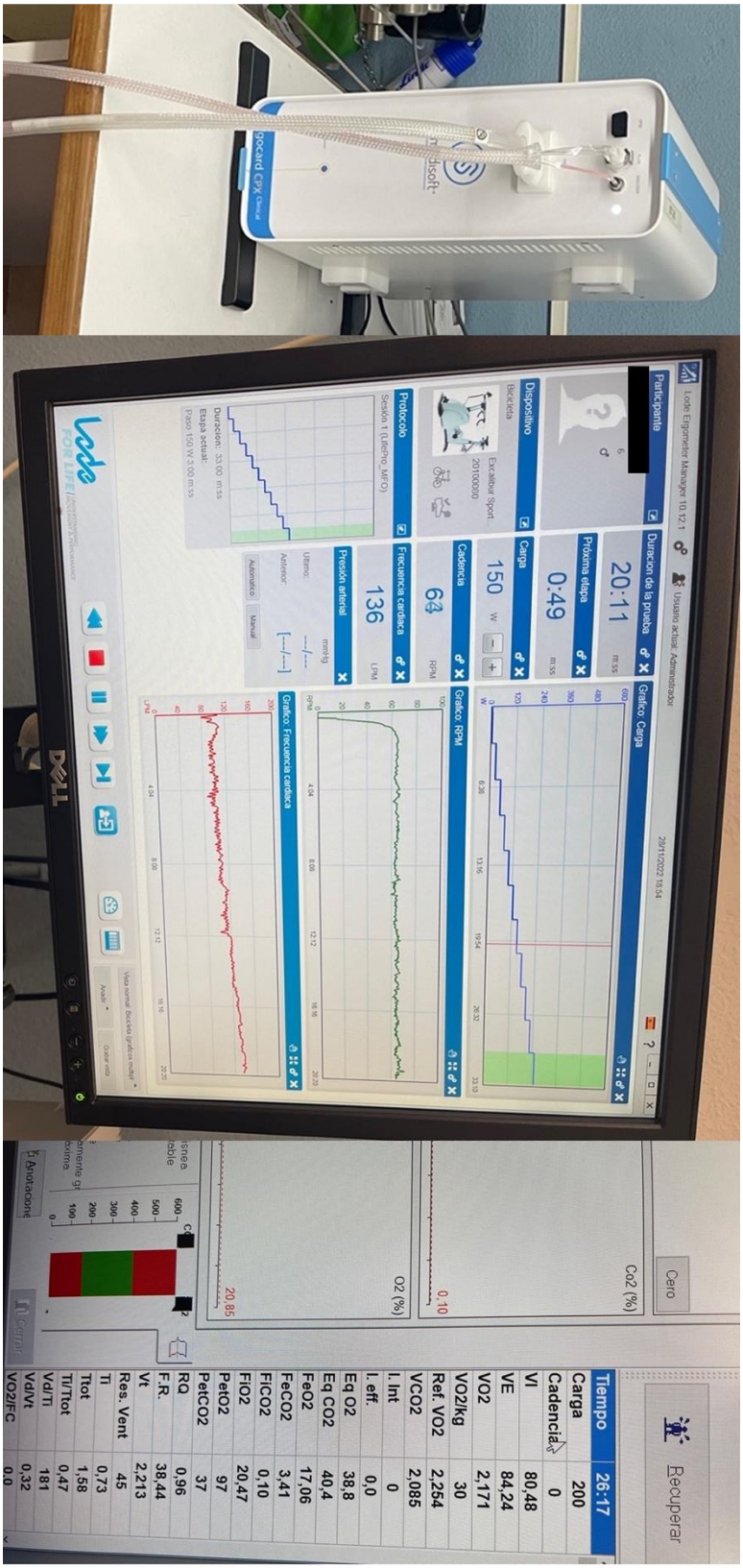
Room used to perform capillary blood extractions and electromyography preparation

Procedure of isometric squat test

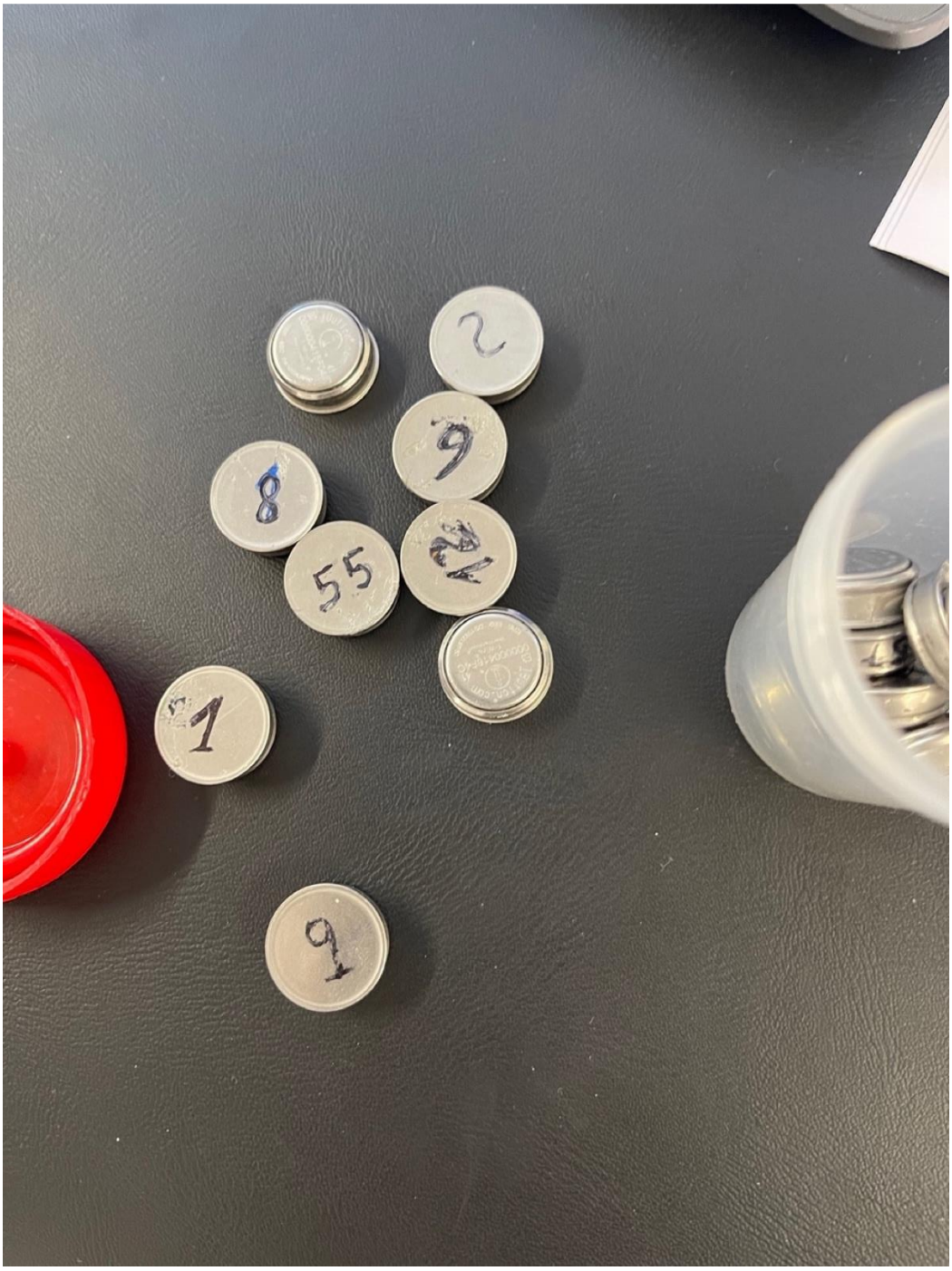
Procedure of dynamic squat test

Body composition assessment during the third study

Breath gas analysis equipment used in the third study



Skin thermometers employed in the third study



Procedures of the third study

Gas analyzer masks used in the third study

Supplements of protein and carbohydrate taken by the participants to standardize their last intake before fasting in the third study

Informed and biological samples consents provided to the participants in the experimental studies

CONSENTIMIENTO INFORMADO DEL PARTICIPANTE

(Los artículos se refieren a la Ley de Investigación Biomédica, BOE 4 de julio de 2007)

D. / Dña. _____ con D.N.I. _____, y

fecha de nacimiento _____

Declaro que:

1. He leído (o me han leído) la hoja de información del proyecto: **Efectos de la suplementación con fenilcapsaicina sobre el rendimiento neuromuscular y metabólico en sentadilla: Un estudio controlado aleatorizado.** Que me ha entregado el investigador principal, el Dr.

2. He comprendido la investigación que se va a realizar con mi participación y he tenido la oportunidad de resolver cualquier duda al respecto.

3. Así mismo, se me ha informado de que:

- Se hace constar que el participante manifiesta expresamente decir la verdad en sus respuestas para garantizar los datos reales sobre su estado físico o salud o los que se le solicitan (art. 23.1. L.I.B).
- Tengo derecho a no otorgar mi consentimiento a participar y a revocarlo en cualquier momento del estudio (art. 4.3. L.I.B.)
- La falta de consentimiento a iniciar el estudio o su revocación una vez iniciado no me supondrá perjuicio alguno (en cualquier otro derecho) o discriminación (art. 4.4 y 6. L.I.B.).
- Seré informado, si así lo deseo, de los datos que se obtengan durante la investigación (art. 4.5 y 27.2. L.I.B.) y de la forma de obtener dicha información (art. 15.2. L.I.B.).
- Tengo derecho a decidir que no se me comuniquen los datos de la investigación (con las excepciones legales pertinentes) (art. 4.5. L.I.B.).
- Los datos que se obtengan con mi participación en la investigación son anónimos y si al publicarlos hay que mencionar mi nombre será precisa mi autorización (art. 15.2. L.I.B).

4. Acepto participar voluntariamente en el proyecto antes mencionado, con lo que doy autorización a que se me realice lo siguiente:

- Evaluación de variables objetivas del rendimiento de fuerza muscular a través de sesiones de prueba supervisadas.
- Evaluación de la máxima contracción voluntaria mediante electromiografía.
- Evaluación de la respuesta metabólica mediante tiras reactivas de lactato, aspartato aminotransferasa y urea tomadas mediante punción en el pulpejo del dedo índice.
- Estandarización de la dieta 24 horas previas a la realización de cada sesión.
- Administración de placebo, y dos dosis diferentes de fenilcapsaicina, de forma aguda y aleatorizada, antes del inicio de cada sesión.

He comprendido que mi participación no tiene ninguna contraprestación económica.

- Autorizo la utilización de imágenes con fines docentes y científicos con absoluto respeto a mi intimidad.

Firma del participante **Fecha y lugar**

Fdo-. _____

Firma del investigador

REVOCACIÓN (la firma anula directamente su participación)

Yo, D. / Dña. _____ con D.N.I. _____, revoco el consentimiento prestado en fecha _____,y no deseo proseguir participando en el estudio: **Efectos de la suplementación con fenilcapsaicina sobre el rendimiento neuromuscular y metabólico en sentadilla: Un estudio controlado aleatorizado,** el cual doy en esta fecha por finalizado.

Firma del participante **Fecha y lugar**

Fdo-. _____

Firma del investigador

CONSENTIMIENTO INFORMADO PARA LA UTILIZACIÓN DE MUESTRAS BIOLÓGICAS

IDENTIFICACIÓN Y DESCRIPCIÓN DEL PROCEDIMIENTO

Durante la intervención/prueba diagnóstica…**de extracción de sangre capilar mediante punción en el pulpejo del dedo**……a la que va a ser sometido se tomarán muestras sanguíneas. El procedimiento que se le propone consiste en donar voluntariamente sangre, sin que ello suponga ningún riesgo añadido para su salud. Dichas muestras podrán ser utilizadas para la investigación que lleva por título: "**Efectos de la suplementación con fenilcapsaicina sobre el rendimiento neuromuscular y metabólico en sentadilla: un estudio controlado aleatorizado**".

Que se realizará en el **Centro de Investigación en Rendimiento Físico y Deportivo de la Universidad Pablo de Olavide.**..... Las muestras que done se almacenarán con los requisitos adecuados para su posterior uso en investigación, cumpliendo los requerimientos establecidos en la normativa vigente. Sus muestras sólo podrán ser utilizadas en proyectos de investigación avalados científicamente y aprobados por un Comité de Ética para la Investigación. Todos los datos seguirán un tratamiento mediante un número idéntico al que se utilizará para identificar la muestra, que no guardará relación con el número de historia, nombre u otros datos que puedan identificarle. Tanto las muestras como los datos asociados a las mismas serán custodiados y en su caso cedidos a terceros con fines de investigación biomédica en los términos previstos en la Ley 14/2007, de 3 de julio, y en el Real Decreto 1716/2011, de 18 de noviembre.

OBJETIVO

El objetivo del estudio es:
"Analizar el impacto de la suplementación con fenilcapsaicina sobre parámetros de rendimiento neuromuscular como la máxima contracción voluntaria isómetrica o un test de fuerza-resistencia y las respuestas metabólicas al ejercicio mediante análisis de sangre capilar de urea, lactato y aspartato aminotransferasa".

GRATUIDAD POR LA PARTICIPACIÓN

Por la donación de las muestras biológicas, usted no percibirá ninguna compensación económica. La donación implica, la renuncia por parte de los donantes a cualquier derecho de naturaleza económica o de otro tipo sobre los resultados que pudieran derivarse de manera directa o indirecta de las investigaciones que se lleven a cabo con las muestras biológicas.

BENEFICIOS ESPERADOS

La investigación que se realizará utilizando las muestras que usted dona voluntariamente, podrían ayudar en el futuro a mejorar el conocimiento en el ámbito del rendimiento físico deportivo.

CONSECUENCIAS PREVISIBLES DE LA DONACIÓN

Sólo si usted lo desea, existe la posibilidad de que pueda ser contactado en el futuro para completar o actualizar la información de la que contamos en este momento y/o de tomar una nueva muestra que pudiera ser interesante en el desarrollo de la investigación biomédica, en cuyo caso volverá a ser informado/a de la situación y tendrá la libertad de participar o declinar dicha participación.

CONSENTIMIENTO INFORMADO PARA LA UTILIZACIÓN DE MUESTRAS BIOLÓGICAS

CONSECUENCIAS PREVISIBLES DE SU NO REALIZACIÓN Y DERECHO DE REVOCACIÓNDEL CONSENTIMIENTO

La decisión de donar sus muestras es totalmente voluntaria, pudiendo negarse a donarlas e incluso pudiendo revocar su consentimiento en cualquier momento, sin tener que dar ninguna explicación y sin que ello tenga ninguna repercusión en la atención médica que recibe. Si decidiera revocar el consentimiento que ahora presta, la parte de las muestras que no se hay utilizado en la investigación, será destruida. Tales efectos, no se extenderán a los datos resultantes de las investigaciones que ya se hayan llevado a cabo con anterioridad.

RIESGOS

El procedimiento que se le propone no supone ningún riesgo añadido para su salud ni compromete el correcto diagnóstico y tratamiento de ninguna enfermedad. No obstante, entre los posibles efectos adversos o riesgos que puede experimentar al realizar la extracción capilar se encuentra dolor, lesión nerviosa, cicatrización, sangrado abundante, infección, disminución de la presión sanguínea, mareo, fatiga.

PROTECCIÓN DE DATOS PERSONALES Y CONFIDENCIALIDAD

a) *Si no se requieren datos personales ni identificación de los sujetos donantes, es decir se tomaran las muestras pero no se identificaran con ningún código, número, etc que pueda permitir identificar al sujeto se debe de indicar.*

b) *Si se requieren datos personales que identifiquen a los donantes, indicar como se van a tratar, para ello consultar el siguiente documento disponible en la página web:*

"Protocolo para el cumplimiento de la normativa de protección de datos en la realización de Prácticas Externas y Trabajos Fin de Estudios".

Se han adoptado las medidas oportunas para garantizar la completa confidencialidad de los datos personales de los sujetos de experimentación que participen en este estudio, de acuerdo con la Ley De Protección de Datos de Carácter Personal (LOPD) 3/2018, de 5 de diciembre.
En el caso que se utilicen los resultados del estudio, con fines de docencia, investigación y/o publicación, se respetará siempre la debida anonimización de los datos de carácter personal, de modo que los sujetos de la investigación no resultarán identificados o identificables.

El titular de los datos personales podrá ejercitar los derechos de acceso, rectificación, cancelación y oposición al tratamiento de datos de carácter personal, y de revocación del consentimiento, en los términos previstos en la normativa aplicable. *(Si lo considera oportuno, puede detallar más esas medidas)*

INFORMACIÓN DE CONTACTO

Si usted tiene cualquier tipo de duda o petición deberá dirigirse al investigador responsable del estudio, Fernando Hipólito Pareja Blanco, en el Centro de Investigación en Rendimiento Físico Deportivo, Ctra. de Utrera, km. 1, 41013, Sevilla o bien con el investigador Pablo Jiménez Martínez con los siguientes datos de contacto:
Tlfno.:671164810. Correo electrónico: pjimmar2@upo.es

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CONSENTIMIENTO INFORMADO PARA LA UTILIZACIÓN DE MUESTRAS BIOLÓGICAS

EJEMPLAR PARA EL PARTICIPANTE

Si la persona es mayor de edad:

D./Dña._____mayor de edad, titular del DNI:_____, por el presente documento manifiesto los siguientes consentimientos:

DECLARO

- Que he leído la hoja de información que se me ha entregado.
- Que he comprendido las explicaciones que se me han facilitado.
- Que he podido realizar observaciones y me han sido aclaradas las dudas que he planteado.
- Que puedo revocar el consentimiento en cualquier momento sin tener que darexplicaciones y sin que esto tenga ninguna repercusión negativa.
- Que de forma libre y voluntaria cedo las muestras biológicas que se me han propuesto ylos datos de información que sean necesarios para el estudio
- Que puedo incluir restricciones sobre el uso de las mismas.

CONSIENTO

Que se utilicen las muestras biológicas obtenidas y los datos que se hallan recopilados en mihistoria clínica para el mencionado estudio.

Que el investigador pueda acceder a mis datos en la medida en que sea necesario y manteniendo siempre su confidencialidad.

Que el personal del centro me contacte en el futuro en caso de que se estime oportuno añadirnuevos datos a los recogidos y/o tomar nuevas muestras. ☐ Sí ☐ No

☐ Deseo incluir la siguiente restricción al uso de mis datos:

CONSENTIMIENTO INFORMADO PARA LA UTILIZACIÓN DE MUESTRAS BIOLÓGICAS

Declaración Investigador:

He informado debidamente al representante legal y/o y al paciente arriba

mencionadoFdo.: ..... DNI

.....

En ..... a ..... de.....de 20…

REVOCACIÓN

Fdo.: D./Dña .....
Revoco el consentimiento cedido para la utilización de los datos de mi hijo/a para el estudiopropuesto

En ..... a..... de.....de 20…