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Exercise-induced hypoalgesia in healthy males is best explained by mechanisms selectively affecting deep-tissue pain sensitivity within exercising body parts

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ABSTRACT

Background: The mechanisms driving the transient reduction in pain sensitivity following a single bout of physical exercise—a phenomenon known as exercise-induced hypoalgesia (EIH)—remain unclear despite proposed contributions from central and peripheral processes. So far it is unknown whether EIH selectively modulates the perception of specific types of nociceptive stimuli and whether this modulation is confined to exercising limbs or generalized to the entire body.

Methods: In 38 healthy males (18–30 years) the effects of a single session of moderate/high-intensity cycling exercise (25 min at 75% of the heart rate reserve) were assessed against a control condition (25 min of light cycling exercise) on the sensitivity to stimuli preferentially activating skin mechano- and heat-sensitive nociceptors versus deep-tissue nociceptors, applied to the exercising lower limbs versus the non-exercising upper limbs. Pressure pain thresholds (PPT), heat pain thresholds (HPT), cold detection thresholds (CDT), and mechanical pinprick ratings (PP), as well as auditory detection thresholds (ADT) serving as control, were recorded before and immediately after the exercise and control conditions.

Results: Linear mixed models revealed that exercise increased the PPT selectively at the exercising limb. No significant effects of exercise were found for other types of stimuli, except for a reduction of CDT at the exercising limb, which was not significant when controlling for skin temperature.

Conclusions: These findings suggest that processes preferentially modulating deep-tissue nociceptor sensitivity within exercising muscles contribute to EIH in young healthy males.

Introduction

Regular exercise reduces pain in individuals with chronic pain (Geneen et al., 2017). In healthy participants, a single bout of exercise can transiently decrease experimentally induced pain for up to 45 min (Tomschi et al., 2022), a phenomenon termed exercise-induced hypoalgesia (EIH) (Vaegter and Jones, 2020). The mechanisms underlying EIH remain poorly understood. Preclinical studies, conducted predominantly in male rodents, have suggested a role for endogenous opioids, yet human studies have reported inconsistent effects of the opioid antagonist naloxone on EIH (Droste et al., 1991; Janal et al., 1985; Koltyn, 2000; Koltyn et al., 2014). In addition to opioidergic pathways, contributions from adrenergic and endocannabinoid systems have also been proposed (Da Silva and Galdino, 2018). Some studies reported an association between EIH and conditioned pain modulation (CPM) (Ellingson et al., 2014; Yang et al., 2024), a phenomenon in which application of a painful conditioning stimulus at one body site attenuates pain perception at remote sites, thought to reflect the activation of descending inhibitory pathways (Nahman-Averbuch et al., 2024). However, the extent to which descending inhibitory pathways may contribute to EIH is unclear (Samuelly-Leichtag et al., 2018; Vaegter et al., 2013).

EIH is typically assessed as a reduction in sensitivity to painful stimuli applied after exercise. Importantly, the magnitude of EIH appears to vary according to the modality of the painful test stimulus, and on whether the stimulus is applied to an exercising vs. a non-exercising

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limb. Aerobic exercise consistently reduces pain induced by blunt pressure (Tomschi et al., 2024b) – a stimulus that predominantly activates deep-tissue nociceptors including muscle nociceptors (Graven-Nielsen et al., 2004) – typically characterized by an increase of the pressure pain threshold (PPT). In contrast, changes in sensitivity to stimuli probing cutaneous nociception, such as mechanical pinprick stimulation (PP) or cutaneous heat stimulation (e.g., heat pain threshold (HPT)), are more variable and potentially smaller than those observed with blunt pressure (Jones et al., 2019; Kortenjann et al., 2020; Schmitt et al., 2020). Furthermore, EIH may be more pronounced at body sites directly involved in the exercise as compared to remote, non-exercising sites (Vaegter and Jones, 2020). Taken together, these observations suggest that EIH may arise from processes preferentially modulating deep-tissue sensitivity within exercising-muscles.

However, evidence for EIH differences as a function of testing site and testing modality is largely drawn from comparisons across separate studies, making it difficult to determine whether observed patterns reflect a genuine feature of EIH or methodological heterogeneity. Even when considering only EIH studies using aerobic exercise, substantial variability persists across studies in factors potentially modulating EIH, including exercise intensity (Tomschi et al., 2024b), comparison to a control condition (Wewege and Jones, 2021), and participant characteristics such as age (Naugle et al., 2016a) and biological sex (Tomschi et al., 2024a; Vaegter et al., 2013, 2015).

In addition, stimuli recruiting superficial skin nociceptors (e.g., HPT, PP) have most often been delivered at non-exercising limbs, which may bias comparisons with blunt pressure measures delivered over exercising muscles (e.g., (Kortenjann et al., 2020; Ruble et al., 2005)). Moreover, the actual involvement of the tested body site in the exercise task has rarely been considered, which may confound the interpretation that local vs. remote differences in EIH reflect a comparison between exercising and non-exercising limbs. For example, cycling exercise can recruit upper-limbs muscles (Turpin et al., 2017), which are typically treated as remote testing sites (Tomschi et al., 2024b). Finally, it remains unclear whether exercise selectively modulates nociception or induces a more general sensory modulation, as findings from studies including non-nociceptive stimuli are inconsistent (Paalasmaa et al., 1991; Ruble et al., 2005).

Data directly comparing EIH using a variety of sensory stimuli, including non-nociceptive stimuli, across exercising and non-exercising testing sites remain scarce in the context of aerobic exercise. Such comparisons could nonetheless help constrain the range of possible mechanisms driving EIH, and guide future mechanistic studies. Accordingly, this study aimed to assess, within-subjects, the changes in sensitivity to stimuli preferentially activating mechano- vs. heat-sensitive nociceptors innervating the skin vs. deep tissues following a single session of cycling exercise compared to a light pedaling control condition, at exercising (lower-limb) vs. non-exercising (immobilized upper-limb) body sites. Changes in innocuous cold detection thresholds (CDT) and auditory detection thresholds (ADT) – respectively innocuous spinothalamic and non-somatosensory modalities – were also examined.

Given the heterogeneity of existing EIH findings and methods, we prioritized internal validity over generalizability and restricted the sample to healthy young males. We hypothesized (1) that EIH would be greater after exercise than after the control condition, supporting an effect of exercise per se rather than contextual or movement-related factors, (2) that exercise would preferentially modulate the sensitivity to nociceptive relative to non-nociceptive stimuli if EIH results from a selective modulation of nociception, (3) that EIH would be greater at exercising vs. non-exercising sites if spatially selective processes such as a peripheral modulation of nociceptor sensitivity contribute to EIH, and (4) that exercise would preferentially affect sensitivity to blunt pressure compared with thermal or mechanical skin stimulation if EIH involves a specific modulation of nociceptive signals originating from deep-tissue nociceptors.

Methods

Study design

In this within-subject cross-over study, participants completed a cycling exercise condition and a light pedaling control condition in a counterbalanced order. The experimental protocol was preregistered on ClinicalTrials.gov (NCT05961449). All experimental procedures were conducted in accordance with the *Declaration of Helsinki*, and ethical approval was obtained from the local ethics committee (Hospital and Departmental Ethics Committee, UCLouvain, Belgium; Approval No. 2023/06MAR/112). Participants were enrolled between April and September 2023.

Participants

Forty healthy male participants – aged 18 to 30 years, with a body mass index (BMI) between 17 and 30 kg/m^2, and literate and fluent in French or English – were recruited by word of mouth, advertisement notes, and social media.

In this study, sex refers to biological sex and was assessed using sex assigned at birth (Finn et al., 2026). Gender identity, defined as the "internal sense of [one's] own gender" (Finn et al., 2026), was not recorded, as no hypotheses were made regarding links between gender identity and EIH.

The decision to recruit only male participants, which limits the external validity of the findings, was motivated by two considerations. First, sex was treated as an important variable to control for, given known sex differences in exercise metabolism (Boisseau and Isacco, 2022), and pain perception and regulation (Mogil, 2020). Whether these differences affect comparisons of EIH across test modalities and sites is unknown, but an interaction between sex, modality, and site could not be ruled out. Second, neither a clear a priori sample size rationale nor sufficient resources were available to adequately power sex-disaggregated analyses. Data comparing EIH across modalities are scarce – only one study has directly compared EIH induced by pressure vs. heat in a mostly male sample (14 males, 2 females;(Jones et al., 2019)). The EIH literature on sex differences is controversial (Bement et al., 2014; Naugle et al., 2014a; Nold et al., 2025; Vaegter et al., 2013, 2015) and estimating the required sample size is further complicated by variability across studies in exercise parameters, testing modalities, and sex differences in pre-exercise pain sensitivity (Bement et al., 2014; Mogil, 2020; Vaegter and Jones, 2020). Including female participants would moreover have required accounting for menstrual cycle-related fluctuations in pain sensitivity and exercise metabolism (Iacovides et al., 2015; Oosthuyse and Bosch, 2010), and restricting testing to a specific cycle phase would have reduced representativeness of findings for females more broadly (Elliott-Sale et al., 2021).

Given these constraints, restricting the sample to healthy males was deemed a pragmatic compromise to prioritize internal validity, and an appropriate first step to guide both future mechanistic work and the planning of sex-disaggregated studies. The sample was further restricted to participants aged 18–30 years, as reduced EIH in older compared with younger adults has been reported (Naugle et al., 2016). The male-only design was made explicit throughout the manuscript in accordance with the Sex and Gender Equity in Research (SAGER) guidelines (Heidari et al., 2016).

Sample size was estimated a priori using PASS (NCSS software) with significance level (alpha) set at 0.05 and power at 80%. Based on prior studies measuring EIH at a remote site using the PPT (Gomolka et al., 2019; Jones et al., 2017; Vaegter et al., 2018), 27 participants were required to detect an effect size of 0.5 using a one-sided paired *t*-test (i.e., H1: PPT increase). To compare EIH induced by thermal vs. pressure stimuli, we assumed an effect size of 0.8 (Jones et al., 2019) and found that 15 participants were needed using a two-sided paired t-test. A total sample size of 40 participants was therefore selected.

Participants were excluded if they met any of the following criteria: tobacco use; inability to comply with the experimental instructions; presence of neurological, cardio-vascular, respiratory, inflammatory, endocrine, psychiatric, oncological, rheumatological, or musculoskeletal condition; any chronic pain condition or recent history thereof (within the preceding 2 years); wounds or skin alterations on testing sites; hearing impairment; surgery within the previous 12 months; any drug intake in the past two weeks preceding the experiment, including antibiotics, herbal medicines or other remedies, with the exception of oral paracetamol or ibuprofen taken up to 48 h before the experiment for a self-limiting condition (e.g., toothache, bruise). Any contraindication to physical activity also led to exclusion.

Participants received a financial compensation (25 euros per session, for a total of 75 euros).

Experimental protocol

Participation began with a 60-min *screening session* conducted between 08:00 and 13:00 at the UCLouvain Faculty of Movement and Rehabilitation Sciences. After obtaining written informed consent, eligibility was assessed, and a sports physician (sex: female; non-disclosed gender) screened for contraindications to physical activity. Eligible participants then completed a set of questionnaires and conducted an incremental maximal effort test on a cycle ergometer. Participants were asked to refrain from caffeine and alcohol intake and from physical activity 24 h prior to the *screening session* and to be in a fed state.

At least 72 h after the *screening session* (median = 8 days, range = 3–32 days), participants completed two 90-min counterbalanced *experimental sessions*. Each session included sensory testing and one of two cycling conditions (moderate-to-high intensity exercise or control). A cycling exercise was selected because it was shown to induce a consistent decrease in pressure pain sensitivity (Tomschi et al., 2024b). Experimental sessions were separated by at least 4 days (median = 7 days, range = 4–21 days) and took place at the Institute of neuroscience (IoNS) of UCLouvain between 08:00 and 13:00, with the time of day kept constant across sessions for each participant (median difference between sessions = 5 min; range = 0–21 min). Participants were offered three time slots to participate in these sessions: 08:00–09:30 (n = 9), 09:45–11:15 (n = 16), 11:30–13:00 (n = 13).

Each experimental session began with questionnaire completion, followed by measurements of blood pressure (BP), heart rate (HR), and breathing rate (BR) after 5 min of quiet rest. Participants were then familiarized with the sensory tests, explained using a standardized script, with practice stimuli applied to the dominant forearm. The pressure pain threshold (PPT), heat pain threshold (HPT), cold detection threshold (CDT), mechanical pinprick rating (PP) and auditory detection threshold (ADT) were subsequently assessed, in a randomized and counterbalanced order, at the non-dominant forearm and the dominant quadriceps, before and after the exercise or the control condition. Near the end of each condition, the rate of perceived exertion (RPE) was recorded. Immediately after the exercise or control condition, HR, BR, and BP were assessed. To ensure that the non-dominant forearm served as a non-exercising testing site, it was immobilized throughout the exercise and control conditions using a shoulder sling, while participants held the handlebar with their dominant hand to maintain stability during cycling.

Room temperature and humidity, as well as skin temperature before and after sensory assessments, were recorded. Accounting for the time required for the HR, BR, BP, and skin temperature measurements, sensory assessments started approximately 3 min after the exercise or control condition. The participants were asked to refrain from caffeine or alcohol intake, high volume music exposure, and moderate to intense physical activity 24 h prior to the experimental sessions, and to have completed an overnight fast. Adherence to the experimental instructions in all sessions was assessed with a standardized checklist. The participants were blinded to the research hypotheses. All sessions were led by a single experimenter (sex: male; gender identity: man). The experimenter was not blinded to the interventions since the changes in skin color, presence of sweat and change in cardiovascular variables would have made the delivered intervention obvious. An outline of the experimental procedures is provided in Fig. 1.

Questionnaires

During the *screening session*, participants completed the French or English versions of the following questionnaires: a custom-made demographics questionnaire (age, weight, height, level of education (i.e., highest official academic degree obtained), type of sports and volume of sports participation); the second part of the State and Trait Anxiety Questionnaire (STAI-Y2; 20 items scored 1–4 with a maximum score of 80) to assess trait anxiety (Spielberger, 1983); the Pain Catastrophizing Scale (PCS; 13 items scored 0–4 with a maximum score of 52) to assess catastrophic thinking in relation to pain experience (Sullivan et al., 1995); the Tampa scale for Kinesiophobia (TSK; 17 items scored 1–4 with a maximum score of 68) to assess fear of movement in relation to pain (Lundberg et al., 2011); the long version of the International Physical Activity Questionnaire (IPAQ; 27 items assessing duration and frequency of physical activity across four domains: work, transport, chores, leisure) to estimate the habitual degree of physical activity in metabolic equivalent (MET)/min/week (Hagströmer et al., 2006); and the Flinders Handedness survey (FLANDERS; 10 items measuring hand preference across a range of daily tasks) to determine the participant's laterality (Nicholls et al., 2013).

Prior to each *experimental session*, the participants were invited to complete: the Stanford Sleepiness Scale (SSS), a single-item 7 points Likert scale ranging from 1 (“Feeling active, alert, wide awake”) to 7 (“No longer struggling to sleep, sleep onset early; having dream-like thoughts”), to measure the current level of self-reported sleepiness (Maclean et al., 1992); and the first part of the State and Trait Anxiety Questionnaire (STAI-Y1), which includes 20 items scored 1 to 4 with a maximum score of 80, to assess state anxiety (Spielberger, 1983).

Maximal incremental effort test

Participants were tested on a cycle ergometer (standard concentric, Cyclus 2, Leipzig, Germany). Starting with an initial load of 70 W incremented by 30 W every 2 min they pedaled at 70 revolution per min (RPM) until volitional exertion. The maximal HR (HR_{max}), peak power output (PPO), and relative PPO (PPO/weight (kg)) were determined. To standardize the procedure, no verbal encouragement was provided (Midgley et al., 2018). HR was continuously monitored with a heart-rate monitor (Polar H10, Polar Electro Oy, Kempele, Finland). Blood lactate from the earlobe (Lactate Pro 2; Arkray, Kyoto, Japan) was collected at the beginning and at the end of the test. A value >6 mmol/l was used as secondary criterion for maximal effort, and the test was rescheduled if this criterion was not reached (n = 0).

Exercise and control interventions

The exercise intervention consisted in 25 min of cycling between 60 and 80 RPM on a cycle ergometer (CST BX40; Cardiostrong; Schleswig, Germany). Resistance was increased progressively until participants reached 75% of the HR reserve (HRR) (mean ± SD time: 4.78 ± 0.66 min) and this intensity was kept for the remaining time. HRR was computed with the Karvonen formula (Karvonen and Vuorimaa, 1988): 0.75 * (HR_{max} - resting HR) + resting HR, using the HR_{max} reached during the maximal incremental effort test. HR and BR were continuously monitored and assessed together with BP before and after exercise (B20 Patient Monitor; GE Healthcare; Chicago, USA). The rate of perceived exertion (RPE) was scored by participants on a Borg RPE 6–20 scale (Chen et al., 2002) near the end of the exercise. The control condition consisted in a similar exercise design except that the resistance

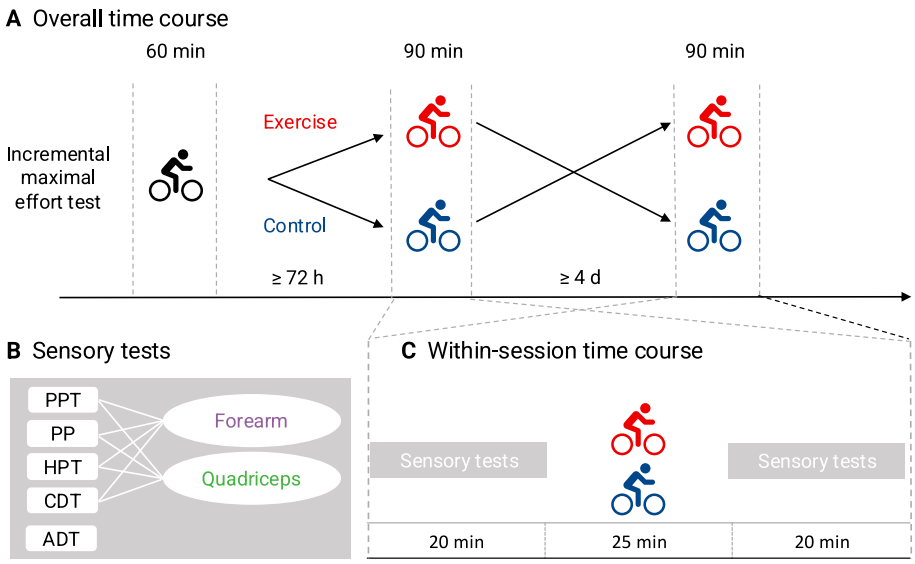

Fig. 1. Study procedures. (A) After a screening/maximal effort test session, participants completed, in a randomized and counter-balanced order, two experimental sessions including an exercise or a control condition. (B) Pressure pain thresholds (PPT), heat pain thresholds (HPT), mechanical pinprick ratings (PP), cold detection thresholds (CDT) were assessed at the quadriceps muscle (exercising limb) and at the volar forearm (non-exercising limb). The auditory detection threshold (ADT) was also recorded. (C) All sensory tests were delivered, in a randomized and counter-balanced order before and immediately after the exercise or the control condition. Abbreviations: min, minutes; h, hours; d, days.

was kept at 25 W and the RPM below 50. The intensity of the exercise was minimized by ensuring HR increase <25% from the resting measurement. The use of a control condition to study EIH has been recommended (Vaegter and Jones, 2020). As previous studies have reported contradictory findings for the impact of low intensity aerobic exercise (i.e., 30% HRR/$VO2_{max}$) on pain (Micalos and Arendt-Nielsen, 2016; Niwa et al., 2022), we opted for a lightly active control condition as per (Jones et al., 2019). Higher forearm muscle activity is associated with increased lower limb power output during cycling (Turpin et al., 2017) and isometric exercise may induce EIH (Vaegter and Jones, 2020). Thus, during both the exercise and the control conditions, physical use of the non-dominant arm was restricted with a shoulder sling to inhibit activity of the non-dominant forearm muscles, preventing any confounding effect on sensory testing.

Sensory tests

All sensory tests were conducted with the participants comfortably seated in a cushioned recliner chair with arm and leg support, in a slightly reclined position. Hip and knee angles were at approximately 80° and 45° of flexion, respectively. Using standardized instructions, each test stimulus was delivered three consecutive times. The order of test modalities in the sequence was randomized, counterbalanced across participants and kept constant within participants. Each modality appeared 8–11 times at each position in the sequence throughout the sample.

All sensory tests except the ADT were conducted alternately on the skin of the non-dominant volar forearm and dominant quadriceps. The site tested first was counterbalanced between participants and kept constant within participant. Before the familiarization procedure, all testing sites were marked with a small cross using a skin marker. On the volar forearm, the testing site was located 5 cm distal to the elbow crease along the forearm midline, overlying the flexor muscles of the wrist and fingers. On the quadriceps, it was located 20 cm proximal to the apex of the patella along the thigh midline, overlying the rectus femoris muscle. For PP, PPT, and CDT/HPT, the first stimulus was applied at the center of the cross, with the two subsequent applications shifted slightly (≤1 cm) from the initial mark, proximally or distally in no particular order, to prevent habituation/sensitization of the area (Nie et al., 2005; Van den Broeke et al., 2015).

The two testing sites were chosen based on pilot testing to ensure accurate stimulus delivery, including perpendicular force application, constant rate of force application for PPT, and full skin-thermode contact for HPT/CDT.

The immobilized non-dominant forearm was selected as the upper-limb, non-exercising, testing site. Similarly, only one lower limb was assessed to maintain consistency across testing sites. Completing all sensory tests at both sites took approximately 20 min. Because EIH may be transient (Vaegter and Jones, 2020), no additional testing sites were included.

Pressure pain threshold

Blunt pressure was used to preferentially activate mechano-sensitive deep-tissue nociceptors (Graven-Nielsen et al., 2004). The stimuli were delivered with a manual digital algometer with a contact surface area of 1 cm^2 (FPIX; Wagner Instruments, Greenwich, USA) connected to a laptop computer. A positive linear relationship between the rate of force application and PPT has been reported (Linde et al., 2018), which highlights the importance of ensuring consistent rate of force application across measurements. A Matlab (The Mathworks, USA, mathworks.com) script was used providing real-time visual feedback (force sampling rate = 10 Hz) on the force application rate, which was set at 50 kPa/s (Rolke et al., 2006). The script is accessible on OSF. A maximal force of 1500 kPa was allowed. The PPT was defined as the pressure at which participants reported pain by pressing on a keyboard key. Measurements were separated by a 30 s interval.

Heat pain threshold and cold detection threshold

Thermal stimuli were applied onto the skin to activate selectively heat-sensitive Aδ- and C-cutaneous fibre afferents (Rolke et al., 2006), at the forearm and quadriceps. The stimuli were produced by a 10 cm^2 rectangular contact thermode based on 10 Peltier elements, each having a surface of 7 × 28 mm (TCS – 2; QST.lab, Strasbourg, France). The temperature change rate was set at 1 °C/s starting from a skin temperature of 32 °C and limited to a range of 0–50 °C; an interval of 6 and 10 s was set between cold and heat stimuli, respectively (Rolke et al., 2006). The HPT and CDT were assessed according to standard operating procedures (Rolke et al., 2006) and defined as the temperature when

participants reported the stimulus as painful (HPT) or cool (CDT). We chose to assess the HPT over the cold pain threshold as the latter has been shown to be difficult to assess and prone to floor effects (Heldestad et al., 2010). CDT was chosen as an innocuous thermosoensory modality to assess the selectivity of EIH for pain. CDT was expressed as the difference in temperature relative to the baseline temperature (32 °C) and assessed systematically before HPT, as recommended (Rolke et al., 2006), since prior assessment of thermal pain thresholds may bias subsequent detection thresholds values (Heldestad et al., 2010).

Mechanical pinprick stimulation

A calibrated mechanical pinprick stimulator (512 mN) with a 0.25 mm diameter flat tip probe was used to preferentially activate mechanosensitive nociceptors of the skin (Rolke et al., 2006) (The PinPrick; MRC Systems GmbH, Heidelberg, Germany). The device consisted in a holding cylinder inside which a calibrated cylindrical weight and a flat tip needle mounted on a plastic rod moved freely. The stimulation was applied for 1 s, perpendicularly to the skin (Rolke et al., 2006). After each stimulation, the participants rated the intensity of the pinprick stimulation (PP) by marking a line with a pen on a visual analogue scale (VAS) anchored between "No perception" and "Maximal imaginable pain" with a visual landmark at the middle of the line denoting the transition from absence to presence of pain. A similar procedure has been used in past studies to assess changes in mechanical pinprick sensitivity (Van den Broeke and Mouraux, 2014). Stimuli were delivered three times with approximately 10 s interstimulus interval. Participants provided a VAS rating for each stimulus. The VAS were displayed on a single evaluation sheet so that the participants could take previous ratings into account. To standardize the direction of effects across all test modalities, the scale of PP was reversed for the analysis (i.e., 100 – individual value); hence '100' corresponds to an absence of perception.

Auditory detection threshold

As a non-somatosensory modality, auditory stimuli were delivered through a custom made Matlab script (The Mathworks, USA, mathworks.com; the script is accessible at OSF). Each auditory stimulus consisted of a 5%/s increase in amplitude (arbitrary unit) of a 50-Hz tone delivered using a pair of commercially available headphones. The ADT was defined as the % of maximal amplitude at which the participant reported hearing the sound by pressing a keyboard key and reported as arbitrary units comprised between 0 and 1. A 5 s interval was set between measurements.

Blood pressure, heart rate, breathing rate, and skin temperature measurements

HR, BR, and BP were measured with a monitoring unit (B20 Patient Monitor; GE Healthcare; Chicago, USA). Resting measures were obtained after 5 min of quiet rest in a seated, standardized position: arms and feet supported. As skin temperature (Tskin) has been found to increase post-aerobic exercise (Rojas-Valverde et al., 2021) and to influence CDT (Hagander et al., 2000), heat sensitivity (Strigo et al., 2000), but not mechanical sensitivity (Strigo et al., 2000), it could mediate the impact of the exercise vs. control conditions on thermal sensory thresholds. Thus, Tskin was measured before and after each sensory assessment at the upper- and lower-limb testing sites and their contralateral equivalent locations, using an infrared thermometer (MediTemp, Peoria, USA) held approximately 2 cm above the skin. For each time point (pre and post conditions), Tskin was defined as the average of one reading taken before and one taken after the sensory assessment.

Statistics

All data analyses were conducted using R (version 4.2.3) in the R studio IDE (Version 2023.06.0 + 421). Significance level (alpha) was set at 0.05. The complete analysis report can be found at OSF. Data distribution was observed using histograms, normal QQ plots, and density plots. In case of deviation from normality, data was $\log_{10}$ transformed or non-parametric statistics were used. Normally distributed data is reported as mean ± SD and median [IQR] otherwise. Log-normal distribution was found for PPT and CDT and a $\log_{10}$ transformation was applied for statistical analyses. Descriptive statistics for sensory thresholds were computed on the average of the three measurements per time point and reported in their original units. Statistical comparisons were conducted using all three individual measurements per time point using linear mixed models, with a participant random intercept (LMM-RI) to account for repeated measurements and increase statistical power.

Manipulation checks

We compared conditions and assessed changes in Tskin across conditions and limbs. The impact of the factors 'timing' (pre vs. post) and 'condition' (control vs. exercise) on HR, BR, and systolic and diastolic BP (SBP, DBP), was assessed with LMM-RI. Scores of RPE, SSS, STAI-Y1, and room temperature and humidity were compared between conditions using two-sided paired *t*-tests or wilcoxon signed-rank test. The effect of the factors 'condition' (control vs. exercise) and 'site' (dominant forearm vs. non-dominant forearm vs. dominant quadriceps vs. non-dominant quadriceps) on Tskin change (ΔTskin: post values - pre values in each condition) were assessed using a LMM-RI.

Comparing EIH across testing sites and test modalities

We used a three-level approach to examine EIH across test modalities and sites. The equations for all models are detailed in the Supplementary material S1.

Level 1 compared a standardized EIH score (EIH_z) between test modalities (PPT vs PP vs HPT vs CDT) and sites (forearm vs quadriceps) using a LMM-RI (Supplementary material S1, eq. 1). EIH_z scores were computed from z-score standardized data to account for differences in average values and variability in sensory thresholds across test modalities and sites (Rolke et al., 2006). Specifically, for each individual observation x_{ijtcsm} for measurement i (1 to 3), participant j (1 to n participant), timing t (pre vs. post), condition c (exercise vs. control), site s (forearm vs. quadriceps), and test modality m (PPT vs. PP vs. HPT vs. CDT), a z_{ijtcsm} score was computed as follows:

$$z_{ijtcsm} = \frac{x_{ijtcsm} - mean_{pre,sm}}{SD_{pre,sm}}$$

where $mean_{pre,sm}$ and $SD_{pre,sm}$ represent the mean and standard deviation computed across pre-exercise and pre-control observations, within each test modality and site. The EIH_z score, reflecting change relative to the baseline variability of each test modality and site, was then calculated as:

$$EIH_{z,ijsm} = \left[\left(z_{ijsm}\right)_{post} - \left(z_{ijsm}\right)_{pre}\right]_{exercise} - \left[\left(z_{ijsm}\right)_{post} - \left(z_{ijsm}\right)_{pre}\right]_{control}$$

To account for potential baseline differences between conditions (Vickers and Altman, 2001) an index of this baseline difference ($baseline_{diff,ijsm} = \left[\left(z_{ijsm}\right)_{pre}\right]_{exercise} - \left[\left(z_{ijsm}\right)_{pre}\right]_{control}$) was included as a continuous covariate. This model was compared with a more complex model additionally including Tskin change across conditions ($\Delta Tskin_{exercise}$ - $\Delta Tskin_{control}$; z-scored) and its interaction with test modality, as skin temperature may differentially affect thermal (CDT, HPT) and mechanical thresholds (Supplementary material S1, eq. 2). A similar LMM-RI (Supplementary material S1, eq. 3) was used to test whether differences in EIH between modalities and sites could have resulted from differential changes in sensory thresholds across modalities and sites in the control condition.

Level 2 tested, for each modality separately, whether exercise

induced a change in sensory threshold. A LMM-RI (Supplementary material S1, eq. 4) modelled the change in sensory threshold ($\Delta_{z,ijcs} = (z_{ijcs})_{post} - (z_{ijcs})_{pre}$) as a function of site (forearm vs quadriceps) and condition (control vs exercise). As in the Level 1 analyses, for thermal modalities, this model was compared with a model additionally including Tskin change from z-scored data ($\Delta Tskin_z$) as a covariate (Supplementary material S1, eq. 5).

Level 3 examined whether condition differences identified at Level 2 were driven by a post-exercise increase in sensory threshold, rather than a pre-exercise difference between conditions. For each site and modality combination where a significant effect of 'condition' was found at Level 2, a LMM-RI with the factors 'timing' (pre vs post) and 'condition' (control vs exercise) was computed (Supplementary material S1, eq. 6). This sequential approach minimized the number of models computed. Again, for thermal modalities, this model was compared with a model additionally including Tskin (original °C units; Supplementary material S1, eq. 7).

As ADT was not assessed at two separate sites, it was excluded from level 1 and 2 analyses.

Model estimation

All LMM-RIs were estimated using restricted maximum likelihood estimation (RMLE) with the `lme4` R package (Bates et al., 2015). Models were compared using likelihood ratio test (LRT). Distributional assumptions of LMMs (Schielzeth et al., 2020) were examined visually (Normal QQ plots, density plots, and histograms), and transformation of data (log, square root) was considered in case of violation. Outliers were considered for removal in case of a cook's distance >1. Simple effect pairwise comparisons were conducted on significant interactions to assess differences of marginal means ($Diff_{MM}$) between each level of each factor with Bonferroni-Holm correction of *p*-values. In the absence of significant interaction, a similar procedure was conducted on significant main effects. The following standardized effect sizes were computed using the 'effectsize' R package (Ben-Shachar et al., 2024): partial omega squared (ω_p^2) for main and interaction effects of LMM-RI, Cohen's d_z for pairwise comparisons of normally distributed data, and rank-biserial correlation (r_{rb}) for non-normally distributed data. ω_p^2 and d_z were estimated from test statistics and degrees of freedom and were interpreted as "Very small", "Small", "Medium", and "Large" for values respectively <0.01, 0.01 to <0.06, 0.06 to <0.14, ≥ 0.14 (Field, 2013) and < 0.2, 0.2 to <0.5, 0.5 to <0.8, ≥0.8 (Cohen, 1988). r_{rb} was computed from sample values and was interpreted as "Very small", "Small", "Moderate", and "Large" for values <0.1, 0.1 to <0.2, 0.2 to <0.3, and ≥ 0.3 (Gignac and Szodorai, 2016).

Results

In total 40 participants were included in the study. Two participants could not complete all the procedures: one was discovered to present a severe forearm supination restriction preventing effective sensory testing and one dropped-out due to time constraints. They matched the rest of the sample in terms of age, BMI, and fitness level. Thus, data from 38 participants was analyzed (Table 1).

Table 1
Participants characteristics.

Variables		$n = 38$
Age (years)		23.8 ± 3.7
BMI (kg/m^2)		22.4 ± 2.6
PPO (W)		258.1 ± 44.3
Relative PPO (W/kg)		3.6 ± 0.6
Maximal HR (bpm)		185 ± 9
STAI-Y2 (/80)		34.3 ± 7.5
PCS total (/52)		13.3 ± 7.1
TSK (/68)		32.8 ± 6.3
IPAQ total mPA (MET/min/wk)		1080 [1530]
IPAQ total vPA (MET/min/wk)		2880 [2880]
IPAQ total PA (MET/min/wk)		6339 [4974]
IPAQ category (n)	Low	1
	Moderate	5
	High	32
Level of education (n)	High school completed	16
	Bachelor's completed	9
	Master's completed	12
	PhD completed	1
FLANDERS (n)	Ambidextrous	1
	Left	3
	Right	34

Data presented as mean ± SD or a as median [IQR]. Units or maximum scores are indicated in parentheses. Abbreviations: BMI, Body mass Index; PPO, Peak power output; HR, Heart rate; STAI-Y2, State-trait anxiety inventory (form 2, trait); PCS, Pain catastrophizing scale; TSK, Tampa scale for kinesiophobia; IPAQ, International physical activity questionnaire; FLANDERS, Flinders handedness survey; kg, kilogram; W, watt; bpm, beats per minute; MET, Metabolic equivalent;min, minute; wk., week; v/mPA, vigorous/moderate physical activity.

Manipulation checks

Table 2 presents summary statistics of the pre- and post- values of HR, BR, SBP, DBP, RPE, SSS, and STAI-Y1 across conditions as well as results of LMM-RI/paired statistical tests.

Pairwise comparisons following significant interactions between the factors 'timing' and 'condition' on HR, BR, SBP, and DBP (Table 2) showed similar baseline values for both conditions (all $|t| \leq 0.92$; all $p \geq 0.362$) and significant increases after exercise (all $t \geq 3.59$; all $p < 0.001$) but not after the control condition (all $|t| \leq 1.94$; all $p \geq 0.0555$), except for HR where a small, yet significant, increase after the control condition was observed ($Diff_{MM} = 7$ bpm; 95%CI [4, 10]; $t_{111} = 4.68$; $p < 0.001$; $d_z = 0.44$, "small").

Along with higher median RPE in the cycling vs. control condition (Difference = 8.5; 95%CI [8, 9]; V = 0; n pairs = 38; $p < 0.001$; paired Wilcoxon-signed rank test; $r_{rb} = 1$, "large"; Table 2), physiological parameters reflecting acute cardio-vascular exercise adaptation (e.g., increase of HR or SBP) showed a clear dissociation between conditions. These results indicate that the control condition, despite involving a cycling motion, can be considered as an adequate "exercise sham". Furthermore, the absence of differences in SSS, STAY-Y1, room temperature, and room humidity (Table 2) suggest similar experimental conditions in both sessions.

Skin temperature changes vary across limbs and conditions

Tskin values are presented in Fig. 2. The LMM-RI revealed a significant 'site' x 'condition' interaction ($F_{3,258} = 17.69$; $p < 0.001$; $\omega_p^2 = 0.16$, "large"; Fig. 2) on ΔTskin. Further analyses revealed significant differences in Tskin changes across body parts in the exercise condition. ΔTskin was significantly greater at the lower limbs and non-dominant forearm compared with the dominant forearm (all $Diff_{MM} \geq 1.1$ °C; all $t \geq 6.98$; all $p < 0.001$; Fig. 2), while no significant differences were found between the lower limbs and the non-dominant forearm (all $Diff_{MM} \leq 0.2$ °C; all $t \leq 1.24$; all $p = 1.000$).

Comparing EIH across test modalities and testing sites

EIH is greatest for blunt pressure at the exercising limb

Descriptive statistics of EIH at each site and for each test modality are presented in Table 3. The Level 1 LMM-RI revealed a significant 'test modality' x 'site' interaction ($F_{3,866} = 5.56$; $p < 0.001$; $\omega_p^2 = 0.015$, "small"; Table 3 and Fig. 3), indicating that exercise differentially affected sensitivity to the different test stimuli at the exercising vs. non-exercising limbs. Change in Tskin across conditions was not included in the final model as its addition did not improve model fit ($\chi^2_4 = 2.53$; $p = 0.640$; LRT).

Table 2
Exercise versus control condition.

Variables	Pre		Post		Timing x Condition interaction / Pairwise comparison	
	Control	Exercise	Control	Exercise	$Stat_{df}$	p
HR (BPM)	62 ± 9	62 ± 11	69 ± 10	154 ± 9	$F_{1,111} = 1605$	< 0.001
BR (CPM)	13 ± 4	13 ± 3	16 ± 5	35 ± 12	$F_{1,111} = 81.58$	< 0.001
SBP (mmHg)	113 ± 10	115 ± 7	115 ± 8	133 ± 11	$F_{1,111} = 65.83$	< 0.001
DBP (mmHg)	65 ± 6	65 ± 6	65 ± 7	69 ± 8	$F_{1,110} = 8.04$	0.00545
RPE (6–20)			6 [1]	15 [2]	$V_{38} = 0$	< 0.001
SSS (/7)	2 [1]	2 [1]			$V_{24} = 148.5$	0.975
STAI-Y1 (/80)	30 [9]	29 [11]			$V_{31} = 176$	0.161
Room humidity (%)	47 ± 8	46 ± 8			$t_{37} = 1.87$	0.069
Room temperature (°C)	22 ± 2	21 ± 2			$t_{37} = 1.51$	0.139

Data from 38 participants presented as mean ± SD or median [IQR]. Units or maximum scores are indicated in parentheses. Abbreviations: HR, Heart rate; BR, Breathing rate; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; BPM, Beats per minute; CPM, Cycles per minute; mmHg, millimeter of mercury; RPE, Rate of perceived exertion; °C, Celsius degrees; Df, Degrees of freedom; STAI-Y1, State-trait anxiety inventory (form 1, state); SSS, Stanford sleepiness scale.

Further Level 1 analyses of the 'test modality' x 'site' interaction revealed that EIH_z at the exercising quadriceps was greatest when assessed using the PPT. It was significantly greater than EIH_z assessed using the HPT ($Diff_{MM} = 0.376$; 95%CI = [0.095, 0.656]; $t_{866} = 3.54$, $p = 0.00208$; $d_z = 0.12$, "very small"; Fig. 3) and the CDT ($Diff_{MM} = 0.521$; 95%CI = [0.239, 0.802]; $t_{866} = 4.89$, $p < 0.001$; $d_z = 0.17$, "very small"; Fig. 3). It also appeared greater than EIH_z assessed using PP but the difference was only close to statistical significance ($Diff_{MM} = 0.263$; 95% CI = [-0.018, 0.543]; $t_{866} = 2.47$, $p = 0.0544$; $d_z = 0.08$, "very small"; Fig. 3). No difference between test modalities was found for EIH_z assessed at the non-exercising forearm (all $|t| \leq 0.774$, all $p = 1.000$). These results are unlikely to be explained by differential changes in the control condition as the LMM-RI on the threshold changes in the control condition revealed only a significant main effect of 'test modality' ($F_{3,866} = 3.84$, $p = 0.00953$; $\omega_p{}^2 = 0.01$, "very small"), with pairwise comparisons showing greater changes for CDT compared to all other modalities (all $t \geq 2.69$, all $p \leq 0.0288$) and no interaction with 'site', in contrast to the significant 'test modality' × 'site' interaction observed for EIH_z.

Level 2 analyses then examined the effects of exercise on each test modality separately. Descriptive statistics for pre, post, and change in sensory threshold for each site and test modality are presented in Table 3.

Pressure pain thresholds are increased by exercise, solely at the exercising limb

The Level 2 LMM-RI revealed a significant 'condition' x 'site' interaction on ΔPPT_z ($F_{1,414} = 10.01$; $p = 0.00167$; $\omega_p{}^2 = 0.02$, "small"; Table 3 and Fig. 4). Pairwise comparisons showed that ΔPPT_z was significantly greater at the quadriceps vs. forearm in the exercise condition ($Diff_{MM} = 0.184$; 95% CI = [0.036, 0.332]; $t_{414} = 2.45$; $p = 0.0147$; $d_z = 0.12$, "very small"; Fig. 4), but not in the control condition. EIH was absent at the forearm where ΔPPT_z was similar between conditions ($Diff_{MM} = 0.027$; 95%CI = [-0.120, 0.175]; $t_{414} = 0.36$; $p = 0.718$; $d_z = 0.02$, "very small"; Fig. 4). Altogether, these results confirm a preferential effect of exercise on PPT at the exercising limb, consistent with the Level 1 findings.

The Level 3 LMM-RI confirmed the presence of EIH for PPT at the quadriceps, revealing a significant 'timing' x 'condition' interaction at that testing site ($F_{1,415} = 12.59$; $p < 0.001$; $\omega_p{}^2 = 0.03$, "small"; Fig. 5). Pairwise comparisons showed that while baseline values of PPT were similar between conditions ($Diff_{MM} = 0.01$ log_{10}; 95%CI [0.01, 0.03]; $t_{415} = 1.13$; $p = 0.260$; $d_z = 0.01$, "very small"; Fig. 5), they significantly increased after exercise in the exercise condition ($Diff_{MM} = 0.02$ log_{10}; 95%CI [0.01, 0.04]; $t_{415} = 2.71$; $p = 0.00705$; $d_z = 0.13$, "very small"; Fig. 5) and significantly decreased in the control condition ($Diff_{MM} = -0.02$ log_{10}; 95%CI [-0.04, -0.00]; $t_{415} = -2.31$; $p = 0.0214$; $d_z = -0.11$, "very small";).

Pinprick ratings are not impacted by exercise, regardless of testing site

For ΔPP_z, the Level 2 LMM-RI revealed no significant main effect of 'condition' ($F_{1,414} = 0.01$; $p = 0.947$; $\omega_p{}^2 < 0.01$, "very small"; Table 3 and Fig. 4) and no significant 'condition' x 'site' interaction ($F_{1,414} = 0.00$; $p = 0.950$; $\omega_p{}^2 < 0.01$, "very small"; Table 3 and Fig. 4). These results indicate that exercise did not modulate sensitivity to mechanical pinprick stimuli at either testing site.

Heat pain thresholds are not impacted by exercise, regardless of testing site

The Level 2 LMM-RI on ΔHPT_z revealed a significant negative effect of $\Delta Tskin_z$ ($\beta = -0.08$; SE = 0.03, $F_{1,448} = 5.71$; $p = 0.0172$) but no significant main effect of 'condition' ($F_{1,435} = 2.99$; $p = 0.0844$; $\omega_p{}^2 < 0.01$, "very small"; Table 3 and Fig. 4) and no significant 'condition' x 'site' interaction ($F_{1,416} = 0.39$; $p = 0.534$; $\omega_p{}^2 < 0.01$, "very small"; Table 3 and Fig. 4), indicating that exercise did not significantly modulate sensitivity to painful heat at either testing site.

Cold detection thresholds are preferentially reduced at exercising limbs

The Level 2 LMM-RI conducted on ΔCDT_z revealed a significant 'site' x 'condition' interaction ($F_{1,414} = 7.87$; $p = 0.00525$; $\omega_p{}^2 = 0.02$, "small"; Table 3 and Fig. 4). The inclusion of $\Delta Tskin_z$ did not improve the model ($\chi^2_1 = 0.39$; $p = 0.532$; LRT). Pairwise comparisons showed that ΔCDT_z was significantly smaller at the quadriceps compared to the forearm, only in the exercise condition ($Diff_{MM} = -0.327$; 95%CI = [-0.495, -0.159]; $t_{415} = -3.83$; $p < 0.001$; $d_z = -0.19$, "very small"; Fig. 4), indicating a preferential *reduction* in cold detection thresholds at the exercising limb.

In contrast, the Level 3 LMM-RI including the factors 'timing' and 'condition' showed a significant negative effect of Tskin on the CDT at the quadriceps ($\beta = -p0.025$; SE = 0.0098; $F_{1,444} = 5.33$; p = 0.0214) and only a trend towards statistical significance for the 'timing' x 'condition' interaction at this testing site ($F_{1,420} = 3.51$; $p = 0.0617$; $\omega_p{}^2 < 0.01$, "very small"; Fig. 5). One observation was removed from the model due to a cook distance >1. These results suggest that exercise *increases* cold sensitivity at exercising vs. non-exercising limbs, independently of Tskin changes. Yet, the impact of exercise per se on the sensitivity to cold at exercising limbs may overlap with the effects of Tskin on cold sensitivity.

Auditory detection threshold is not affected by exercise

The LMM-RI assessing the effects of 'timing' and 'condition' on ADT did not reveal any significant effect (all $F \leq 1.94$; all $p \geq 0.165$; Table 3 and Fig. 5) suggesting that neither exercise nor the control condition modulated auditory perception. Due to a technical issue, three measurements were removed from this analysis.

Discussion

In this study, we compared EIH induced by a single session of cycling

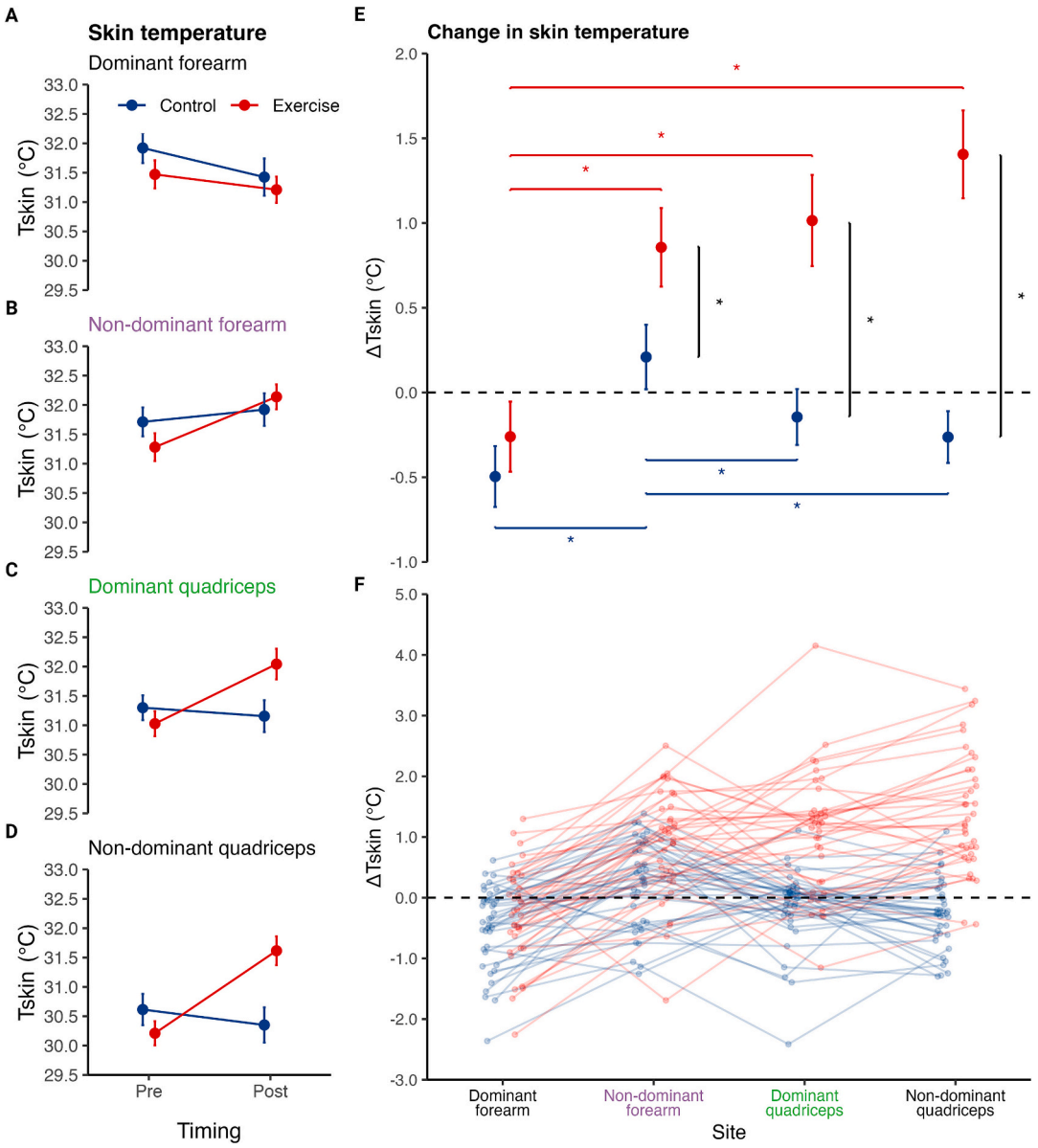

Fig. 2. Skin temperature across limbs and conditions. (A,B,C,D) Skin temperature (Tskin) at the dominant and non-dominant forearms and quadriceps, before and after the control or the exercise condition. Sensory assessments were conducted on the non-dominant forearm (immobilized with a sling) and the dominant quadriceps—color coded in purple and green, respectively. (E,F) Change (post-pre) in Tskin (ΔTskin) in each condition across limbs. A significant 'site' x 'condition' interaction was found on ΔTskin ($p < 0.05$). *Significant pairwise comparisons ($p < 0.05$). Lines and points denote (F) individual and (A,B,C,D,E) mean data. Error bars represent 95% confidence interval. Abbreviations: °C, Celsius degrees. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

exercise – relative to a light pedaling control condition – across sensory test modalities and at an exercising (quadriceps) and non-exercising (immobilized forearm) body site in healthy young males. Cycling exercise reduced sensitivity to blunt pressure at the exercising limb but not at the non-exercising limb. Exercise also increased cold sensitivity at the exercising limb compared to the non-exercising limb. In contrast, no effect of exercise was observed on the cutaneous sensitivity to noxious heat or mechanical pinprick stimulation, or the sensitivity to auditory stimulation.

Effects of test modality and site

Altogether our results are consistent with previous findings and indicate that the impact of aerobic exercise on pain in pain-free young males is greater (1) at exercising versus non-exercising limbs, (2) for blunt pressure compared with heat or mechanical skin stimulation. This pattern supports the view that EIH in this population is driven predominantly by processes that preferentially modulate perceptual sensitivity to stimulation of deep-tissue nociceptors within exercising muscles.

Consistent with previous reports (Tomschi et al., 2024b), we observed an increase in PPT at the exercising limb. However, the absence of remote effects on pressure pain sensitivity contrasts with some previous studies (Tomschi et al., 2024b). This could be explained by differences in sample characteristics and experimental design. First, our sample consisted of young males with above-average levels of self-reported physical activity (Bauman et al., 2009). In this context, an absence of remote EIH has been reported in trained runners (Peterson et al., 2019). However, this explanation should be interpreted with caution, as our participants were predominantly untrained recreational

Table 3
Pre/post condition values and exercise-induced hypoalgesia.

Test modality	Site	Control			Exercise			Site x Condition interaction		$Baseline_{diff}$	EIH	Test modality x Site interaction	
		Pre	Post	Δ	Pre	Post	Δ	$F_{df1,df2}$	p			$F_{df1,df2}$	p
PPT (kPa)	Forearm	441 ± 146	439 ± 138	-2 ± 70	433 ± 131	440 ± 158	7 ± 101	$10.01_{1,414}$	0.00167	-8 ± 107	10 ± 113	$5.56_{3,866}$	< 0.001
	Quadriceps	680 ± 175	651 ± 182	-30 ± 92	664 ± 184	710 ± 228	46 ± 101			-16 ± 118	75 ± 131		
PP (100−0)	Forearm	64 ± 16	62 ± 18	-2 ± 10	64 ± 16	63 ± 19	-2 ± 11	$0_{1,414}$	0.95	1 ± 9	0 ± 13		
	Quadriceps	70 ± 16	69 ± 15	-1 ± 7	72 ± 14	70 ± 17	-2 ± 11			1 ± 11	-1 ± 12		
HPT (°C)	Forearm	43.2 ± 2.5	43.0 ± 2.4	-0.2 ± 1.7	42.5 ± 2.7	42.9 ± 2.5	0.3 ± 1.7	$0.39_{1,418}$	0.534	-0.7 ± 1.9	0.5 ± 2.6		
	Quadriceps	45.8 ± 2.9	45.6 ± 2.7	-0.2 ± 1.7	45.4 ± 3.2	45.2 ± 2.9	-0.2 ± 1.6			-0.4 ± 1.6	0.1 ± 2.4		
CDT (ΔT, °C)	Forearm	1.4 ± 0.6	1.5 ± 0.6	0.0 ± 0.4	1.5 ± 0.7	1.5 ± 0.7	0.1 ± 0.6	$7.87_{1,415}$	0.00525	0.0 ± 0.4	0.0 ± 0.7		
	Quadriceps	2.0 ± 0.9	2.1 ± 1.0	0.1 ± 0.7	2.2 ± 1.2	2.0 ± 1.1	-0.2 ± 0.7			0.2 ± 0.8	-0.3 ± 0.9		
ADT (/1)	No site	0.35 ± 0.02	0.35 ± 0.01	0.00 ± 0.02	0.35 ± 0.01	0.35 ± 0.01	0.00 ± 0.01	$^{\#}1.11_{1,412}$	$^{\#}0.294$	0.00 ± 0.01	0.00 ± 0.02		

Data from 38 participants, reported as mean ± SD. Units or maximum scores are indicated in parentheses. # F statistics and *p* value reported from timing x condition interaction. Abbreviations: PPT, Pressure pain threshold; PP, Mechanical pinprick rating; HPT, Heat pain threshold; CDT, Cold detection threshold; Δ, Difference in post and pre values in each condition; kPa, kilopascal; °C, Celsius degree; ΔT, difference from baseline temperature (32 °C); df, degrees of freedom; $Baseline_{diff}$, $pre_{exercise}$ - $pre_{control}$; EIH, Exercise-induced hypolagesia computed as $(Post - pre)_{exercise}$ - $(Post - pre)_{control}$.

cyclists (Pauw et al., 2013). Second, some EIH studies did not include a control condition (Koltyn, 2000), limiting the specificity of their findings to exercise per se. In contrast, our light pedaling control condition tended to induce a local *decrease* in thresholds across all nociceptive test modalities, suggesting a non-specific sensitization effect, potentially due to repeated nociceptive stimulation, low intensity exercise (Micalos and Arendt-Nielsen, 2016), or the experimental context itself. Third, and most importantly, we immobilized the tested forearm during both conditions, unlike previous studies reporting some amount of remote EIH (e.g., (Vaegter et al., 2013)). As cycling can recruit upper-limb muscles (Turpin et al., 2017), body sites previously considered as non-exercising may in fact have been actively involved in the exercise task.

We found no effect of exercise on heat pain sensitivity (HPT), in line with most previous studies examining the local and remote effects of *aerobic* exercise on HPT (Antonio et al., 2024; Jones et al., 2019; Kodesh and Weissman-Fogel, 2014; Kortenjann et al., 2020; Ruble et al., 2005; Thomas et al., 2020). An apparent discrepancy remains, however, as aerobic exercise has been shown to reduce heat pain ratings during sustained or repeated stimulation (Kodesh and Weissman-Fogel, 2014; Naugle et al., 2014b; Zheng et al., 2021), in contrast with its absence of effect on HPT. Notably, these reductions in perceived heat pain appear to develop progressively over sustained or repeated heat stimulation (e.g., emerging only during the final 10 s of a 30-s stimulus (Naugle et al., 2014b)). This temporal pattern suggests that exercise may influence nociceptor sensitization or habituation processes (Koyama et al., 2004), which could also account for previously reported reductions in heat pain temporal summation (Naugle et al., 2014b; Vierck et al., 2001).

Consistent with prior work, we also observed no effect of exercise on mechanical pinprick sensitivity (PP) (Kortenjann et al., 2020), or innocuous cold sensitivity (CDT) (Kortenjann et al., 2020; Ruble et al., 2005) assessed at *non-exercising* limbs. However, as discussed below, exercise may increase innocuous cold sensitivity at *exercising* limbs.

Mechanisms that could explain a preferential modulation of deep-tissue pressure pain by exercise

The selective modulation of PPT at the exercising limb constrains the range of possible mechanisms underlying EIH. Below, we consider the extent to which central and peripheral processes could account for this pattern.

Central processes

Descending inhibitory pathways are modulated by exercise (Scheef et al., 2012), somatotopically organized (Crawford et al., 2025), and may exert stronger effects on nociceptive input from muscle vs. skin (Yu and Mense, 1990). Such pathways could therefore preferentially modulate blunt pressure sensitivity within exercising muscles. Yet, in the context of EIH, the exact circuitry of these pathways and how they are engaged by exercise remains largely unknown. The PPT increase observed solely at the exercising limb is also compatible with a contribution of segmental anti-nociceptive processes modulating the transmission of muscle input, possibly through the activation of muscle spindle afferents (Lambertz et al., 2008). However, the low intensity cycling motion of the control condition would also be expected to activate muscle spindle afferents, which is difficult to reconcile with the PPT *decrease* observed in that condition.

Peripheral processes

An involvement of peripheral α2 adrenoreceptors (α2-AR) is supported by findings in male rats showing that systemic, but not central, blockade of α2-AR inhibits EIH, suggesting a peripheral site of action (de Souza et al., 2013). α2-AR could modulate peripheral muscle nociception through the activation of ATP-sensitive potassium channels (Guo et al., 2022; Romero et al., 2012).

The increased blood flow in exercising muscles could facilitate local delivery of endogenous analgesic compounds released during exercise – such as beta-endorphins (BE) and endocannabinoids (ECB) – thereby modulating deep nociceptive afferents at exercising body sites (Jones et al., 2017; Schwarz and Kindermann, 1992; Matei et al., 2023). BE can induce peripheral analgesia in skin primary afferents by modulating K^+ and Ca^{2+} conductance (Kapitzke et al., 2005), though their effects on muscle nociceptive afferents remain unexplored, and correlations between plasmatic BE and EIH have been inconsistent (Droste et al., 1991; Hughes and Patterson, 2020). ECB are endogenous ligands of cannabinoid receptors (CBR) that modulate muscle nociception (Gonçalves et al., 2021) and elicit peripheral analgesia through mechanisms that remain to be fully elucidated (Akopian et al., 2009). A rodent study in male rats suggested a peripheral contribution of cannabinoid signaling to EIH (Fuss et al., 2015), though human data on the link between ECB and EIH remain scarce (Hughes et al., 2021; Koltyn et al., 2014).

Finally, exercising muscles release myokines with diverse metabolic functions (Martin et al., 2020). A myokine of particular interest is kynurenic acid (KynA), a metabolite of the kynurenine pathway

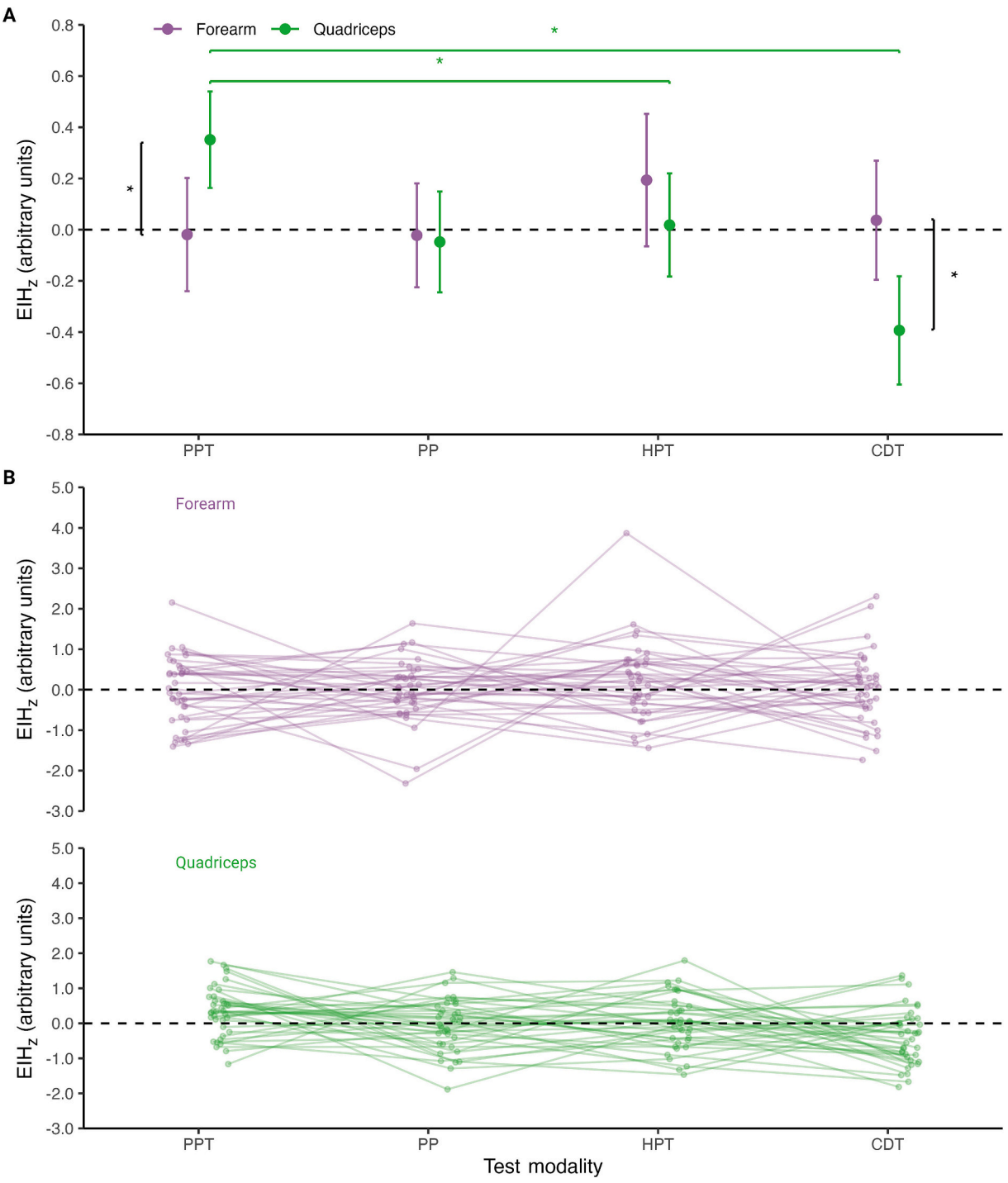

Fig. 3. Effects of test modality and testing site on exercise-induced hypoalgesia. (A) Averages of exercise-induced hypoalgesia scores [EIH_z: $(Post - Pre)_{exercise}$ - $(Post - Pre)_{control}$ computed from z-scored data standardized by pre-exercise and pre-control conditions values, for each testing site and test modality]. Values greater than 0 reflect decreased sensitivity. A linear mixed model revealed a significant 'site' x 'test modality' interaction on EIH_z ($p < 0.05$). (B) Individual EIH_z scores, averaged from three measurements. Lines and point denote condition mean (A) and individual (B) data. Error bars represent 95% confidence interval. *Significant pairwise comparisons ($p < 0.05$). Abbreviations: PPT, Pressure pain threshold. PP, Mechanical pinprick rating; HPT, Heat pain threshold; CDT, Cold detection threshold.

predominantly synthesized in skeletal muscles, that transiently increase after aerobic exercise (Joisten et al., 2020). KynA acts as an antagonist of the endogenous *N*-methyl-D-aspartate receptors (NMDAR) (Martin et al., 2020), which are involved in muscle peripheral nociceptive transmission (Cairns et al., 2003).

Impact of exercise on cold perception

We found that exercise increased innocuous cold sensitivity preferentially at the exercising limb, contrasting with previous studies reporting either no effect or *decreased* cold sensitivity following exercise (Ouzzahra et al., 2012; Ruble et al., 2005).

When controlling for changes in skin temperature, the effect of exercise on cold sensitivity at the exercising quadriceps was no longer

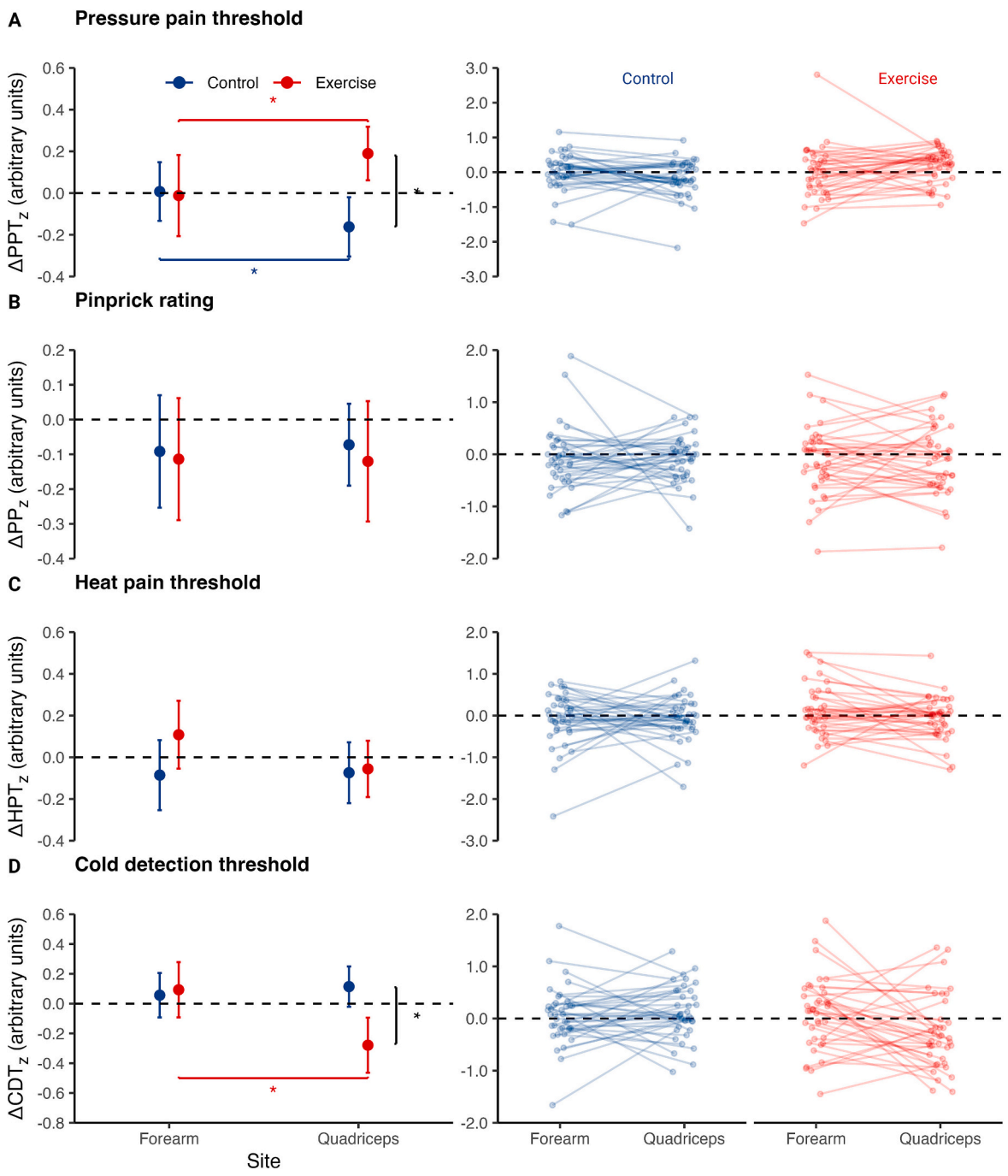

Fig. 4. Effects of testing site and condition on change in sensory thresholds. Linear mixed models investigating the effects of the factors 'site' and 'condition' on the change (difference post – pre each condition, computed from z-scored data, standardized by pre-exercise and pre-control conditions values, for each testing site (Δ_z)) in (A) pressure pain threshold (PPT), (B) mechanical pinprick rating (PP), (C) heat pain threshold (HPT), and (D) cold detection threshold (CDT) revealed significant interactions solely for PPT and CDT ($p < 0.05$). Lines and points denote condition mean (enlarged) and individual mean of three values data. Error bars represent 95% confidence interval. *Significant pairwise comparisons ($p < 0.05$).

significant, suggesting a potential mediating role of skin temperature (Hagander et al., 2000). However, this would predict a comparable effect at the non-exercising forearm, where skin temperature also increased, yet no such effect was observed. This discrepancy suggests that changes in skin temperature alone cannot fully account for the exercise-related modulation of cold sensitivity.

This difference between sites might be explained by the different sources of the skin temperature increase at each location. At the non-exercising forearm, skin temperature likely increased due to reduced evaporative cooling associated with the immobilizing sling, whereas at the exercising quadriceps, the increase may have been driven predominantly by convective heat transfer from the underlying active muscle (Rojas-Valverde et al., 2021). Although the contribution of muscle temperature to cold sensitivity is currently unknown, cutaneous thermoreceptors and thin thermosensitive muscle afferents project to similar dorsal horn laminae (Craig and Kniffki, 1985; Mense and Meyer, 1985). Therefore, if muscle temperature were to increase cold sensitivity through central integration of convergent thermal inputs, it could have acted as a confounding factor independently of skin temperature. This remains speculative and warrants further investigation.

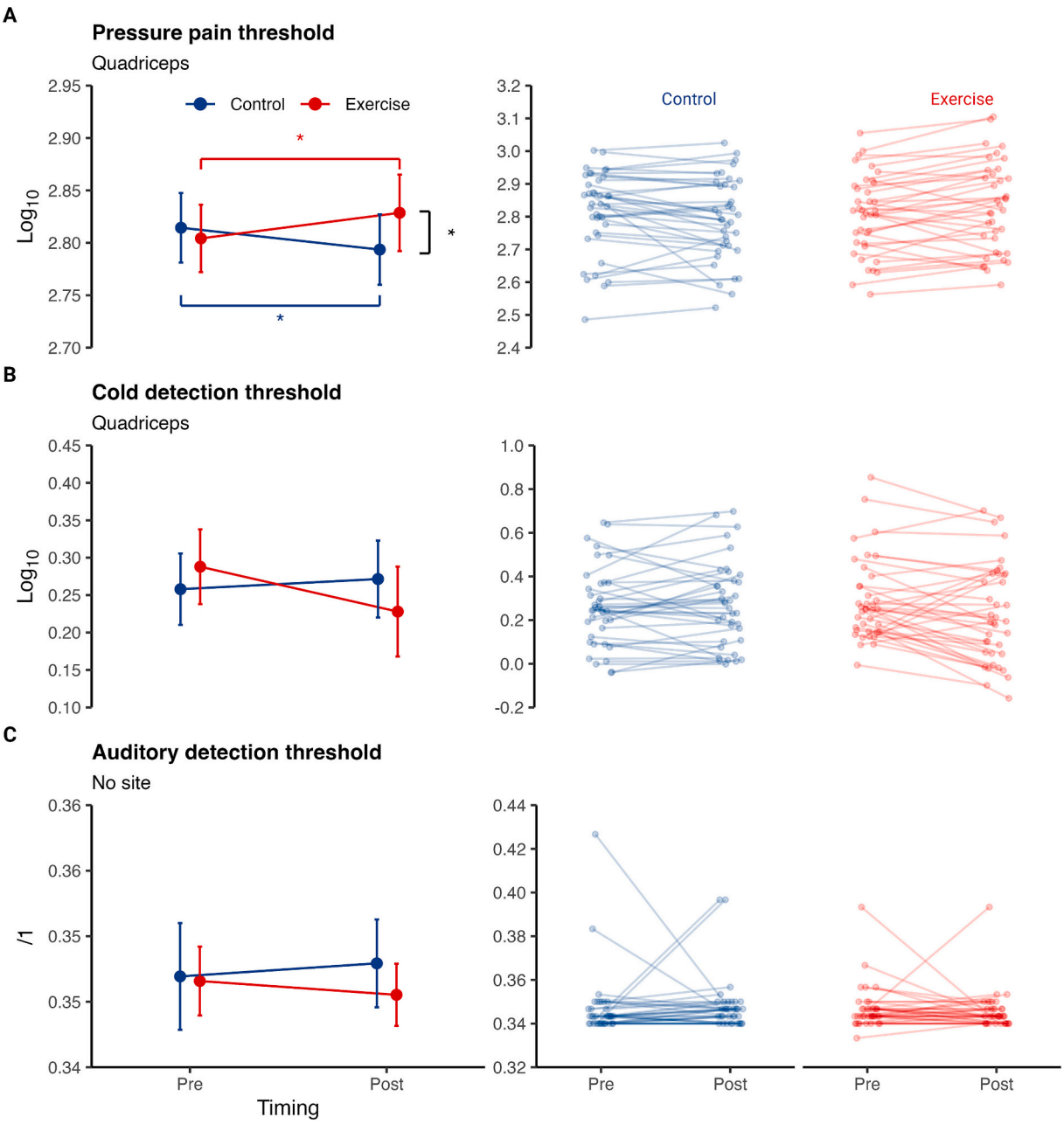

Fig. 5. Effects of timing and condition on sensory thresholds. Linear mixed models examining the impact of the factors 'timing' and 'condition' on (A) pressure pain threshold (PPT) at the quadriceps, (B) cold detection threshold (CDT) at the quadriceps (model adjusted for skin temperature), and (C) auditory detection threshold (ADT) revealed a significant interaction for PPT ($p < 0.05$). Lines and point denote condition mean (enlarged) and individual mean of three values data. Error bars represent 95% confidence interval. *Significant pairwise comparisons ($p < 0.05$). ADT is expressed in arbitrary units (0–1). Abbreviations: Log, Log_{10}-transformed values.

Study limitations and future directions

Several limitations of the present study should be acknowledged and may inform future research.

First, the study only included pain-free young males, limiting the generalizability of the findings to females, older individuals, and clinical populations. As the mechanisms of EIH may vary between sexes, future studies should perform sex-disaggregated analyses. Evidence for sex-differences in EIH magnitude is inconsistent: aerobic exercise studies report greater EIH in males (Nold et al., 2025), females (Sternberg et al., 2001; Vaegter et al., 2013), or no differences (Naugle et al., 2014b; Vaegter et al., 2015). One study found that isometric exercise reduced heat pain ratings in females but not males despite comparable PPT increases in both sexes (Naugle et al., 2014a), which may reflect an interaction between sex and test modality, though this was not formally tested. In contrast, sex differences in the potential mechanisms of EIH have been more consistently reported. Pain sensitivity is generally higher in females, while males often show greater conditioned pain modulation and stronger descending inhibitory activity in both human and rodent studies (Hermans et al., 2016; Mogil, 2012, 2020). Although aerobic exercise does not appear to differentially affect circulating BE, ECB, or KynA in humans (Desai et al., 2021; Goldfarb et al., 1998; Joisten et al., 2020), rodent studies report sex differences in central CBR expression (Blanton et al., 2021) and in peripheral processes, including opioid analgesia (Ji et al., 2006) and muscle afferent response to NMDAR activation (Dong et al., 2007). Sex differences in the peripheral

effects of ECB, KynA, and α2-AR remain largely unexplored. The reasons for these differences are not fully understood but likely involve genetic, hormonal, and psychosocial factors as well as contextual influences such as the sex/gender of the examiner (Levine and Lee De Simone, 1991; Mogil, 2020). Interactions between gonadal hormones and opioid, adrenergic, and cannabinoid analgesia have been widely documented in humans and rodents (Blanton et al., 2021; Martin, 2009; Thompson et al., 2008) but the direction of these effects remains controversial (Iacovides et al., 2015).

Second, we did not include a resting control condition. While this does not affect our main conclusions, it limits our ability to isolate the effects of cycling motion per se, which occurred in both experimental conditions.

Third, all test modalities except mechanical pinprick stimulation were assessed using the method of limits, which may *over*estimate sensory thresholds (Mücke et al., 2021). Nonetheless, this method has been used extensively in EIH studies (Vaegter and Jones, 2020) and alternatives are time consuming (Mücke et al., 2021). In addition, auditory detection thresholds were assessed using a non-standardized procedure designed to match the other sensory assessments, whose psychometric properties are unknown. Finally, neither participants nor the assessor were blinded to the conditions as the physiological changes induced by exercise made blinding impossible.

Beyond these limitations, future studies should directly examine the central and peripheral processes that may explain a preferential effect of exercise on deep-tissue pain sensitivity within exercising body parts, building on the present findings to inform both mechanistic hypotheses and power calculations. The proposed peripheral processes could be examined using pharmacological probes, such as the α2-AR antagonist yohimbine (Vo and Drummond, 2016), or analysing the association between EIH and exercise-induced changes in circulating ECB, BE, and KynA. Furthermore, the state and trait variables collected to characterize the sample and compare the control and exercise conditions could be used in future work integrating datasets from various experimental paradigms and populations to analyse the contribution of these variables to EIH.

Conclusions

In this study, we showed in young healthy males that a single bout of aerobic exercise reduces pain induced by blunt pressure at the exercising limb but not the non-exercising limb. In contrast, exercise had no effect on the sensitivity to stimuli activating cutaneous mechano-sensitive or heat sensitive nociceptors, except for a reduced sensitivity to innocuous cold at the exercising limb which was not significant when accounting for changes in skin temperature. Together, these findings suggest that EIH in this population is driven by mechanisms selectively affecting deep-tissue pain sensitivity within exercising body parts. Future studies are needed to directly investigate these mechanisms and to replicate these findings in more diverse populations including females and older individuals.

Use of artificial intelligence

Generative artificial intelligence (AI) was used in the preparation of this manuscript (**ChatGPT; GPT-5 mini, OpenAI).** It was used to assist with **grammar refinement, clarity, and conciseness of text.** All content, interpretations, and conclusions were **reviewed and validated by the authors**, and the AI did not generate original scientific data or analyses.

CRediT authorship contribution statement

Vladimir Aron: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Thomas Graven-Nielsen:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Conceptualization. **Louise Deldicque:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Conceptualization. **André Mouraux:** Writing – review & editing, Validation, Supervision, Software, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Vladimir Aron reports financial support was provided by Fond National pour la Recherche Scientifique. Thomas Graven-Nielsen reports financial support was provided by Danish National Research Foundation (DNRF121). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.ynpai.2026.100221.

Data availability

The data supporting the findings of this study are publicly available on the project OSF repository.

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