

Reviewed Preprint

v1 • May 28, 2026

Not revised

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Competing interests: Christian Büchel is a senior editor at eLife. The other authors declare no other competing interests.

Reviewing editor: José Biurrún Manresa, National Scientific and Technical Research Council (CONICET), National University of Entre Ríos (UNER), Argentina

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Exercise-Induced Hypoalgesia Following High- vs. Low-Intensity Aerobic Exercise in Fit Females

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eLife Assessment

This study proposes that fitness level influences exercise-induced hypoalgesia in women. However, the evidence to support this claim is **incomplete**: the conclusions rely on a small interaction that emerges only under specific conditions and are incongruent with the title, the findings are inconsistent across pain modalities and stimulus intensities, the analysis approach does not fully exploit the continuous pain ratings collected, and the absence of a baseline condition limits the interpretability of results as reflecting true hypoalgesia. Additionally, the methods by which fitness level was categorized across cohorts can be questioned, and the results and figures do not clearly illustrate how between-group comparisons were conducted. With a proper revision, it could be **useful** for sports medicine practitioners to consider how they administer exercise protocols to help those experiencing pain.

<https://doi.org/10.7554/eLife.109061.1.sa2>

Abstract

In a previous study by our group (Nold et al., 2025), we investigated exercise-induced pain modulation after high-intensity compared to low-intensity exercise in a heterogeneous sample of diverse fitness levels. Exploratory analyses suggested an interaction of sex, fitness level, and drug treatment, indicating that males showed increasing hypoalgesia after high-compared to low-intensity exercise with increasing fitness levels, which was diminished when naloxone was administered. In contrast, these effects were not evident in females. These exploratory findings warranted further investigation to determine if and to what extent exercise-induced hypoalgesia depends on fitness level and/or sex. In this current study, we investigated an all-female sample ($N = 21$) of high fitness levels using a similar paradigm as in the previous study, comparing heat and pressure pain ratings after high-intensity and low-intensity exercise. Our data show an interaction of exercise intensity and stimulus intensity in heat pain, with greater pain relief following high-intensity exercise, especially at the highest stimulus intensity. Despite results for pressure pain not reaching significance, a similar trend was evident. These results suggest that females at a high fitness level also show exercise-induced hypoalgesia for high-intensity compared to low-intensity exercise. Together with our previous findings, this suggests that exercise-induced hypoalgesia depends on fitness level but not on sex.

Introduction

In a previous study (Nold et al., 2025 [DOI](#)), we investigated the effect of high-(HI) and low-intensity (LI) aerobic exercise on pain perception in a heterogeneous sample of both sexes and diverse fitness levels ($N = 39$, 21 females), where exploratory analyses revealed a three-way interaction of sex, fitness level, and drug treatment. Males showed greater hypoalgesia after HI compared to LI exercise with increasing fitness levels. This effect was reduced when the μ -opioid antagonist

naloxone was administered. These effects were not evident in females. Taken together, these results point to the nuanced interplay of individual factors such as fitness level and sex underlying exercise-induced hypoalgesia.

Correspondingly, previous research in humans (Geva and Defrin, 2013 [↗](#); Schmitt et al., 2020 [↗](#); Sluka et al., 2018 [↗](#)) suggests that fitness level plays a crucial role in exercise-induced pain modulation, where subjects with higher fitness levels showed increased hypoalgesia following exercise compared to subjects with lower fitness levels (Schmitt et al., 2020 [↗](#)). Furthermore, higher self-reported fitness levels were shown to be associated with reduced pain ratings (Geva and Defrin, 2013 [↗](#)), reduced temporal summation (Naugle and Riley, 2014 [↗](#)), and greater opioid release in the orbitofrontal cortex and insula (Saanijoki et al., 2022 [↗](#)). Crucially, most of these studies (Saanijoki et al., 2022 [↗](#); Schmitt et al., 2020 [↗](#)) have been conducted in healthy all-male samples, limiting the translation to females, where the association of physical fitness and pain perception after exercise remains less clear (de Bruijn et al., 2011 [↗](#)).

Despite the majority of chronic pain patients being female (Fillingim et al., 2009 [↗](#); Mogil, 2012 [↗](#); Rice et al., 2019 [↗](#)), evidence on potential sex differences in exercise-induced pain modulation is surprisingly limited (Koltyn et al., 2014 [↗](#)). Of those studies that have considered potential sex differences in exercise-induced pain modulation, some (Brellenthin et al., 2017 [↗](#); Koltyn et al., 2014 [↗](#); Niwa et al., 2022 [↗](#); Smith, 2004 [↗](#)) could show that there are no sex differences, whereas others (Koltyn, 2000 [↗](#); Sternberg et al., 2001 [↗](#); Vaegter et al., 2014 [↗](#)) suggest that hypoalgesia following exercise is more robust in females, but overall results remain inconclusive (Rice et al., 2019 [↗](#)).

This current behavioural study aims to build on our previous findings (Nold et al., 2025 [↗](#)) by investigating exercise-induced pain modulation in an independent all-female sample ($N = 21$) with a high fitness level using the same paradigm as in the previous study, without a pharmacological intervention. Importantly, fitness levels (quantified by the weight-corrected functional threshold power (FTP)) in the previous study (Nold et al., 2025 [↗](#)) differed significantly ($P = 0.003$) between females ($M = 1.58$, $SD = 0.44$) and males ($M = 2.03$, $SD = 0.40$), thus, warranting further investigation into whether exercise-induced hypoalgesia after HI compared to LI aerobic exercise can also be observed in females with a higher fitness. Disentangling if and to what extent exercise-induced hypoalgesia depends on fitness level and sex is a crucial step to determine exercise-based interventions for (chronic) pain populations.

Results

The final sample included $N = 21$ healthy female participants with high fitness (weight-corrected FTP: $M = 2.25$ W/kg, $SD = 0.41$; Fig. 1A [↗](#)). The distribution of menstrual cycle phases (based on self-report and divided into three phases: follicular, ovulatory, and luteal as well as hormonal contraceptives) was equal across ($\chi^2(3) = 1.66$, $P = 0.65$) as well as within days (Day 1: $\chi^2(3) = 2.26$, $P = 0.52$; Day 2: $\chi^2(3) = 0.40$, $P = 0.94$; Supplemental Fig. S1A [↗](#)). Furthermore, the expectation of how acute exercise affects different pain domains was assessed (Lindheimer et al., 2020 [↗](#)) and yielded no significant effects overall (joint pain: $t(20) = -1.78$, $P = 0.09$, muscle pain: $t(20) = 0.15$, $P = 0.88$). However, participants indicated that they expect acute aerobic exercise to reduce whole-body pain ($t(20) = -2.83$, $P = 0.01$; Supplemental Fig. S1B [↗](#)). Finally, mood ratings were assessed using the Profile of Mood States (POMS) questionnaire (Curran et al., 1995 [↗](#)) before the experiment commenced (pre) and after it was completed (post). There were no significant differences in pre- and post-levels of fatigue ($t(20) = -0.50$, $P = 0.62$), discontent ($t(20) = 1.84$, $P = 0.08$), and drive ($t(20) = 0.58$, $P = 0.57$). However, participants rated significantly reduced dejection post compared to pre experimental testing ($t(20) = 3.61$, $P = 0.002$; Supplemental Fig. S1C [↗](#)).

Comparison with the previous study sample

We compared the weight-corrected FTP values of the current sample with those of the previous sample (Nold et al., 2025 [↗](#)) ($N = 39$, 21 females) for males and females separately using two-sample t -tests. The females employed in the current study (Fig. 1A [↗](#); dark blue) had a significantly

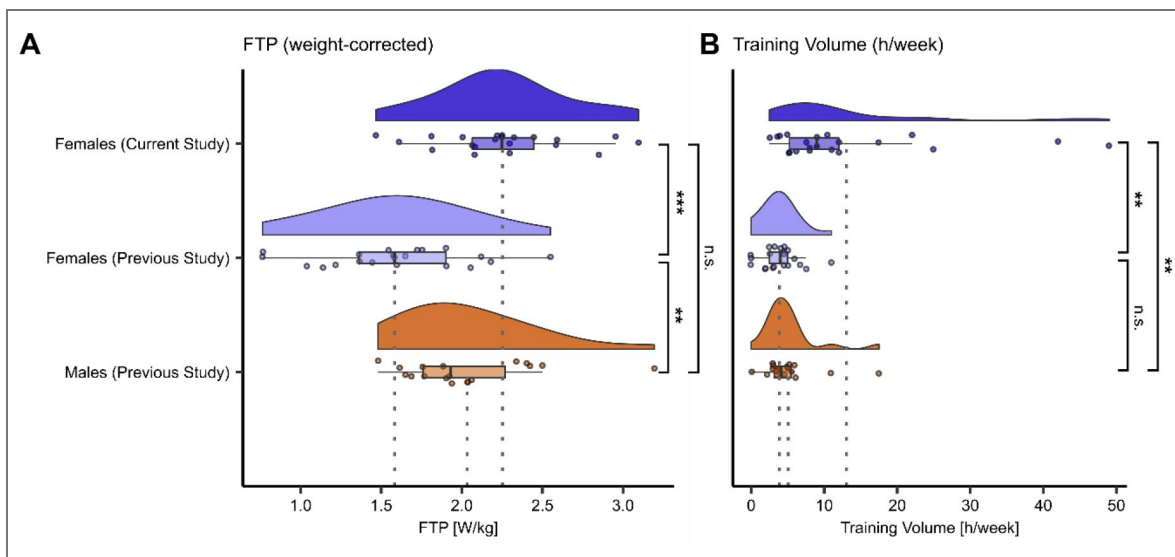


Figure 1. Comparing participant characteristics of the females of the current study ($N = 21$; dark blue) with the females ($N = 21$; light blue) and the males ($N = 18$; red) of the previous study (Nold et al., 2025).

(A) Weight-corrected Functional Threshold Power (FTP) differed significantly between the females in the current study and the females in the previous study ($P = 1.23 \times 10^{-5}$) but not between the females in the current study and males in the previous study ($P = 0.11$). (B) Training volume (hours per week) differed significantly between the females in the current study and the females ($P = 0.003$) and males ($P = 0.009$) of the previous study. n.s. = not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

higher FTP compared to the females of the previous study (light blue) ($t(40) = -5.00, P = 1.23 \times 10^{-5}$; Fig. 1A [↗](#)). There was no significant difference in the FTP between the females in this study (dark blue) and males in the previous study (red) ($t(36) = 1.65, P = 0.11$; Fig. 1A [↗](#)). The self-reported training volume was significantly higher in the females of the current study (dark blue) compared to the females (light blue) ($t(22) = 3.45, P = 0.003$, Fig. 1B [↗](#)) and males (red) ($t(24) = 2.82, P = 0.009$; Fig. 1B [↗](#)) of the previous study. Further, analyses comparing the females of the previous study and the females of the current study concerning height, weight, and BMI revealed no significant differences but a significant age difference ($t(32.98) = 3.27, P = 0.003$) (Supplemental Fig. S2 [↗](#)). Overall, this suggests that this current study included females of overall higher fitness levels compared to the previous study.

Successful pain calibration and exercise intervention

We calibrated pain intensity levels corresponding to 30, 50, and 70 on a Visual Analog Scale (VAS), where VAS 0 marks the pain threshold (Supplemental Fig. S3A–B [↗](#)). On the experimental day, we introduced an online rating scale where the pain threshold corresponded to VAS 50 (Supplemental Fig. S3C–D [↗](#)) to more accurately track the temporal dynamics of pain perception through online ratings and incorporate non-painful sensation. Participants perceived the stimulus intensities as significantly different for pressure ($\beta = 1.13, SE = 0.07, t(553.13) = 16.84, P < 2 \times 10^{-16}$; Supplemental Table S6 [↗](#)) and heat pain ($\beta = 1.75, SE = 0.08, t(532.61) = 21.46, P < 2 \times 10^{-16}$; Supplemental Table S8 [↗](#)). The anticipated intensities of VAS 50 and VAS 70 were rated above the pain threshold on the online scale, indicating that they were perceived as painful (Supplemental Fig. S4 [↗](#)), despite the ratings being lower than the anticipated intensities, potentially due to habituation effects between days (Ellerbrock et al., 2015 [↗](#); May et al., 2012 [↗](#)). We achieved a significant difference between HI and LI exercise conditions in absolute power ($t(20) = 19.34, P = 2.043e-14, d = 0.97$; Fig. 2A [↗](#)), relative power (%FTP; $t(20) = 42.35, P < 2.2e-16, d = 7.97$; Fig. 2B [↗](#)), HR ($t(13) = 20.98, P = 2.073e-11, d = 3.45$; Fig. 2C [↗](#)), and ($t(20) = 17.17, P = 1.942e-13, d = 4.61$; Fig. 2D [↗](#)), confirming the successful implementation of the exercise intervention at the anticipated intensities (55% FTP at LI exercise and 100% at HI exercise).

Greater pain relief after high-intensity exercise at higher stimulus intensities

In the current study, participants provided online ratings throughout the stimulus duration. We calculated the maximum pain ratings (in the following referred to as pain ratings) across the whole stimulus duration (0–17 seconds), which closely correspond to the pain ratings provided in the previous study (Nold et al., 2025 [↗](#)), where participants were asked to rate the most painful sensation after the stimulus concluded. To statistically test the effect of exercise intensity (HI and LI) on pain ratings, we calculated separate Linear Mixed Effect models (LMER) with the dependent variables overall pain ratings, pressure pain ratings, and heat pain ratings, respectively. All models included exercise intensity or the interaction of exercise intensity and stimulus intensity as a fixed effect, as well as subject, trial, and block as random effects.

There was no main effect of exercise intensity on overall pain ratings ($\beta = -3.07, SE = 2.14, t(1119.79) = -1.43, P = 0.15$) as well as no main effect of exercise intensity on pressure pain ratings ($\beta = -2.96, SE = 2.15, t(556.23) = -1.37, P = 0.17$; Fig. 3A [↗](#)) or heat pain ratings ($\beta = -3.99, SE = 2.92, t(540.57) = -1.37, P = 0.17$; Fig. 3A [↗](#)). However, an interaction of exercise intensity and stimulus intensity on overall pain ratings was evident ($\beta = -0.22, SE = 0.10, t(1114.27) = -2.21, P = 0.03$). This interaction was driven by the interaction of exercise intensity and stimulus intensity during heat pain ($\beta = -0.35, SE = 0.11, t(531.93) = -3.183, P = 0.002$; Fig. 3B–C [↗](#)). Post-hoc *t*-tests revealed that the effect was especially evident at the highest stimulus intensity ($P = 0.004$; Fig. 3B–C [↗](#)). For pressure pain, this interaction of exercise intensity and stimulus intensity was not significant ($\beta = -0.10, SE = 0.09, t(548.88) = -1.05, P = 0.29$; Fig. 3B–C [↗](#)) but a similar trend was evident when considering the post-hoc *t*-tests (Fig. 3B–C [↗](#)), where pain relief following HI compared to LI was greater at the highest stimulus intensity ($P = 0.07$). The full model outputs and post-hoc *t*-tests can be found in Supplemental Tables S1–S9 [↗](#).

Figure 2. Implementation of high-intensity (HI) and low-intensity (LI) exercise.

(A) Absolute power (Watts), (B) Relative power (%FTP), (C) heart rate in beats per minute (bpm), and (D) rating of perceived exertion (RPE; BORG scale) during LI (green) and HI (purple) cycling were all significantly different. *P*-values were calculated using paired *t*-tests (two-tailed, (absolute/relative) power: *N* = 21, heart rate: *N* = 14, BORG rating: *N* = 21). n.s. = not significant, * *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001.

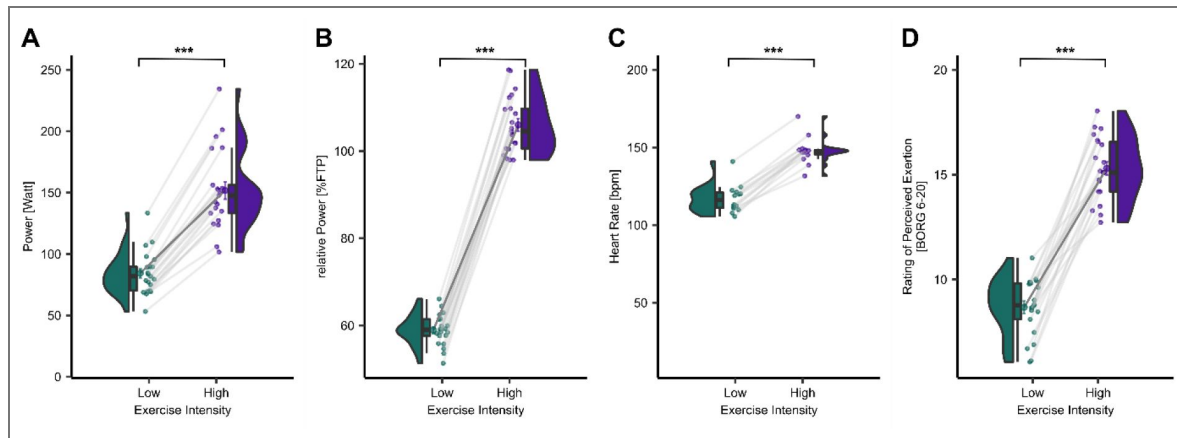
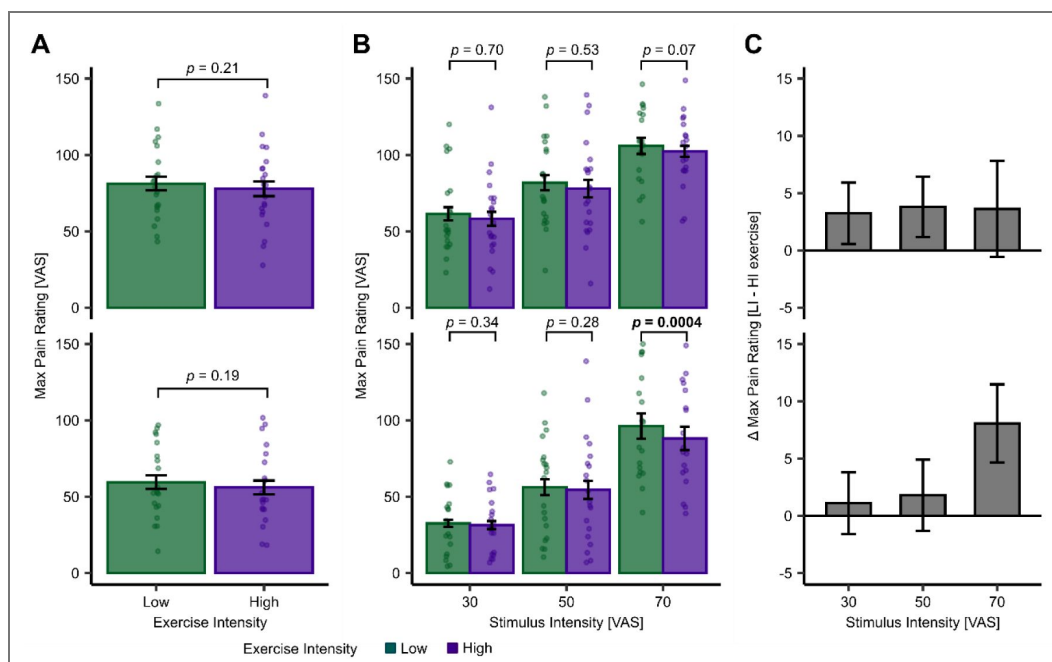


Figure 3. The effect of exercise intensity and stimulus intensity on pain ratings.

(A) Pressure (top; *P* = 0.17) and heat pain ratings (bottom; *P* = 0.17) for LI (green) and HI (purple) exercise. (B) A significant interaction between exercise intensity and stimulus intensity was observed in heat (*P* = 0.002) but not pressure pain (*P* = 0.29). Post-hoc *t*-tests revealed that this interaction in heat pain was driven by the highest stimulus intensity (VAS 70; *P* = 0.004). (C) Differences between HI and LI exercise (LI - HI) for pressure (top) and heat (bottom) pain ratings visualised at each stimulus intensity. Dots depict subject-specific pain ratings averaged across trials. *P*-values were calculated using post-hoc *t*-tests for the respective LMER models. Error bars depict the SEM (pressure: *N* = 21, heat: *N* = 20).



Discussion

In this study, we investigated exercise-induced pain modulation between high-intensity (HI) and low-intensity (LI) exercise in a sample of fit females ($N = 21$). In a previous study by our group (Nold et al., 2025 [↗](#)), exploratory analyses suggested a three-way interaction of fitness levels, sex, and drug treatment, where males showed hypoalgesia after HI compared to LI exercise with increasing fitness levels, which was diminished when naloxone was administered. These effects were not evident in females. In this current study, we could show that, for heat pain, fit females showed a similar pain relief after HI compared to LI exercise, especially for highly painful stimuli. A similar trend was observed for pressure pain. These results suggest that fit females show a hypoalgesic response to HI compared to LI exercise. Together with our previous findings (Nold et al., 2025 [↗](#)), this underscores that the hypoalgesic response to exercise depends on fitness level but not on sex.

Comparison with the previous study (Nold et al., 2025 [↗](#))

We confirmed that the females of the current sample showed significantly higher fitness level (as measured in weight-corrected FTP) than the females of the previous study (Nold et al., 2025 [↗](#)). Furthermore, the absolute training volume was significantly higher compared to the males and females of the previous study, suggesting overall higher fitness levels of the females of the current study. Interestingly, although the training volume of the females of the current study was significantly higher compared to the males of the previous study, the FTP did not significantly differ. Furthermore, as pain is a dynamic experience, we utilised real-time pain ratings in the current study as opposed to static post-stimulus pain ratings (Nold et al., 2025 [↗](#)) to better capture the temporal dynamics of pain. Previous research (Koyama et al., 2004 [↗](#)) has shown that, in heat pain, real-time ratings account for $R^2 = 0.89$ of the variability of post-stimulus rating, where much of the variability for the post-stimulus intensity pain rating is accounted for by the peak response during the online ratings (adj. $R^2 = 0.3 - 0.4$), suggesting good comparability of both pain measures.

Effect of exercise and stimulus intensity on pain

Corresponding to our previous study (Nold et al., 2025 [↗](#)), there was no overall effect of exercise intensity on heat or pressure pain. However, we identified an interaction of stimulus intensity and exercise intensity that was driven by heat pain, with greater pain relief after HI compared to LI exercise at high stimulus intensity (VAS 70). A similar trend was evident in pressure pain, but it did not reach significance. These findings align with the results of our previous study (Nold et al., 2025 [↗](#)), where exercise-induced pain modulation after HI compared to LI exercise was more prominent in heat than pressure pain.

A multitude of findings on exercise-induced hypoalgesia have been derived from earlier studies with male-dominated samples with higher fitness levels (Geisler et al., 2019 [↗](#); Haier et al., 1981 [↗](#); Janal et al., 1984 [↗](#); Scheef et al., 2012 [↗](#)), and results on sex dependent effects in exercise-induced hypoalgesia are still equivocal (Rice et al., 2019 [↗](#)). Of those studies that have included both sexes in their samples (Naugle et al., 2014 [↗](#); Niwa et al., 2022 [↗](#)), a dose-response relationship of aerobic exercise intensity and the analgesic response could be shown in the overall sample, where high-intensity (70% heart rate reserve (HRR)) aerobic exercise produced larger hypoalgesic effects compared to moderate-intensity (50% HRR) exercise (Naugle et al., 2014 [↗](#); Niwa et al., 2022 [↗](#)) and low-intensity (30% HRR) exercise (Niwa et al., 2022 [↗](#)). Importantly, despite including both sexes, these studies did not include sex as a factor in their analyses, potentially confounding the observed effects (Tesarz et al., 2012 [↗](#)). Of those studies that have explicitly accounted for sex as a moderating variable in exercise-induced hypoalgesia, some studies could show a hypoalgesic response from exercise in both sexes (Koltyn et al., 2014 [↗](#), 2001 [↗](#)), whereas others (Gajsar et al., 2017 [↗](#); Lemley et al., 2016 [↗](#)) found a stronger hypoalgesic response in females. When administering an aerobic exercise protocol, one study found the hypoalgesic response following exercise to be more pronounced in females compared to males (Vaegter et al., 2014 [↗](#)).

Despite some studies showing comparable or greater hypoalgesic effects in females, these studies rarely account for fitness levels or athletic status (Tesarz et al., 2012). Generally, it has been shown that athletes exhibit increased pain tolerance and decreased pain intensity compared to non-athletic controls (Geva and Defrin, 2013; Tesarz et al., 2012). Among the few studies that have explicitly considered both fitness levels and sex in the hypoalgesic response to exercise, one study (Sternberg et al., 2001) found that treadmill running induced hypoalgesia in the cold pressor test in females, regardless of athletic status, but not in males. Notably, both male and female athletes showed a significant decrease in forearm withdrawal latency from a radiant heat device after treadmill exercise (Sternberg et al., 2001). Further corroborating this, female athletes experienced more pronounced hypoalgesia following aerobic exercise, especially during cold pressor and thermal pain tests (Smith, 2004). For isometric exercise, one study (Black et al., 2017) found that females demonstrated analgesic responses regardless of their physical activity levels, which is in contrast to our previous findings (Nold et al., 2025), where females did not show an analgesic response after aerobic exercise. Thus, the extent of sex differences might also vary depending on the type of exercise, the pain stimulus administered, and the measurement method employed (Tesarz et al., 2012), emphasising the complexity of sex-specific pain modulation mechanisms. Overall, female athletes tend to report pain similarly to their male counterparts (Tesarz et al., 2012), suggesting that athletic training may mitigate typical sex differences in the hypoalgesic response after exercise and potentially reduce disparities observed in the general population. Our results advance this by showing that fitter females show a hypoalgesic response, and even after cycling for 10 minutes at HI compared to LI in response to heat pain.

Limitations

On the experimental day, heat stimuli with a target rating of VAS 30 were rated below the pain threshold. The anticipated intensities of VAS 50 and VAS 70 were rated above the pain threshold, suggesting that they were perceived as painful. However, the lower pain ratings on the experimental day as opposed to the calibration day are likely due to habituation effects between days (Ellerbrock et al., 2015; May et al., 2012). Moreover, as we compared HI and LI exercise but no sedentary rest condition, we cannot draw conclusions on the overall effect of exercise as opposed to no exercise on pain. Thus, it could be possible that even LI exercise suffices to evoke a hypoalgesic response (Niwa et al., 2022) or that individuals with higher fitness levels can show a distinction in the analgesic response based on the exercise intensity. However, previous research has suggested that HI exercise produces greater hypoalgesia compared to LI exercise (Jones et al., 2019; Naugle et al., 2014; Niwa et al., 2022; Smith, 2004; Vaegter et al., 2014, p. 201). Therefore, we chose the LI exercise as the control condition instead of rest to contribute to the understanding of the dose-response relationship between exercise intensities and hypoalgesic effects. Future research should investigate whether LI exercise suffices to induce an analgesic response.

Conclusion

In this study, we could show that fit females experience pain relief following high-intensity compared to low-intensity exercise, especially at high pain intensities. Together with our previous findings, this indicates that fitness level, rather than sex, plays a central role in modulating exercise-induced hypoalgesia.

Methods

Participants

Overall, $N = 22$ female participants were recruited for this study from local cycling clubs and by (online) advertisements. Participants were required to be aged between 18 – 50 years and have a body mass index (BMI) ranging from 18 – 30, and to be physically active. One participant was excluded due to a technical failure on the experimental day, resulting in incomplete data. For one

participant, heat pain calibration failed, resulting in $N = 20$ participants for heat pain data and $N = 21$ participants for pressure pain data (for participant characteristics, see [Table 1](#)). It is crucial to note that the $N = 21$ females included in the current study did not participate in the previous study (Nold et al., 2025).

Experimental Paradigm

The experimental paradigm was similar to that of the original study (Nold et al., 2025), using the same study setup and equipment (CPAR and TSA 2 and Wahoo KICKR bike). Furthermore, the same calibration procedures were used to calibrate heat and pressure pain as well as the functional threshold power using the FTP₂₀ test (Allen and Coggan, 2012, 2006) as implemented and validated in previous studies (Borszcz et al., 2018; McGrath et al., 2019; Nold et al., 2025). However, participants in the current study did not receive a pharmacological intervention and did not undergo fMRI scans; therefore, they received the painful stimuli outside the MR scanner whilst lying in a supine position. To monitor the painful stimuli more closely, participants provided online ratings whilst the pain stimulus was ongoing on a VAS scale ranging from “no sensation” (0) to “almost unbearably painful” (150), with VAS 50 marking “minimally painful” (pain threshold). Furthermore, we administered the same questionnaires as described in the previous study. For a detailed description of the study procedures and experimental paradigm, please refer to Nold et al. (2025). Overall, the current study consisted of one calibration day (Day 1) and one experimental day (Day 2).

Behavioural Data Acquisition

During cycling, heart rate (HR), maintained power (in watts), and cadence (in revolutions per minute (RPM)) were acquired continuously. In $N = 7$ participants, HR data were not recorded due to a technical failure, but were monitored throughout the cycling. The rating of perceived exertion (RPE) on the BORG scale, ranging from “no exertion” (6) to “maximal exertion” (20), was provided after each cycling block. Furthermore, the elapsed time between each cycling and pain within each block was five minutes to correspond with the mean time required in the previous study. Following the cycling, participants received a total of 18 pain stimuli, with 9 heat and 9 pressure stimuli applied in an alternating fashion and with their respective intensities (30, 50, 70 VAS) applied in a randomised order. Whilst the pain was ongoing, participants were asked to rate their currently perceived pain/non-painful sensation using the left and right buttons of a button box (Logitech). When their perception did not change, the cursor should remain at the same position. The online rating time exceeded the stimulus length by 2 seconds to capture changes in perception when the stimulus ramped down. The online ratings were sampled at 70 – 75 samples per second and interpolated at 0.9 seconds.

Statistical Analyses

The behavioural statistical analyses were performed in MATLAB and RStudio (Version 2021.09.1). We used the *lmer* and *emmeans* functions from the *lme4* package (Version 1.1-35.1) to conduct linear mixed effect (LMER) (Bates et al., 2014; Hox et al., 2010) and post-hoc *t*-tests in R, respectively. Furthermore, the *rstatix* package (Kassambara, 2019) was used to conduct one and two-sample *t*-tests.

To investigate whether the distribution of menstrual cycle phases as well as hormonal contraceptives differed within and between days, χ^2 -tests were calculated. Furthermore, we evaluated whether there was a significant effect of expectation about acute aerobic exercise on different pain dimensions and calculated one-sample *t*-tests (two-tailed). Furthermore, we conducted paired samples *t*-tests comparing pre- and post-mood ratings on the dimensions of dejection, fatigue, discontent, and drive. To compare the FTP, self-reported fitness levels, training volume, age, weight, height, and BMI between the previous sample (males and females) and the current sample, we calculated two-sample *t*-tests. To evaluate the exercise parameters within the current study, watts and HR were averaged across time and blocks for each participant and for

	Previous Study (Nold et al., 2025)			Current Study
	Overall (<i>M</i> ± <i>SD</i>)	Females (<i>M</i> ± <i>SD</i>)	Males (<i>M</i> ± <i>SD</i>)	Females (<i>M</i> ± <i>SD</i>)
<i>N</i>	39	21	18	21
Age (years)	26.03 (4.83)	25.33 (5.10)	26.83 (4.35)	32.29 (7.64)
Weight (kg)	70.95 (12.14)	63.33 (7.53)	79.83 (10.38)	63.6 (8.09)
Height (cm)	177.10 (9.08)	170.52 (6.35)	184.78 (4.58)	170 (5.95)
BMI	22.51 (2.64)	21.80 (2.59)	23.33 (2.53)	21 (1.93)
FTP (Watts/kg)	1.79 (0.48)	1.58 (0.44)	2.03 (0.40)	2.25 (0.41)
Training Volume (h/w)	4.44 (3.24)	3.88 (2.63)	5.08 (3.81)	13.10 (12.28)

M = Mean; *SD* = Standard deviation; kg = kilogram; cm = centimetre; FTP = Functional Threshold Power.
h/w = hours per week.

Table 1. Participant characteristics from the previous (Nold et al., 2025 [DOI](#)) and current study.

both exercise intensities. The RPE was also averaged across blocks for participants and exercise intensity. We calculated one-sample *t*-tests comparing the respective measures between HI and LI exercise.

For visualisation purposes, we displayed the online ratings (averaged across subjects, blocks, trials, and stimulus intensities) and the SEM following HI and LI exercise for heat and pressure pain separately (Supplemental Fig. S4 [↗](#)). For a more comprehensive understanding, the averaged online ratings at each stimulus intensity were visualised in the Supplemental Fig. S5 [↗](#). To statistically evaluate the online pain ratings and improve consistency with the post-stimulus pain ratings reported in the previous study (Nold et al., 2025 [↗](#)), we calculated the maximum rating by averaging the online rating across the whole stimulus duration (0 – 17 seconds) and extracting the peak rating. The peak pain ratings across the whole stimulus duration would most closely correspond to the post-stimulus pain ratings provided in the previous study, where participants were asked to rate the most painful sensation throughout the stimulus duration. We calculated separate LMER models with the dependent variables overall pain ratings, heat pain ratings, and pressure pain ratings, respectively. All LMER models included subject, trial, and block as random effects. In a first LMER model, we included exercise intensity as a fixed effect; in a second LMER model, the stimulus intensity and exercise intensity, as well as their interaction, served as fixed effects. Finally, we conducted post-hoc *t*-tests and applied a Tukey adjustment to correct for multiple comparisons.

Data availability [↗](#)

The dataset for the raw behavioural data generated in the current study is available from the corresponding author on reasonable request and after publication. All necessary data to evaluate the results of the study are included in the manuscript and supplementary materials.

Acknowledgements

We thank the participants for taking part in the study. Further, we thank Alexandra Tinnermann for her helpful comments.

Additional information

Funding

C.B. and J.N. are supported by ERC-AdG-883892-PainPersist. C.B. is supported by DFG SFB 289 Project A02 (Project-ID 422744262–TRR 289).

Author Contributions

Conceptualisation, C.B. and J.N.; Methodology, C.B. and J.N.; Investigation, C.B., J.N., T.F., and Z.G.; Visualisation, J.N.; Writing – Original Draft, C.B., J.N., and Z.G.; Funding Acquisition, C.B.; Resources, C.B.; Supervision, C.B.

Ethics

Human subjects: All participants gave informed written consent. The study was approved by the Ethics Board of the Hamburg Medical Association (PV7456/2020-10144-BO-ff). We support inclusive, diverse, and equitable conduct of research.

Code availability


This study was programmed using MATLAB 2021b and Psychophysics Toolbox (Version 3.0.19). For data visualisation, we used RStudio (Version 2021.09.1). The custom behavioural pipelines are available on the public repository https://github.com/jannenold/hotspin_analyses_behavioural_after_publication [↗](#).

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Additional files

Supplementary file 1.  Supplemental Figures S1–S5 and Supplemental Tables S1–S9.

References

- Allen H, Coggan A. (2012) *Training and Racing with a Power Meter*, 2nd VeloPress.
- Allen H, Coggan A. (2006) *Racing and training with a power meter* Boulder, CO: VeloPress.
- Bates D, Mächler M, Bolker B, Walker S. (2014) Fitting Linear Mixed-Effects Models using lme4. *Journal of Statistical Software* <https://doi.org/10.18637/jss.v067.i01>
- Black CD, Huber JK, Ellingson LD, Ade CJ, Taylor EL, Griffeth EM, Janzen NR, Sutterfield SL (2017) Exercise-Induced Hypoalgesia Is Not Influenced by Physical Activity Type and Amount. *Medicine & Science in Sports & Exercise* **49**:975 <https://doi.org/10.1249/MSS.0000000000001186> | PubMed
- Borszcz FK, Tramontin AF, Bossi AH, Carminatti LJ, Costa VP (2018) Functional Threshold Power in Cyclists: Validity of the Concept and Physiological Responses. *Int J Sports Med* **39**:737-742 <https://doi.org/10.1055/s-0044-101546> | PubMed
- Brellenthin AG, Crombie KM, Cook DB, Sehgal N, Koltyn KF (2017) Psychosocial Influences on Exercise-Induced Hypoalgesia. *Pain Medicine* **18**:538-550 <https://doi.org/10.1093/pm/pnw275> | PubMed
- Curran SL, Andrykowski MA, Studts JL (1995) Short Form of the Profile of Mood States (POMS-SF): Psychometric information. *Psychological Assessment* **7**:80-83 <https://doi.org/10.1037/1040-3590.7.1.80>
- de Bruijn ST, van Wijck AJM, Geenen R, Snijders TJ, van der Meulen WJTM, Jacobs JWG, Veldhuijzen DS (2011) Relevance of physical fitness levels and exercise-related beliefs for self-reported and experimental pain in fibromyalgia: an explorative study. *J Clin Rheumatol* **17**:295-301 <https://doi.org/10.1097/rhu.0b013e31822c5196> | PubMed
- Ellerbrock I, Wiehler A, Arndt M, May A. (2015) Nocebo context modulates long-term habituation to heat pain and influences functional connectivity of the operculum. *Pain* **156**:2222 <https://doi.org/10.1097/j.pain.0000000000000297> | PubMed
- Fillingim RB, King CD, Ribeiro-Dasilva MC, Rahim-Williams B, Riley JL (2009) Sex, Gender, and Pain: A Review of Recent Clinical and Experimental Findings. *The Journal of Pain* **10**:447-485 <https://doi.org/10.1016/j.jpain.2008.12.001> | PubMed
- Gajjar H, Titze C, Hasenbring MI, Vaegter HB (2017) Isometric Back Exercise Has Different Effect on Pressure Pain Thresholds in Healthy Men and Women. *Pain Med* **18**:917-923 <https://doi.org/10.1093/pm/pnw176> | PubMed
- Geisler M, Eichelkraut L, Miltner WHR, Weiss T. (2019) An fMRI study on runner's high and exercise-induced hypoalgesia after a 2-h-run in trained non-elite male athletes. *Sport Sciences for Health* <https://doi.org/10.1007/s11332-019-00592-8>
- Geva N, Defrin R. (2013) Enhanced pain modulation among triathletes: A possible explanation for their exceptional capabilities. *Pain* **154**:2317-2323 <https://doi.org/10.1016/j.pain.2013.06.031> | PubMed
- Haier RJ, Quaid K, Mills JSC (1981) Naloxone Alters Pain Perception After Jogging. *Psychiatry Research* **5**:231-232 [https://doi.org/10.1016/0165-1781\(81\)90052-4](https://doi.org/10.1016/0165-1781(81)90052-4) | PubMed
- Hox JJ, Moerbeek M, van de Schoot R (2010) *Multilevel Analysis: Techniques and Applications, Second Edition* (2nd) New York: Routledge.

- Janal MN**, Colt EWD, Crawford W, Glusman M. (1984) Pain Sensitivity, Mood and Plasma Endocrine Levels in Man Following Long-Distance Running: Effects of Naloxone. *Pain* **19**:13-25 [https://doi.org/10.1016/0304-3959\(84\)90061-7](https://doi.org/10.1016/0304-3959(84)90061-7) | PubMed
- Jones MD**, Nuzzo JL, Taylor JL, Barry BK (2019) Aerobic Exercise Reduces Pressure More Than Heat Pain Sensitivity in Healthy Adults. *Pain Medicine* **20**:1534-1546 <https://doi.org/10.1093/pm/pny289> | PubMed
- Kassambara A.** (2019) rstatix: Pipe-Friendly Framework for Basic Statistical Tests. CRAN. <https://doi.org/10.32614/CRAN.package.rstatix>
- Koltyn KF** (2000) Analgesia Following Exercise. *Sports Medicine* **29**:85-98 <https://doi.org/10.2165/00007256-200029020-00002> | PubMed
- Koltyn KF**, Brellenthin AG, Cook DB, Sehgal N, Hillard C. (2014) Mechanisms of exercise-induced hypoalgesia. *Journal of Pain* **15**:1294-1304 <https://doi.org/10.1016/j.jpain.2014.09.006> | PubMed
- Koltyn KF**, Trine MR, Stegner AJ, Tobar DA (2001) Effect of isometric exercise on pain perception and blood pressure in men and women. *Medicine and Science in Sports and Exercise* **33**:282-290 <https://doi.org/10.1097/00005768-200102000-00018> | PubMed
- Koyama Y**, Koyama T, Kroncke AP, Coghill RC (2004) Effects of stimulus duration on heat induced pain: the relationship between real-time and post-stimulus pain ratings. *Pain* **107**:256 <https://doi.org/10.1016/j.pain.2003.11.007> | PubMed
- Lemley KJ**, Senefeld J, Hunter SK, Hoeger Bement M. (2016) Only women report increase in pain threshold following fatiguing contractions of the upper extremity. *Eur J Appl Physiol* **116**:1379-1385 <https://doi.org/10.1007/s00421-016-3389-8> | PubMed
- Lindheimer JB**, Szabo A, Raglin JS, Beedie C, Carmichael KE, O'Connor PJ (2020) Reconceptualizing the measurement of expectations to better understand placebo and nocebo effects in psychological responses to exercise. *European Journal of Sport Science* **20**:338-346 <https://doi.org/10.1080/17461391.2019.1674926> | PubMed
- May A**, Rodriguez-Raecke R, Schulte A, Ihle K, Breimhorst M, Birklein F, Jürgens T p. (2012) Within-session sensitization and between-session habituation: A robust physiological response to repetitive painful heat stimulation. *European Journal of Pain* **16**:401-409 <https://doi.org/10.1002/j.1532-2149.2011.00023.x> | PubMed
- McGrath E**, Mahony N, Fleming N, Donne B. (2019) Is the FTP Test a Reliable, Reproducible and Functional Assessment Tool in Highly-Trained Athletes?. *Int J Exerc Sci* **12**:1334-1345 <https://doi.org/10.70252/rqoo7391> | PubMed
- Mogil JS** (2012) Sex differences in pain and pain inhibition: multiple explanations of a controversial phenomenon. *Nat Rev Neurosci* **13**:859-866 <https://doi.org/10.1038/nrn3360> | PubMed
- Naugle KM**, Naugle KE, Fillingim RB, Samuels B, Riley JLI (2014) Intensity Thresholds for Aerobic Exercise-Induced Hypoalgesia. *Medicine & Science in Sports & Exercise* **46**:817-825 <https://doi.org/10.1249/MSS.000000000000143> | PubMed
- Naugle KM**, Riley JL (2014) Self-reported Physical Activity Predicts Pain Inhibitory and Facilitatory Function. *Med Sci Sports Exerc* **46**:622-629 <https://doi.org/10.1249/MSS.0b013e3182a69cf1> | PubMed
- Niwa Y**, Shimo K, Ohga S, Tokiwa Y, Hattori T, Matsubara T. (2022) Effects of Exercise-Induced Hypoalgesia at Different Aerobic Exercise Intensities in Healthy Young Adults. *J Pain Res* **15**:3615-3624 <https://doi.org/10.2147/JPR.S384306> | PubMed
- Nold JI**, Fadai T, Büchel C. (2025) Acute aerobic exercise does not modulate pain potentially due to differences in fitness levels and sex effects – results from a pharmacological fMRI study. *eLife* **14** <https://doi.org/10.7554/eLife.102392.1>
- Rice D**, Nijs J, Kosek E, Wideman T, Hasenbring MI, Koltyn K, Graven-Nielsen T, Polli A. (2019) Exercise-Induced Hypoalgesia in Pain-Free and Chronic Pain Populations: State of the Art and Future Directions. *The Journal of Pain* **20**:1249-1266 <https://doi.org/10.1016/j.jpain.2019.03.005> | PubMed

Saanijoki T, Kantonen T, Pekkarinen L, Kalliokoski K, Hirvonen J, Malén T, Tuominen L, Tuulari JJ, Arponen E, Nuutila P, *et al.* (2022) Aerobic Fitness is Associated with Cerebral mu-Opioid Receptor Activation in Healthy Humans. *Medicine & Science in Sports & Exercise*
<https://doi.org/10.1249/MSS.0000000000002895> | PubMed

Scheef L, Jankowski J, Daamen M, Weyer G, Klingenberg M, Renner J, Mueckter S, Schürmann B, Musshoff F, Wagner M, *et al.* (2012) An fMRI study on the acute effects of exercise on pain processing in trained athletes. *Pain* **153**:1702-1714 <https://doi.org/10.1016/j.pain.2012.05.008> | PubMed

Schmitt A, Wallat D, Stangier C, Martin JA, Schlesinger-Irsch U, Boecker H. (2020) Effects of fitness level and exercise intensity on pain and mood responses. *European Journal of Pain* **24**:568-579
<https://doi.org/10.1002/ejp.1508> | PubMed

Sluka KA, Frey-Law L, Hoeger Bement M. (2018) Exercise-induced pain and analgesia? Underlying mechanisms and clinical translation. *Pain* **159**:S91-S97
<https://doi.org/10.1097/j.pain.0000000000001235> | PubMed

Smith LD (2004) The effects of competition and exercise on pain perception.

Sternberg WF, Boka C, Kas L, Alboyadjia A, Gracely RH (2001) Sex-Dependent Components of the Analgesia Produced by Athletic Competition. *The Journal of Pain* **2**:65-74
<https://doi.org/10.1054/jpai.2001.18236> | PubMed

Tesarz J, Schuster AK, Hartmann M, Gerhardt A, Eich W. (2012) Pain perception in athletes compared to normally active controls: a systematic review with meta-analysis. *Pain* **153**:1253-1262
<https://doi.org/10.1016/j.pain.2012.03.005> | PubMed

Vaegter HB, Handberg G, Graven-Nielsen T. (2014) Similarities between exercise-induced hypoalgesia and conditioned pain modulation in humans. *Pain* **155**:158-167
<https://doi.org/10.1016/j.pain.2013.09.023> | PubMed

Peer reviews

Reviewer #1 (Public review):

Summary:

The current study is a follow-up to a previously published study by the same research group (Nold *et al.* 2025). In the previous study, the authors had included a set of exploratory analyses which assessed the effects of fitness level (denominated by a relative FTP), sex, and drug treatment (Naxolone versus placebo). In this previous study, the authors state that "exploratory analysis showed a significant main effect of fitness level on differences in pain ratings in the [saline] condition... suggesting increased hypoalgesia with increasing fitness levels, pooled across all stimulus intensities".

In the current study, the authors have recruited an additional 22 female participants (21 included in analysis) from local cycling clubs to assess if fitness level does indeed impact exercise-induced hypoalgesia responses to experimental thermal and pressure pain models.

Strengths:

The current study has the potential to present a convincing argument about the effect of fitness level and potentially other factors (e.g., sex) on exercise-induced hypoalgesia responses. Combining data across two of their primary studies would be highly fruitful to the research community interested in this area. Specifically, it has the potential to inform sports medicine practitioners and how they administer exercise protocols to help those experiencing pain with a further consideration for the fitness level (and maybe sex) of their patients.

Weaknesses:

However, the current study makes several bold claims about the role of fitness level and sex on exercise-induced hypoalgesia, which I do not believe that this study on its own - or in conjunction with the previously published study by the same authors - can make at present. Namely, the current study does not appear to conduct any specific analyses between the cohorts from either study (current and present). The results mention a difference in the group mean values in "fitness level" between cohorts, but the analysis itself on pain responses/exercise-induced hypoalgesia is limited only to the cohort from the current study. If the authors wanted to provide a convincing argument that fitness level has an effect on exercise-induced hypoalgesia, then the analysis of this study would have to include an analysis between the groups considered to be of "high" and "low" fitness level. I do not think the current study does this. Instead, it makes an assumption from the previous study (Nold et al. 2025) which only states that "exploratory analysis showed a significant main effect of fitness level on differences in pain ratings in the [saline] condition... suggesting increased hypoalgesia with increasing fitness levels, pooled across all stimulus intensities". The analysis of this study would have to include fitness level "high fitness" versus "low fitness" of participants across both studies in its statistical model to properly discern if fitness level has an impact on exercise-induced hypoalgesia.

A similar comment can be made with respect to sex differences, as these have not been assessed in the analysis of this study either.

Another area of weakness in this study is how "fitness level" has been demarcated across participants. One issue is how authors have assumed that the current cohort is 'fit', whereas the previous cohort was 'less fit', meaning that the authors could be coming to false conclusions about fitness level. In detail, figures within the current study show a large overlap between the 'fit' and 'less fit' cohorts, where some participants have a higher relative functional threshold power (FTP) in the 'less fit' cohort than the 'fit' cohort and vice versa. Therefore, I believe the authors should better demarcate between those that are in the 'more fit' and 'less fit' groups according to a validated and well-established criterion from the kinesiology and sport science literature. That being said, I think this may be problematic in some ways as FTP is considered a relatively poor measure to denote fitness levels, a limitation highlighted in the previous study's review.

Altogether, whilst I commend the researchers on their body of work across the two studies, the current methods and analysis provide an incomplete assessment of their primary research question, and therefore, I would urge the authors to reconsider some of their methods/analysis and the framing of their results to better reflect the main research question they have attempted to answer. Likewise, I would recommend that readers ensure they consider the current results with caution until the authors have addressed some areas of concern which currently limit their main conclusions.

<https://doi.org/10.7554/eLife.109061.1.sa1>

Reviewer #2 (Public review):

This study addresses an important question regarding exercise-induced modulation of pain in women, but the conclusions appear to be based on relatively limited and selective evidence. The authors report an interaction between exercise intensity and stimulus intensity, which they interpret as evidence for exercise-induced hypoalgesia and conclude that fitness, but not sex, modulates this effect. However, this main result relies on a relatively small interaction that emerges only under specific conditions, with inconsistent findings across pain modalities and stimulus intensities, and an analysis approach that does not fully exploit the continuous pain ratings collected. The lack of a baseline condition further limits the interpretability of the findings as reflecting hypoalgesia, and overall, the data provide a rather constrained basis for drawing broader conclusions.

Strengths:

(1) The focus on women is important and timely, particularly given the ambiguity in prior findings and the historical bias toward male-dominated samples.

(2) The attempt to revisit previous findings in a new cohort is valuable in principle.

Weaknesses:

(1) The core interpretation may not be fully supported by the data

The central claim—that the results demonstrate exercise-induced hypoalgesia and its dependence on fitness but not sex—does not appear to be fully supported by the evidence presented.

1.1 Lack of baseline condition

The absence of a no-exercise baseline substantially limits interpretation. The study compares high- and low-intensity exercise, but without a baseline, it is not possible to determine whether either condition produces hypoalgesia or hyperalgesia relative to calibration. The observed HI-LI difference, therefore, reflects only a relative contrast between exercise intensities, not an absolute reduction in pain. As a result, attributing the findings to "hypoalgesia" may be difficult to justify fully.

1.2 Lack of internal replication across conditions

The reported effect is highly specific and does not clearly generalise across the experimental design. It emerges significantly only for heat pain at the highest stimulus intensity, with no clear effects for other intensities and for pressure pain. Moreover, the main statistical result is a relatively small interaction effect with a modest *p* value, which translates into a difference of approximately 6-8 VAS units on a 150 scale. This combination—a small effect size, limited statistical strength, and restriction to a single condition—substantially weakens the evidence for a robust or generalisable effect.

1.3 Deviations from the original study and selective use of data

Although framed as a follow-up to previous work, the current study introduces substantial methodological changes, particularly in the acquisition and scaling of pain ratings (continuous vs post-hoc ratings, modified VAS with sub-threshold range). Despite collecting rich continuous data, the analysis focuses on peak responses to approximate the previous study. While this may aid comparability, it results in a strong emphasis on a single data point (highest intensity), rather than leveraging the full dataset. This limits both interpretability and comparability.

1.4 Over-reliance on null results regarding sex differences

The conclusion that fitness, but not sex, modulates exercise-induced pain may not be directly supported by the data presented. The current study includes only highly fit women, and comparisons with men or less-fit women rely on non-significant differences in a previous cohort. The absence of a significant difference does not provide evidence for equivalence, and no formal statistical support for a null effect is provided. As such, conclusions about the absence of sex differences would unfortunately benefit from more cautious interpretation.

(2) Limited sample and lack of diversity

The dataset is narrow in scope, comprising a small sample (*N* = 21) of healthy, highly fit women. Key demographic characteristics (e.g. age range, BMI distribution) are not fully presented, explored or discussed. This limits generalisability and makes it difficult to draw

broader conclusions about exercise-induced pain modulation in women, as the main focus of the study.

(3) Methodological choices limit the interpretability of the data

Several methodological decisions would benefit from stronger justification:

3.1 The use of a non-standard VAS scale (0-150 with a fixed pain threshold at 50) is unconventional and may influence how participants report pain, while limiting comparability with related literature.

3.2 Participants explicitly reported expecting exercise to reduce pain, introducing a potential confound that is not presently addressed.

3.3 A more comprehensive use of the full time series of pain ratings would provide a stronger and more transparent basis for interpretation of the present findings.

<https://doi.org/10.7554/eLife.109061.1.sa0>