Remote Ischaemic Preconditioning Increase Tolerance to Experimentally Induced Cold Pain.

Ryan Norbury*, Lieben Dickens, Ian Grant, Alison Emery, Stephen D Patterson

Faculty of Sport, Allied Health and Performance Science. St Mary's University, Twickenham.

Corresponding Author:

Dr Ryan Norbury

Faculty of Sport, Allied Health and Performance Science, St Mary's University, Twickenham, London, TW1 4SX.

Email: ryan.norbury@stmarys.ac.uk

Telephone: (+44) 020 8240 4000 (ext. 4838)

Funding details: No funding was received for this project.

Disclosure Statement: No authors declare any conflict of interest with this work.

Abstract

Ischaemic preconditioning (IPC) applied locally and remotely has been shown to reduce pain which may underpin its ergogenic effect on exercise performance, however it is unclear how many IPC cycles are needed to induce hypoalgesia. Therefore the purpose of this study was to examine the number of cycles of IPC on experimental pain perception. Sixteen healthy participants underwent four, randomised, experimental sessions where they either underwent a sham protocol (1 × 5 minutes at 20 mmHg), and 1, 2 or 3 cycles × 5 minutes of remote IPC at 105% of limb occlusion pressure. Ten minutes post-intervention, participants underwent a cold-pressor test where pain threshold, pain tolerance and pain intensity were examined and compared between conditions with a one-way repeated measure analysis of variance. Pain threshold was not different between conditions ($P = 0.065$); but pain tolerance was increased by ~30% in the 1 × 5 condition, 2 × 5 condition, and 3 × 5 condition compared to the sham condition. No differences in pain tolerance were seen between the different number of cycles (all $P > 0.05$). There was also no difference in the perception of pain 30 s into the cold pressor test ($P = 0.279$). Remote IPC appears to significantly improve tolerance to pain which may have significant

implications for endurance performance and exercise rehabilitation, but this warrants further investigation.

Highlights:

- We found that one, two or three cycles of ischaemic preconditioning improved cold pain tolerance by 30% in compared to a sham protocol, but there was no clear effect of IPC on pain threshold or pain intensity.
- The pain reported during IPC decreased from cycle one to cycle three in the three cycle condition, suggesting a potential conditioned pain modulation effect.
- An increase in pain tolerance may explain why IPC can improve exercise performance and IPC itself could be used as a tool to improve tolerance to pain.

Key Words: ischemic preconditioning; passive blood flow restriction; pain; hypoalgesia; cold pressor test

Introduction

Ischaemic preconditioning (IPC), also known as passive blood flow restriction (Patterson et al., 2019), involves intermittently occluding blood flow to the limb(s) whilst at rest through the application of a tourniquet. This method typically involves one to four cycles of several minutes of inflation and deflation (O'Brien & Jacobs, 2021; Patterson et al., 2019). Originally, the purpose of IPC was to protect cardiac tissue against ischemia reperfusion injury during surgery (Tapuria et al., 2008). However, in the last decade, IPC has been found to enhance exercise performance (Crisafulli et al., 2011; De Groot et al., 2010; Santos de Oliveira Cruz et al., 2015), exercise recovery (Franz et al., 2018; Page et al., 2017; Patterson et al., 2021) and post-operative outcomes (Memtsoudis et al., 2010, 2014; Pereira et al., 2016). Pain, which is defined as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" (Raja et al., 2020) is a common sensation that is experienced across these different physiological perturbations, and a reduction in pain perception may be one of the mechanisms which underpins the ergogenic effect of IPC (Astokorki & Mauger, 2017; Behrens et al., 2020; O'Brien & Jacobs, 2022; Pereira et al., 2020)..

To date, there is a paucity of data investigating whether IPC and remote IPC (occluding tissues non-local to the ones of interest) can produce a hypoalgesic response to a range of nociceptive stimuli (e.g.,

exercise-induced pain, delayed onset muscle soreness or post-operative pain). Initial work in patients undergoing surgery has found that IPC has reduced the intensity of post-operative pain with one cycle of occlusion (Memtsoudis et al., 2010, 2014). Furthermore, IPC has been consistently shown to reduce the amount of soreness (a sensory component of pain) in response to damaging physical exercise (Daab et al., 2021; Franz et al., 2018; Page et al., 2017). More recently, IPC has been shown to reduce subsequent experimental pain from post-exercise muscle ischemia, but did not increase mechanical pain pressure threshold or exercise-induced pain (Angius et al., 2022). Mechanical pressure pain intensity has also been shown to be reduced after remote IPC in males, but not females, which warrants further investigation on potential sex-differences(Pereira et al., 2020)., Finally, work by Slysz & Burr (2021) found that IPC of the upper limb reduced the total time under pain after two minutes of cold-water immersion of the same hand, and IPC has also been shown to increase vasoconstriction during cold-water immersions, but unfortunately no measures of pain were assessed (Horiuchi et al., 2015) Therefore, it is still not well established if remote IPC can induce hypoalgesia. It is also currently unclear as to whether remote IPC can induce a robust hypoalgesic response. This is important to establish as the physiological responses which cause IPC to improve exercise performance are not well understood (O'Brien & Jacobs, 2022).

Hypoalgesic effects have been induced just a single 5-minute cycle of IPC (Memtsoudis et al., 2010, 2014), but three to four cycles are typically employed (Patterson et al., 2019) and no further improvement in exercise performance has been observed with 8 × 5 minute versus 4 × 5 minute cycles (Cocking et al., 2018). It is also currently unknown how many cycles are needed to induce a hypoalgesic effect. Furthermore, due to the widespread use of arbitrary occlusion pressures (i.e. 220 mmHg) (O'Brien & Jacobs, 2021), research should be performed with the applied pressure being fully occlusive (e.g. ≥100% of limb occlusion pressure [LOP]) to standardise the intervention and allow for better comparisons between studies Therefore, the purpose of this study was threefold. To investigate the effect of remote IPC on cold-pain perception, to determine if there is a difference in the hypoalgesic response with a differing number of IPC cycles, and to determine if there is a sex-difference response in pain perception with remote IPC. Based on previous work by Slysz and Burr (Slysz & Burr, 2021), we hypothesised that remote IPC would delay the onset of pain, increase the tolerance to cold pain, and decrease pain intensity experienced during a cold pressor test. Additionally, we hypothesised that three cycles of IPC would induce a greater pain tolerance compared to a sham condition and one cycle of IPC.

Methods

Participants

Sixteen healthy participants (8 female, 8 male) total mean ± SD age: 27.8 ± 4.5 years; height: 172 ± 7 cm, mass; 74.9 ± 12.4 kg) volunteered to take part in the study after providing written informed consent.

All experimental procedures were approved by the ethics committee at St Mary's University, Twickenham.

Sample Size Calculation

The sample size recruited was based on an α-priori calculation for a repeated measures ANOVA (four conditions). To detect our minimally interesting large effect size of partial eta squared (η_p^2) 0.16 (Cohens f = 0.4) with a 95% chance (power of 0.95), at and alpha level of 0.05, a total of 15 participants would be needed (actual power = 0.957).

Experimental Design

Participants visited the lab on five separate occasions, separated by a minimum of 72 hours and at a similar time of day (± 2 hours) for one familiarisation session and four experimental visits. Prior to each lab visit, participants were instructed to abstain from caffeine (8 hours), analgesics (8 hours) and strenuous exercise (24 hours). Participants were informed that IPC could modulate pain sensitivity, but they were not informed of the specific hypothesis of the research.

Familiarisation. Participants were initially familiarised with the pain intensity scale (see 'pain intensity' below) and then underwent one five-minute cycle of IPC at 105% of LOP in the seated upright position. Participants were then familiarised with the cold pressor test and the measures associated with it.

Experimental Visits. Participants initially arrived at the laboratory and were seated for five minutes in an upright position and the blood flow restriction tourniquet was fitted to their right leg. After five minutes of rest, LOP was automatically determined with the personalised tourniquet system. Whilst still seated, participants then completed one of the four experimental interventions in which the order was randomised. These conditions were a sham protocol (participants were informed it was a lower pressure intervention) which was 1 cycle of 5 minutes of cuff inflation at a pressure of 20 mmHg (SHAM), one cycle of IPC at 105% of LOP (1 × 5), two cycles of IPC with 5 minutes of reperfusion at 105% LOP (2 × 5), or three cycles of IPC with 5 minutes of reperfusion at 105% LOP (3 × 5). Participants were asked to rate the intensity of muscle pain in their leg in the final 20 s of cuff inflation. Ten minutes after the deflation of the final IPC cycle, participants then completed the cold-pressor test (see 'cold pressor test'). A schematic of the experimental visits can be seen in figure 1.

Equipment and Measures

Remote Ischaemic Preconditioning. A 11.5 cm wide and 86 cm long contoured cuff (Delfi Medical Systems, Vancouver, Canada) was placed at the most proximal part of the right leg. The cuff was inflated by a pneumatic personalised tourniquet system (Delfi Medical Systems, Vancouver, Canada) which automatically calculates LOP and maintains the desired pressure. Once LOP was determined in

the seated upright position, the cuff was inflated to 105% of LOP to ensure that arterial inflow was completely stopped. Inflation and reperfusion times were set at 5 minutes each.

Cold Pressor Test. A container of water and ice between 0 and 2 ºC was used for induction of experimental pain. Participants were stood next to the container and instructed to submerge their entire hand right within the water and move it around in a gentle circular motion to prevent the formation of a micro-climate around the hand. Participants were told to keep their hand in the water for as long as they could tolerate, or until they reached the maximum cut off time of 8 minutes (which was not revealed to the participants). Participants were asked to verbally indicate the onset of pain, their pain intensity every 30 s and the offset of pain once they had reached their tolerance and removed their hand from the water. No verbal encouragement was provided by the investigator during the test.

Pain Intensity. The intensity of muscle pain was recorded on the 0 - 10 Cook pain scale (Cook et al., 1998) with 0 at 'no pain at all' and 10 at 'worst imaginable pain (almost intolerable)'. Participants were asked to anchor the worst imaginable pain the greatest intensity of exercise-induced pain they have ever experienced and were able to rate above 10 in order of magnitude to how much greater the pain was to the worst exercise-induced pain they ever felt, in order to prevent a ceiling effect.

Data Analysis

For the cold pressor test, the onset time in seconds was taken when the participants first felt pain. Pain tolerance was taken as the number of seconds from hand submersion in the water to the point where the participants removed their hand from water. Pain offset was the time taken from removal of the hand from the water until no more pain was felt in the right hand.

Statistical Analysis

All data was analysed with a One-way repeated measures analysis of variance (ANOVA) with condition (1 × 5, 2 × 5, 3 × 5 and SHAM) as a within subject factors and Sex (Male, Female) as between subject factors for temporal data (pain threshold, pain tolerance and pain offset). Pain intensity data (pain during IPC and pain during cold-pressor test were analysed with a Friedman ANOVA. Data was initially checked for the assumption of normality with a Shapiro-wilk test on the standardised residuals. If reasonable normality could not be assumed then the data was Log10 transformed. Data was also checked for the assumption of sphericity with Mauchly's test and was Greenhouse-Geiser corrected if the assumption was violated. If a significant Condition or Sex × condition effect was observed, subsequent post-hoc paired samples t-tests were performed which were Bonferroni-Holm(Holm, 1979) corrected were used to establish differences between conditions. Statistical significance was set at $P < 0.05$.

Effect sizes were reported for ANOVAs as partial eta squared (η_p^2) with small, medium, and large effects denoted by values of ≥0.01, ≥0.06 and ≥0.14 respectively. For post-hoc paired samples t-tests, effect size was determined with Cohen's D with ≥0.2, ≥0.5, and ≥0.8 for small, medium and large effects respectively.(Cohen, 2013) All data is presented as mean ± SD unless stated otherwise.

Results

All participants were able to tolerate and complete each IPC protocol without any complications. The average LOP was 202 ± 19 mmHg.

Pain During the IPC

The median and interquartile range for pain intensity reported at the end of each IPC cycle in the 3 × 5 condition was 3 [2 – 3], 2 [1 - 3] and 2 [1 – 3] (between 'mild pain' and 'moderate pain') from cycles one to three, respectively. A Friedman ANOVA revealed an effect of cycle condition ($\chi^2 = 8.450$, $P = 0.015$), with cycle 2 ($P = 0.021$) and cycle 3 ($P = 0.018$) pain being lower than cycle one with no difference between cycle 2 and cycle 3 ($P = 0.900$).

Pain Threshold

There was no condition × sex interaction ($F_{3,\ 42} = 0.241$, $P = 0.867$, $\eta_p^2 = 0.017$), no main effect of condition ($F_{3,\ 42} = 2.599$, $P = 0.065$, $\eta_p^2 = 0.156$) and no main effect of sex ($F_{1,\ 14} = 0.032$, $P = 0.861$, $\eta_p^2 = 0.002$) on pain threshold (see figure 2 and 3 b).

Pain Tolerance

Two participants were able to make it to cut-off time of 8 minutes in at least one condition, therefore their tolerance data was excluded. There was no condition × sex interaction ($F_{3,\ 36} = 0.235$, $P = 0.871$, $\eta_p^2 = 0.019$) or main effect of Sex ($F_{1,\ 12} = 2.725$, $P = 0.125$, $\eta_p^2 = 0.185$). However, there was a main effect of condition ($F_{3,\ 36} = 11.890$, $P < 0.001$, $\eta_p^2 = 0.498$). Post-hoc tests revealed that cold pain tolerance time was longer in 1 × 5 ($P = 0.006$, $d = 1.11$), 2 × 5 ($P = 0.003$, $d = 1.27$) and 3 × 5 ($P = 0.002$, $d = 1.41$) in comparison to SHAM. No difference in pain tolerance was seen between 1 × 5 and 2 × 5 ($P = 0.807$, $d = 0.07$) or 1 × 5 and 3 × 5 ($P = 0.428$, $d = 0.36$), and there was no difference between 2 × 5 and 3 × 5 ($P = 0.396$, $d = 0.45$) (see figure 2 and 3a).

Pain Intensity

For pain intensity, two participants were unable to reach 30 s in the cold pressor test in at least one condition, therefore the analysis was performed on $n = 14$ (6 male, 8 female). There was no condition × sex interaction ($F_{1.85,\ 22.27} = 0.595$, $P = 0.548$, $\eta_p^2 = 0.047$) and no main effect of sex ($F_{1,\ 12} = 0.146$, $P = 0.709$, $\eta_p^2 = 0.012$) and no effect of condition ($F_{1.85,\ 22.27} = 1.345$, $P = 0.279$, $\eta_p^2 = 0.101$).

Pain Offset

Two participants did not reach pain tolerance in at least one experimental condition, which confounds their pain offset time, therefore these participants data was removed for the subsequent analysis. For pain offset, there was no condition × sex interaction ($F_{3,\ 36} = 0.110$, $P = 0.945$, $\eta_p^2 = 0.009$) or main effect of sex ($F_{1,\ 12} = 5.728$, $P = 0.589$, $\eta_p^2 = 0.025$), but there was a main effect of condition ($F_{3,\ 36} = 5.728$, $P = 0.003$, $\eta_p^2 = 0.323$). Post-hoc tests revealed that pain offset was not different between SHAM and 1 × 5 ($P = 0.682$, $d = 0.28$) but pain offset was longer in 2 × 5 ($P = 0.043$, $d = 0.88$) and 3 × 5 ($P = 0.043$, $d = 0.90$) compared to SHAM. No other differences in pain offset were seen between any other condition ($P > 0.05$)(see figure 2 and 3d).

Discussion

The aim of the present study was to investigate whether IPC induced a hypoalgesic effect and reduce the perception to cold-induced experimental pain. In consonance with our primary hypothesis, the main finding of the present study demonstrates that remote IPC can improve tolerance to cold pain, whereas threshold and pain intensity was unaffected which was in contrast to our primary hypothesis. Our secondary hypothesis was that three cycles of IPC would provide a greater hypoalgesic effect than one cycle which the findings of this study did not confirm.

The IPC protocol induced a mild to moderate amount of pain (i.e. 2-3 points out of 10) within each cycle which was reduced in the second and third cycle in the 3 × 5 condition. This reduction in pain intensity during the IPC may indicate a conditioned pain modulation effect (Damien et al., 2018) which has previously been demonstrated with cuff algometry (Cummins et al., 2020) and occurs within remote tissues (Kennedy et al., 2016; Mertens et al., 2021).

Pain threshold was not different between sham and any of the IPC conditions (figure 2b), although there was a large effect size ($\eta_p^2 = 0.156$) for a condition effect, which may indicate some effect of remote IPC. The finding of no difference in pain threshold is in agreement with the findings of Angius et al. (Angius et al., 2022) who saw no difference in local or contralateral pain pressure threshold measured with an algometer after 3 × 5 minute cycles of IPC at a pressure of 220 mmHg in comparison to a SHAM condition (20 mmHg). The threshold to pain perception likely reflects the responsiveness of nociceptors to noxious stimuli (Smith & Lewin, 2009), with an increased pain threshold mediated by a desensitisation of the nociceptors. Based on the current evidence, it is unlikely that IPC desensitises the group III/IV afferent nociceptors as previously postulated (De Oliveira Cruz et al., 2017; O'Brien & Jacobs, 2022). However, if a conditioned pain modulation effect was present (as suggested by a reduction in pain during the IPC) then it would be expected that there would also be an increase in pain threshold as is observed with typical conditioned pain modulations paradigms (Kennedy et al., 2016).

This may have occurred due to the large effect size but, it is also not well established how sensitive the threshold from cold pain is to a given intervention.

Pain tolerance was increased with a large effect (approximately 30 s; d = 1.11-1.41) with one, two and three cycles of IPC in comparison to sham (see figure 2a), and this hypoalgesic effect was observed in thirteen out of fourteen of the analysed participants. This is the first study to demonstrate an increase cold pain tolerance after IPC, and this was unlikely driven by a lower intensity of pain perceived as after the first 30 s of hand submersion pain intensity was not different between conditions, indicating that the perception during the test may not have been altered which is in agreement with previous work using cold pain (Slysz & Burr, 2021). However, it should be noted that due to short tolerance times (30 – 60 s) in multiple participants, we were unable to analyse data over more than one time point, and a higher resolution of pain intensity assessment (e.g. every 5 s)(Graven-Nielsen et al., 1997) may have revealed altered pain kinetics in response to IPC. Improvements in pain tolerance after just one cycle of IPC is partially in alignment with previous studies which have utilised a single cycle and observed reductions post-operative pain (Memtsoudis et al., 2014). Interestingly, the increase in pain tolerance was not different between males and females which is in contrast to similar work (Pereira et al., 2020) who only found hypoalgesic effects of remote IPC in Males. However, more research is needed to verify potential sex differences with IPC on pain perception. As a likely consequence of an increased pain tolerance, there was an increase in time taken for the pain to subside in the 2 × 5 and 3 × 5 condition in comparison to sham (figure 2d). Interestingly, this effect did not persist for the 1 × 5 condition despite a longer tolerance time, but it is not certain whether this indicates and advantage to one cycle over two to three cycles. Nevertheless, this further supports the notion that remote IPC was able to improve pain tolerance. The mechanism(s) which appear to underpin an increase in pain tolerance may be conditioned pain modulation and/or psychological factors. In particular, we speculate that prior exposure to pain and discomfort (i.e. from the remote IPC interventions) may have altered the psychophysiological state of the participants (Marocolo et al., 2022) and 'primed/habituated' them to cope better with the unpleasant sensory and affective components of the cold-pressor test (Savitha et al., 2022) . This would explain why pain tolerance increased with no other apparent change, and why one cycle of IPC was sufficient to improve tolerance.

Methodological Considerations

Participants were informed that all conditions could impact pain sensitivity, but the sensory difference of the cuff pressure between 20 mmHg and 105% LOP may have altered the expectation of the effect and induced a placebo or nocebo effect. This could have been verified with the inclusion of a control (i.e. quiet rest) condition, or the sham procedure could have utilised a short period of high pressure occlusion (e.g., 1 × 5 minute at 105% LOP).

Female participants were not tested at a similar phase of the menstrual cycle. Whilst we acknowledge that the different phases of the menstrual cycle may have impacted results, there is lacking evidence that suggests different phases meaningfully influence pain perception (Iacovides et al., 2015; Klatzkin et al., 2010; Kowalczyk et al., 2006). Furthermore, it would be difficult to fully control for the menstrual cycle phases as there was multiple experimental visits which would make it impractical to delay some experimental visits by multiple weeks to ensure that female participants were in a similar phase of the menstrual cycle.

Conclusion

In summary, remote ischemic preconditioning of one cycle of five minutes above the LOP was able to improve pain tolerance by approximately 30 s (large effect) which could explain why IPC has previously been shown to improve exercise performance during high intensity endurance exercise which induces high levels of muscle pain (De Groot et al., 2010; Kido et al., 2018; Santos de Oliveira Cruz et al., 2015). There was no further improvement in pain tolerance with two or three cycles, but pain offset was longer in two and three cycles (but not one cycle) compared to sham. The mechanism(s)which IPC might improve pain tolerance is through a conditioned pain or pain habituation effect, but further research is warranted to establish the mechanisms responsible..

References

Angius, L., Pageaux, B., Crisafulli, A., Hopker, J., & Marcora, S. M. (2022). Ischemic preconditioning of the muscle reduces the metaboreflex response of the knee extensors. *European Journal of Applied Physiology*, *122*(1), 141–155. https://doi.org/10.1007/s00421-021-04815-0

Astokorki, A. H. Y., & Mauger, A. R. (2017). Transcutaneous electrical nerve stimulation reduces exercise-induced perceived pain and improves endurance exercise performance. *European Journal of Applied Physiology*, *117*(3), 483–492. https://doi.org/10.1007/s00421-016-3532-6

Behrens, M., Zschorlich, V., Mittlmeier, T., Bruhn, S., & Husmann, F. (2020). Ischemic Preconditioning Did Not Affect Central and Peripheral Factors of Performance Fatigability After Submaximal Isometric Exercise. *Frontiers in Physiology*, *11*. https://doi.org/10.3389/fphys.2020.00371

Cocking, S., Wilson, M. G., Nichols, D., Cable, N. T., Green, D. J., Thijssen, D. H. J., & Jones, H. (2018). Is there an optimal ischemic-preconditioning dose to improve cycling performance? *International Journal of Sports Physiology and Performance*, *13*(3), 274–282.

Cohen, J. (2013). *Statistical power analysis for the behavioral sciences*. Routledge.

Cook, D. B., O'connor, P. J., Oliver, S. E., & Lee, Y. (1998). Sex differences in naturally occurring leg muscle pain and exertion during maximal cycle ergometry. *International Journal of Neuroscience*, *95*(3–4), 183–202.

Crisafulli, A., Tangianu, F., Tocco, F., Concu, A., Mameli, O., Mulliri, G., & Caria, M. A. (2011). Ischemic preconditioning of the muscle improves maximal exercise performance but not maximal oxygen uptake in humans. *Journal of Applied Physiology*, *111*(2), 530–536. https://doi.org/10.1152/japplphysiol.00266.2011

Cummins, T. M., Kucharczyk, M. M., Graven-Nielsen, T., & Bannister, K. (2020). Activation of the descending pain modulatory system using cuff pressure algometry: Back translation from man to rat. *European Journal of Pain (United Kingdom)*, *24*(7), 1330–1338. https://doi.org/10.1002/ejp.1580

Daab, W., Bouzid, M. A., Lajri, M., Bouchiba, M., & Rebai, H. (2021). Brief cycles of lower-limb occlusion accelerate recovery kinetics in soccer players. *Physician and Sportsmedicine*, *49*(2), 143–150. https://doi.org/10.1080/00913847.2020.1785260

Damien, J., Colloca, L., Bellei-Rodriguez, C.-É., & Marchand, S. (2018). Pain modulation: from conditioned pain modulation to placebo and nocebo effects in experimental and clinical pain. *International Review of Neurobiology*, *139*, 255–296.

De Groot, P. C. E., Thijssen, D. H. J., Sanchez, M., Ellenkamp, R., & Hopman, M. T. E. (2010). Ischemic preconditioning improves maximal performance in humans. *European Journal of Applied Physiology*, *108*(1), 141–146. https://doi.org/10.1007/s00421-009-1195-2

De Oliveira Cruz, R. S., Pereira, K. L., Lisbôa, F. D., & Caputo, F. (2017). Could small-diameter muscle afferents be responsible for the ergogenic effect of limb ischemic preconditioning? *Journal of Applied Physiology*, *122*(3), 718–720. https://doi.org/10.1152/japplphysiol.00662.2016

Franz, A., Behringer, M., Harmsen, J. F., Mayer, C., Krauspe, R., Zilkens, C., & Schumann, M. (2018). Ischemic Preconditioning Blunts Muscle Damage Responses Induced by Eccentric Exercise. *Medicine and Science in Sports and Exercise*, *50*(1), 109–115. https://doi.org/10.1249/MSS.0000000000001406

Graven-Nielsen, T., Arendt-Nielsen, L., Svensson, P., & Jensen, T. S. (1997). Quantification of local and referred muscle pain in humans after sequential i.m. Injections of hypertonic saline. *Pain*, *69*(1–2), 111–117. https://doi.org/10.1016/S0304-3959(96)03243-5

Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics*, 65–70.

Horiuchi, M., Endo, J., & Thijssen, D. H. J. (2015). Impact of ischemic preconditioning on functional sympatholysis during handgrip exercise in humans. *Physiological Reports*, *3*(2), 1–9. https://doi.org/10.14814/phy2.12304

Iacovides, S., Avidon, I., & Baker, F. C. (2015). Does pain vary across the menstrual cycle? A review. *European Journal of Pain*, *19*(10), 1389–1405.

Kennedy, D. L., Kemp, H. I., Ridout, D., Yarnitsky, D., & Rice, A. S. C. (2016). Reliability of conditioned pain modulation: A systematic review. *Pain*, *157*(11), 2410–2419. https://doi.org/10.1097/j.pain.0000000000000689

Kido, K., Suga, T., Tanaka, D., Honjo, T., Fujita, S., Hamaoka, T., & Isaka, T. (2018). Remote ischemic preconditioning accelerates systemic O 2 dynamics and enhances endurance during work-to-work cycling exercise . *Translational Sports Medicine*, *1*(5), 204–211. https://doi.org/10.1002/tsm2.36

Klatzkin, R. R., Mechlin, B., & Girdler, S. S. (2010). Menstrual cycle phase does not influence gender differences in experimental pain sensitivity. *European Journal of Pain*, *14*(1), 77–82.

Kowalczyk, W. J., Evans, S. M., Bisaga, A. M., Sullivan, M. A., & Comer, S. D. (2006). Sex differences and hormonal influences on response to cold pressor pain in humans. *The Journal of Pain*, *7*(3), 151–160.

Marocolo, M., Hohl, R., Arriel, R. A., & Mota, G. R. (2022). Ischemic preconditioning and exercise performance: are the psychophysiological responses underestimated? *European Journal of Applied Physiology*. https://doi.org/10.1007/s00421-022-05109-9

Memtsoudis, S. G., Della Valle, A. G., Jules-Elysse, K., Poultsides, L., Reid, S., Starcher, B., Ma, Y., & Sculco, T. P. (2010). Perioperative inflammatory response in total knee arthroplasty patients: Impact of limb preconditioning. *Regional Anesthesia and Pain Medicine*, *35*(5), 412–416. https://doi.org/10.1097/AAP.0b013e3181e82e8e

Memtsoudis, S. G., Stundner, O., Yoo, D., Gonzalez Della Valle, A., Boettner, F., Bombardieri, A. M., Jules-Elysee, K., Poultsides, L., Ma, Y., & Sculco, T. P. (2014). Does limb preconditioning reduce pain after total knee arthroplasty? A randomized, double-blind study. *Clinical Orthopaedics and Related Research*, *472*(5), 1467–1474. https://doi.org/10.1007/s11999-013-3106-4

Mertens, M. G., Hermans, L., Crombez, G., Goudman, L., Calders, P., Van Oosterwijck, J., & Meeus, M. (2021). Comparison of five conditioned pain modulation paradigms and influencing personal factors in healthy adults. *European Journal of Pain*, *25*(1), 243–256.

O'Brien, L., & Jacobs, I. (2021). Methodological Variations Contributing to Heterogenous Ergogenic Responses to Ischemic Preconditioning. In *Frontiers in Physiology* (Vol. 12). Frontiers Media S.A. https://doi.org/10.3389/fphys.2021.656980

O'Brien, L., & Jacobs, I. (2022). Potential physiological responses contributing to the ergogenic effects of acute ischemic preconditioning during exercise: A narrative review. *Frontiers in Physiology*, *13*(November), 1–12. https://doi.org/10.3389/fphys.2022.1051529

Page, W., Swan, R., & Patterson, S. D. (2017). The effect of intermittent lower limb occlusion on recovery following exercise-induced muscle damage: A randomized controlled trial. *Journal of Science and Medicine in Sport*, *20*(8), 729–733. https://doi.org/10.1016/j.jsams.2016.11.015

Patterson, S. D., Hughes, L., Warmington, S., Burr, J., Scott, B. R., Owens, J., Abe, T., Nielsen, J. L., Libardi, C. A., Laurentino, G., Neto, G. R., Brandner, C., Martin-Hernandez, J., & Loenneke, J. (2019). Blood flow restriction exercise position stand: Considerations of methodology, application, and safety. In *Frontiers in Physiology* (Vol. 10, Issue MAY). Frontiers Media S.A. https://doi.org/10.3389/fphys.2019.00533

Patterson, S. D., Swan, R., Page, W., Marocolo, M., Jeffries, O., & Waldron, M. (2021). The effect of acute and repeated ischemic preconditioning on recovery following exercise-induced muscle damage. *Journal of Science and Medicine in Sport*, *24*(7), 709–714. https://doi.org/10.1016/j.jsams.2021.02.012

Pereira, F. E. C., Mello, I. L., Pimenta, F. H. D. O. M., Costa, D. M., Wong, D. V. T., Fernandes, C. R., Lima Junior, R. C., & Gomes, J. M. A. (2016). A clinical experimental model to evaluate analgesic effect of remote ischemic preconditioning in acute postoperative pain. *Pain Research and Treatment*, *2016*, 1–7. https://doi.org/10.1155/2016/5093870

Pereira, H. M., de Lima, F. F., Silva, B. M., & Kohn, A. F. (2020). Sex differences in fatigability after ischemic preconditioning of non-exercising limbs. *Biology of Sex Differences*, *11*(1). https://doi.org/10.1186/s13293-020-00338-z

Raja, S. N., Carr, D. B., Cohen, M., Finnerup, N. B., Flor, H., Gibson, S., Keefe, F., Mogil, J. S., Ringkamp, M., & Sluka, K. A. (2020). The revised IASP definition of pain: Concepts, challenges, and compromises. *Pain*, *161*(9), 1976.

Santos de Oliveira Cruz, R., Alves de Aguiar, R., Turnes, T., Leonardo Pereira, K., Caputo, F., Rso, C., & Aguiar, de R. (2015). Effects of ischemic preconditioning on maximal constant-load cycling performance. *J Appl Physiol*, *119*, 961–967. https://doi.org/10.1152/japplphysiol.00498.2015.-This

Savitha, D., Anto, T., & Thomas, T. (2022). Effects of repeated exposures to experimental cold pain

stimulus on pain perception in healthy young Indian men. *Medical Journal Armed Forces India*, *78*, S238–S245.

Slysz, J. T., & Burr, J. F. (2021). Ischemic Preconditioning: Modulating Pain Sensitivity and Exercise Performance. *Frontiers in Physiology*, *12*. https://doi.org/10.3389/fphys.2021.696488

Smith, E. S. J., & Lewin, G. R. (2009). Nociceptors: a phylogenetic view. *Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology*, *195*(12), 1089–1106. https://doi.org/10.1007/s00359-009-0482-z

Tapuria, N., Kumar, Y., Habib, M. M., Amara, M. A., Seifalian, A. M., & Davidson, B. R. (2008). Remote ischemic preconditioning: a novel protective method from ischemia reperfusion injury—a review. *Journal of Surgical Research*, *150*(2), 304–330.