## ABSTRACT

**Objectives:** Exercise-induced hypoalgesia (EIH) is a decrease in the pain sensitivity after exercise. Individuals with chronic pain show less EIH after one exercise session compared with pain-free individuals possibly due to pain in exercising muscles. The primary aim of this randomized controlled cross-over study was to compare the EIH response at the exercising thigh muscle following exercises performed with painful vs. non-painful muscles. Secondary aims were to explore if a reduced EIH response was confined to the painful muscle, and whether the muscle pain intensity and the EIH responses were negatively associated.

**Methods:** In two sessions, 34 pain-free participants received a painful (hypertonic saline, 5.8%) injection and a control (isotonic saline, 0.9%) injection in the right thigh muscle before performing a 3 min isometric wall squat exercise. Pressure pain thresholds (PPTs) were assessed at both thighs and the left neck/shoulder at baseline, after injections and after exercise. Pain intensities in the thighs were rated on numerical rating scales (NRS: 0-10).

**Results:** Hypertonic saline induced moderate thigh pain at rest (NRS: 4.6 ± 2.1) compared to the control injection (NRS: 0.3 ± 0.4; $p<0.001$). EIH at the thighs and neck/shoulder were not different between sessions (Injected thigh: 0 kPa; 95% CI: -51 to 52; Contralateral thigh: -6 kPa; 95% CI: -42 to 30; neck/shoulder: 19 kPa; 95% CI: -6 to 44). No significant associations between pain intensity ratings immediately after the Painful injection and EIH responses at any assessment sites were found (right thigh: $\beta$=0.08, 95% CI: -12.95 to 20.64, p=0.64, left thigh: $\beta$=-0.33, 95% CI: -27.86 to 0.44, p=0.06; neck/shoulder: $\beta$=-0.18, 95% CI: -15.11 to 4.96, p=0.31).

**Conclusions:** Pain in the area of an exercising muscle did not reduce local or systemic EIH responses.

**Trial registration number:** NCT04354948.

**Keywords:** exercise; exercise-induced hypoalgesia; experimental pain; isometric exercise; pain modulation; saline injection.