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AUTHOR
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ORIGINAL ARTICLE

Intra- and interindividual reliability of muscle pain induced by an intramuscular injection of hypertonic saline injection into the quadriceps

Samuel A. Smith[1] | **Ryan Norbury**[1,2] | **Adam J. Hunt**[1] | **Alexis R. Mauger**[1]

[1]School of Sport and Exercise Sciences, University of Kent, Canterbury, UK

[2]Faculty of Sport, Allied Health, and Performance Science, St Mary's University, Twickenham, UK

Correspondence
Alexis R. Mauger, School of Sport and Exercise Sciences, University of Kent, Canterbury, UK.
Email: l.mauger@kent.ac.uk

Abstract

Background: Intramuscular injections of hypertonic saline are commonly used to induce experimental muscle pain, but reliability data on this technique are lacking. This study investigated the intra- and interindividual reliability of pain measures from a hypertonic saline injection into the vastus lateralis.

Methods: Fourteen healthy participants (6 female) attended three laboratory visits where they received an intramuscular injection of 1 mL hypertonic saline into the vastus lateralis. Changes in pain intensity were recorded on an electronic visual analogue scale, and pain quality was assessed after pain had resolved. Reliability was assessed with the coefficient of variation (CV), minimum detectable change (MDC) and intraclass correlation coefficient (ICC) with 95% CIs.

Results: Mean pain intensity displayed high levels of intraindividual variability (CV = 16.3 [10.5–22.0]%) and 'poor' to 'very good' relative reliability (ICC = 0.71 [0.45–0.88]) but had a MDC of 11 [8–16] au (out of 100). Peak pain intensity exhibited high levels of intraindividual variability (CV = 14.8 [8.8–20.8]%) with 'moderate' to 'excellent' levels of relative reliability (ICC = 0.81 [0.62–0.92]), whereas the MDC was 18 [14–26] au. Measures of pain quality exhibited good reliability. Interindividual variability in pain measures was high (CV > 37%).

Conclusions: Intramuscular injections of 1 mL of hypertonic saline into the vastus lateralis display substantial levels of interindividual variability, but MDC is below the clinically important changes in pain. This model of experimental pain is suitable for studies involving repeated exposures.

Significance: Many pain research studies have performed intramuscular injections of hypertonic saline to investigate responses to muscle pain. However, the reliability of this technique is not well established. We examined the pain response over three repeated sessions of a hypertonic saline injection. The pain induced by hypertonic saline has considerable interindividual variability but has largely acceptable intraindividual reliability. Therefore, the injections of hypertonic saline to induce muscle pain are a reliable model of experimental muscle pain.

1 | INTRODUCTION

Pain 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). Arising from the stimulation of Group III and IV nociceptive afferents in response to a noxious stimulus (Mense, 2008), muscle pain is deep-tissue, diffuse and often described as a 'throbbing', 'aching', 'cramping' and 'taut' (Coppieters et al., 2005; Ford et al., 2021; Graven-Nielsen & Arendt-Nielsen, 2003; Rubin et al., 2010; Schabrun & Hodges, 2012; Schilder et al., 2014; Smith et al., 2020). With its experience associated with impairments in muscle function, exercise participation and overall quality of life (De Groot & Fagerström, 2011; Graham et al., 1986; Mense, 2008), muscle pain can have notable clinical, societal and economic implications. It is therefore important to have a comprehensive understanding of its impact (and the underlying mechanisms) on function and to develop knowledge of pain management interventions (Olesen et al., 2012).

In the study of muscle pain, experimental pain models provide a standardized means to activate the nociceptive system and stimulate acute muscle pain in healthy populations (Olesen et al., 2012; Staahl & Drewes, 2004). This allows for the assessment of the psychophysiological, behavioural and neurophysiological responses to be recorded (Graven-Nielsen & Arendt-Nielsen, 2003; Staahl & Drewes, 2004). One particular method of pain induction is the intramuscular injection of hypertonic saline. When injected, hypertonic saline activates Group III/IV nociceptive afferents and is suggested to induce an acute experience of pain that is comparable to clinical muscle pain (Graven-Nielsen et al., 1997a). It is considered safe, does not impair peripheral function and can be placebo-controlled in the form of isotonic saline (Graven-Nielsen et al., 1997b; Graven-Nielsen & Arendt-Nielsen, 2003; Norbury et al., 2022a).

Previously, pain induction from hypertonic saline has been broadly employed in basic science investigations (Martinez-Valdes et al., 2020, 2021), clinical trials (Arendt-Nielsen et al., 1996; Graven-Nielsen et al., 2000; Sörensen et al., 1998) and exercise physiology research. Here, it has been commonly applied as a single bolus during single-limb isometric or whole-body dynamic exercise tasks to study the impact of acute elevated muscle pain on muscle function, and to understand the mechanisms that underpin both exercise tolerance and changes in motor control (Canestri et al., 2021; Ciubotariu et al., 2004; Farina et al., 2004; Graven-Nielsen et al., 1997c; Khan et al., 2011; Norbury et al., 2022b; Smith et al., 2020, 2021).

However, in experimental settings that require repeated hypertonic saline injections, it is important to evaluate the within-individual and between-individual response. Despite the extensive use of this technique, there is limited evidence for its reliability (Graven-Nielsen et al., 1997a). Whilst the within-individual reliability of hypertonic saline infusion into the tibialis anterior has previously been examined (Graven-Nielsen et al., 1997a), a reliability analysis in another commonly used site, is warranted. The vastus lateralis (VL) is a large, monoarticular muscle that has a primary role in the production of force in a range of daily activities and tasks (e.g. locomotion, stair climbing, running, cycling) (Raasch et al., 1997; Sasaki & Neptune, 2006). Based on this, a number of recent investigations have applied the hypertonic saline model of muscle pain in the VL (Canestri et al., 2021: Norbury et al., 2022a; Norbury et al., 2022b; Smith et al., 2020; Smith et al., 2021), providing evidence for the impact of acute muscle pain in a context that is translatable and functionally relevant. Therefore, the aim of this study was to quantify the intra- and interindividual reliability of pain intensity and pain quality measures after the singular bolus of 1 mL (a volume that is commonly used and induces a robust pain response (Norbury et al., 2022b; Smith et al., 2020; Smith et al., 2021)) 5.85% hypertonic saline injected into the VL. We hypothesized that hypertonic saline-evoked pain would have acceptable test–retest reliability (i.e. intraclass correlation coefficient >0.5, coefficient of variation <10%) for measures of pain intensity but would have a large interindividual variability (i.e. coefficient of variation >10%).

2 | METHODS

2.1 | Ethics statement

This study was ethically approved by the School of Sport & Exercise Sciences (University of Kent) Research Ethics Advisory Group (Ref no: Prop 24_2018_19) and conducted in conformity with the Declaration of Helsinki (but without being registered). Written informed consent was provided by participants prior to their voluntary participation.

2.2 | Participants

A convenience sample of 14 healthy individuals (8 male, 6 female; mean ± SD: age, 25 ± 5 years; stature 172.9 ± 8.5 cm; body mass 71.9 ± 12.7 kg) volunteered to take part in the study. Six of the participants indicated that they had experience of receiving at least one hypertonic saline injection within the last year, whilst the remaining eight had not experienced the technique. The estimated sample size was calculated using a 'hypothesis approach' for the intraclass correlation coefficient (Borg et al., 2022). With the minimum ICC considered above poor (0.5; Koo & Li, 2016)

with an expected reliability of 0.85 (between good and excellent) for the primary outcome measure (mean pain intensity), with an alpha level of 0.05 and 80% power, a total of 12 participants were required. To account for a 10% dropout rate, an $n = 14$ was recruited for the study.

Participants with a phobia of needles, blood-borne diseases (e.g. HIV and hepatitis B), lower limb injuries, cardiovascular disease, neurological disorders, any food allergy and anyone taking medication for pre-existing pain were excluded from the study. Prior to each visit, participants were asked to refrain from consuming caffeine, alcohol and analgesics (e.g. paracetamol) 8, 48 and 6 h before their visit, respectively. Participants confirmed compliance with these prerequisites at the start of each visit.

2.3 | Experimental procedures

Participants were required to attend the laboratory on three separate occasions separated by a minimum of 1 week. In the first visit, anthropometric measures were recorded and participants were familiarized with all perceptual measures (see Section 2.4). Each laboratory visit consisted of participants receiving an intramuscular injection of hypertonic saline into the vastus lateralis of the right leg (see Section 2.3.1) with participants required to assess the experience of muscle pain in terms of pain intensity and pain quality (see Section 2.4.1). A schematic of the experimental design and protocol is outlined in Figure 1.

2.3.1 | Hypertonic saline injection

Participants received the intramuscular injections whilst seated at rest with their knees positioned at a 90° angle. In each visit, a single bolus of 1 mL of 5.85% sterile hypertonic saline solution (B Braun Medical Industries) was injected into the vastus lateralis of the right leg (middle third of the lateral aspect of the thigh between the greater trochanter and the lateral femoral condyle of the femur) at an angle of 90° (i.e. perpendicular to the muscle belly) and at a depth of ~25 mm. To mitigate tissue damage and scarring, the injection site was moved 2–4 mm proximally or distally from the previous injection site on the second and third visits. Injections were performed manually over a 20-s period (5 s pause after needle insertion, 10 s of solution infusion [infusion rate 0.1 mL/s], followed by 5 s pause prior to needle removal) using a 3 mL Luer-Lok plastic syringe attached to a 25 G × 38 mm SurGuard2 disposable stainless-steel needle (Terumo). Prior to any injection, the injection site and surrounding area were inspected and palpated to confirm the absence of local tenderness or muscle soreness and then cleaned with an alcohol swab. Upon the start of the saline infusion, participants were asked to continuously rate the intensity of the muscle pain experienced (see Section 2.4.1) until the return to the state of 'no pain'. The quality of muscle pain was reported through the completion of the Short-form McGill Pain Questionnaire (SF-MPQ) and the Long-form McGill Pain Questionnaire (LF-MPQ).

2.4 | Perceptual and psychological measurements

2.4.1 | Assessment of muscle pain

At the start of each visit, participants were asked to rate pain expectation (0 = 'no pain' to 10 = 'worst possible pain') and the confidence to cope with the expected

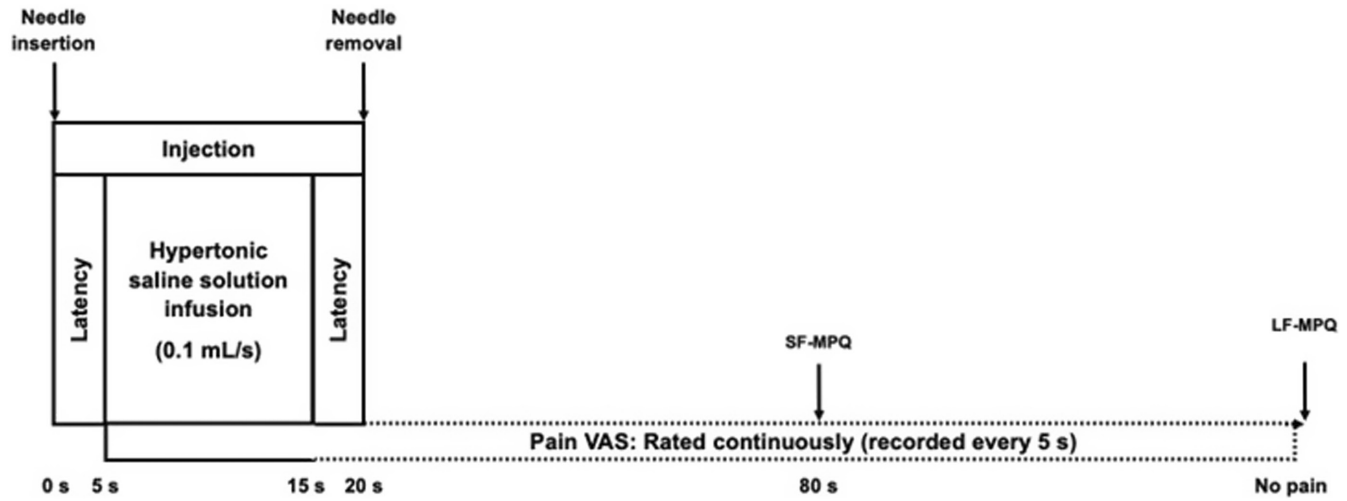

FIGURE 1 Schematic of the experimental protocol. LF-MPQ, long-form McGill pain questionnaire; SF-MPQ, short-form McGill pain questionnaire.

level of pain (0 = 'not confident at all' to 10 'completely confident') on a visual analogue scale (VAS). Muscle pain was assessed in terms of pain intensity and pain quality. Pain intensity was continuously measured on a moment-to-moment basis using an electronic VAS ranging from 0 ('no pain') to 100 ('most intense pain') based on the numeric perceived pain scale (Cook et al., 1997). Participants were instructed to anchor the rating of pain intensity to prior experiences of naturally occurring EIP (Astokorki & Mauger, 2017). The VAS device automatically recorded the pain intensity reading every 5 s onto an external SD card. The data recorded from this were then used to calculate various measurements including mean pain intensity (average of all pain ratings) from pain onset to pain offset (time at first and last value above zero), peak pain intensity (highest value), time to peak intensity (from the start of infusion to peak pain), pain duration (time from first to last measurement above zero), time spent above 50% peak intensity and area under the VAS curve.

Pain quality was measured at two time points using two different questionnaires: the long-form McGill pain questionnaire (LF-MPQ) and the short-form McGill pain questionnaire (SF-MPQ). The LF-MPQ, a comprehensive questionnaire commonly utilized within studies employing the hypertonic saline model of pain (see Ford et al., 2021), quantifies the sensory, affective, evaluative and miscellaneous components, as well as an overall intensity of the pain (Melzack, 1975). This questionnaire can be inefficient in a time-sensitive research setting (Olesen et al., 2012), so the LF-MPQ was administered upon the return to 'no pain'. The SF-MPQ (Melzack, 1987), which allows for a more rapid assessment of pain quality, quantifies the sensory and affective dimensions, as well as an overall intensity of pain. The SF-MPQ was verbally reported at 60-s postinjection, which provided the participant with sufficient time to ruminate about the quality of the pain experienced and corresponds with the point at which the pain from a resting hypertonic saline injection in the VL is reported to be at, or approaching peak pain intensity (Smith et al., 2020). Words that were selected by at least one-third of participants were included as a commonly selected word in the results.

2.4.2 | Self-reported psychological data

The positive and negative affect schedule (PANAS; Watson et al., 1988) was recorded prior to each experimental visit to check whether participants arrived in a similar psychological state.

2.5 | Statistical analysis

Systematic error for each variable was assessed with a one-way repeated-measures analysis of variance (ANOVA) with an alpha level of 0.05. Data were considered to have systematic error when a significant main effect of session was observed ($p < 0.05$). Uncorrected post-hoc tests were used to determine systematic error between specific sessions. A one-way repeated-measures ANOVA was also performed to determine whether there were pretest differences in PANAS scores, pain expectation and pain coping confidence across visits and whether a main effect was observed, subsequent post-hoc tests were Holm–Bonferroni corrected.

Absolute variability was quantified with the coefficient of variation (CV) for each participant, which was calculated as: (SD/mean × 100). A CV value of <10% was considered good (Stokes, 1985). The typical error was calculated from an online Excel spreadsheet, and the minimum detectable change was derived as the typical error × 1.96 × √2 (Hopkins, 2017; Weir, 2005).

Relative reliability of pain VAS measures was calculated with the intraclass correlation coefficient (ICC) using a 3.1 model (fixed effects). Intraclass correlation coefficient scores of <0.5, 0.5–0.75, >0.75–0.9 and >0.9 were considered as poor, moderate, good and excellent, respectively (Koo & Li, 2016). Reliability of pain quality measures was analysed with Cronbach's alpha where values of >0.75 indicate good consistency (Tavakol & Dennick, 2011). Repeated-measures ANOVAs, Cronbach's α and ICCs were calculated in SPSS Statistics v28.0 (SPSS, IBM). The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions. Data are reported as the mean point estimate and 95% CI.

3 | RESULTS

All participants attended each visit in a similar psychological state as indicated by the PANAS positive affect ($F_{2,26} = 0.163$, $p = 0.850$) and negative affect ($F_{2,26} = 1.318$, $p = 0.285$). There was also no significant difference in pain expectation ($F_{1.253,16.293} = 3.177$, $p = 0.086$) and pain coping confidence over the three testing sessions ($F_{1.432,18.616} = 2.545$, $p = 0.118$). Hypertonic saline injections into the vastus lateralis produced a peak pain intensity of approximately 48/100 au (between 'somewhat strong' pain and 'strong pain') and the total pain experience lasted for approximately 5.4 min with a mean intensity of 28/100 au (between 'mild pain' and 'moderate pain'; see Table 1 and Figure 2a). Commonly selected

TABLE 1 Mean ± SD of key pain variables across visits and the associated reliability statistics.

Variable	Visit 1	Visit 2	Visit 3	Interindividual CV (%)	Intraindividual CV (%)	ICC (3, 1)	TE (nrs)	MDC (nrs)
Mean pain	29 ± 19	31 ± 8	27 ± 10	37.4 (28.6–54.0)	16.3 (10.5–22.0)	0.71 (0.45–0.88)	4 (3–6)	11 (8–16)
Peak pain	49 ± 18	50 ± 16	46 ± 18	38.0 (29.3–55.3)	14.8 (8.8–20.8)	0.81 (0.62–0.92)	6 (5–9)	18 (14–26)
Pain duration (s)	355 ± 129	306 ± 91*	307 ± 127*	41.3 (31.8–60.1)	15.0 (9.3–24.9)	0.75 (0.49–0.90)	49 (38–71)	136 (105 – 198)
Time to peak (s)	91 ± 56	92 ± 43	90 ± 37	40.7 (31.4–59.2)	25.5 (18.8–32.2)	0.71 (0.45–0.88)	25 (19–36)	68 (53–99)
Time spent > 50% peak (s)	204 ± 76	201 ± 54	181 ± 68	37.3 (28.7–54.2)	15.4 (9.9–20.9)	0.70 (0.44–0.88)	29 (22–42)	80 (62–117)
Area under curve	10,205 ± 4591	9581 ± 4060	8963 ± 5464	62.9 (48.5–91.5)	20.9 (11.9–29.9)	0.80 (0.60–0.92)	1721 (1383–2353)	4770 (3675 6937)

Note: All data are presented as point estimates and 95% CI. Typical error averaged from the two values from visits 1–2 and 2–3. Inter-individual CV presented for visit 3.

Abbreviations: ICC, intraclass correlation coefficient; MDC, minimum detectable change; NRS, numeric rating scale TE, typical error.

*Denotes significantly differed from visit 1 ($p < 0.05$).

words describing the pain experience were 'cramping' (69%); 'aching' (57%); and 'throbbing' (33%), which can be seen in Table 2 (percentage number indicates the proportion of participants selecting that descriptor). Reliability of the whole sample can be observed in Table 1.

3.1 | Systematic bias

3.1.1 | Pain VAS

Pain duration revealed a main effect of visit ($F_{2,26} = 4.630$, $p = 0.026$) whereby pain duration was longer in visit one versus two ($p = 0.024$) and visit one versus three ($p = 0.023$; see Table 1). No other pain VAS-dependent variable displayed systematic error (all $p > 0.05$).

3.1.2 | SF-MPQ

Present pain intensity revealed a main effect of session (Friedman ANOVA $p = 0.024$) and follow-up Wilcoxon signed-rank tests revealed that present pain intensity was lower in visit 3 compared with visit 1 (Wilcoxon $p = 0.038$) and also lower in visit 3 compared with visit 2 (Wilcoxon $p = 0.038$; see Table 3). No other measure for the SF-MPQ indicated systematic error (all $p > 0.05$).

3.1.3 | LF-MPQ

Total pain rating index changed significantly between sessions (Friedman ANOVA $p = 0.006$). Post-hoc tests revealed that the total pain rating index was lower in visit 3 compared with visit 1 (Wilcoxon $p = 0.009$) and visit 2 (Wilcoxon $p = 0.013$), but there was no difference between visit 1 and 2 (Wilcoxon $p = 0.323$). Present pain index also revealed a main effect of visit (Friedman ANOVA $p = 0.039$), but follow-up tests revealed no significant differences. Miscellaneous total also revealed a main effect of session (Friedman ANOVA $p = 0.019$) where visit 3 was lower than visit 1 (Wilcoxon $p = 0.023$) and visit 3 was lower than visit 2 (Wilcoxon $p = 0.039$; see Table 3).

4 | DISCUSSION

The data from the present study suggest that the pain response from an intramuscular injection of hypertonic saline has 'moderate' to 'good' test-rest reliability for ICCs of intensity (e.g. peak pain, mean pain; see Table 1), whereas

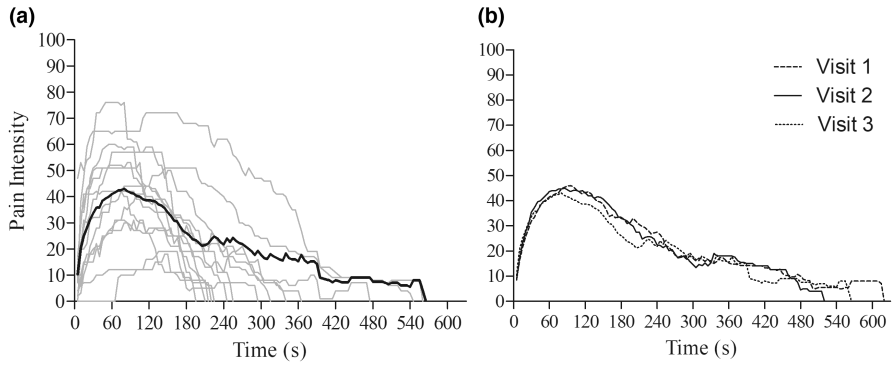

FIGURE 2 (a) Individual (grey lines) and mean (black line) pain intensity response to the hypertonic saline injection in the third visit. (b) Mean pain response at each 5-s interval for each visit. Dashed line denotes visit 1; solid line denotes visit 2; and dotted line denotes visit 3.

TABLE 2 Most commonly selected words (>33% selection) from the short-form McGill and long-form McGill.

Pain subclass	Visit 1	Visit 2	Visit 3	Total
Short-form McGill				
Sensory	Aching (71%)	Cramping (79%)	Cramping (64%)	Cramping (69%)
	Cramping (64%)	Aching (50%)	Aching (50%)	Aching (57%)
	Boring (36%)	Throbbing (36%)	Throbbing (36%)	Throbbing (33%)
	Tender (36%)	Pulsing (36%)	Tender (36%)	–
Affective	–	–	–	–
Long-form McGill				
Sensory	Aching (71%)	Cramping (79%)	Cramping (64%)	Cramping (69%)
	Cramping (64%)	Aching (50%)	Aching (50%)	Aching (57%)
	Boring (36%)	Throbbing (36%)	Throbbing (36%)	Throbbing (33%)
	Tender (36%)	Pulsing (36%)	Tender (36%)	–
Affective	–	–	–	–
Evaluative	–	–	–	–
Misc.	Spreading (36%)	–	Tight (36%)	–

temporal characteristics (e.g. pain duration, time to peak) exhibit 'poor' to 'moderate reliability' (see Table 1). The quality of the pain experience assessed with the SF-MPQ and LF-MPQ has acceptable to very good reliability (Cronbach's $\alpha > 0.75$). However, the total present pain intensity and pain rating index may decrease on the third visit.

To our knowledge, there are currently no studies that have formally assessed the test–retest reliability of other commonly employed experimental models of tonic muscle pain, such as the cold-pressor test (Angius et al., 2015; Slysz & Burr, 2021) and ischaemic contractions (Jones et al., 2014; O'Leary et al., 2017). Conversely, the reliability of the pain response elicited from techniques such as cuff and point pressure algometry, which provide a measure of pain pressure threshold have been well established (for example, Chesterton et al., 2007; Graven-Nielsen et al., 2015; Kvistgaard Olsen et al., 2017; Nussbaum & Downes, 1998). However, the ability of these methods to assess muscle pain specifically is confounded by the unavoidable stimulation of cutaneous nociceptors and low-threshold non-nociceptors alongside muscle nociceptors (Graven-Nielsen & Arendt-Nielsen, 2003). As such, these mechanical and nonspecific techniques of pain stimulation are considered to be notably different in terms of processing and experience when compared with the tonic muscle pain induced by hypertonic saline. Therefore whilst useful in its own application and context, it is not appropriate to directly compare the reliability of these techniques with the hypertonic saline model of muscle pain.

4.1 | Intraindividual reliability

No systematic change in pain scores was observed across sessions for pain intensity variables, but pain duration significantly decreased after the first visit (Table 1). An explanation for the systematic decrease in pain duration after the first visit could be that participants initially have difficulty in determining the offset of pain due to a low amount of sensory feedback (i.e. very low pain, which is changing in intensity slowly). However, after one experience of pain

TABLE 3 Mean ± SD and the reliability statistics of the pain quality measures derived from the short-form McGill pain questionnaire and the long-form McGill pain questionnaire.

Pain quality measure	Visits			Cronbach's alpha
	Visit 1	Visit 2	Visit 3	
Short-form McGill				
T-PRI	6.9 ± 4.7	6.5 ± 3.4	5.5 ± 3.7	0.882
PPI	2.1 ± 0.9	2.1 ± 0.7	1.6 ± 0.7*,$	0.806
Sensory	6.7 ± 4.7	6.4 ± 3.4	5.4 ± 3.7	0.889
Affective	0.1 ± 0.4	0.1 ± 0.3	0.1 ± 0.3	0.397
Long-form McGill				
T-PRI	14.4 ± 6.3	13.9 ± 5.3	11.1 ± 5.7*,$	0.933
PPI	2.2 ± 0.7	2.1 ± 0.7	1.9 ± 0.5	0.849
Sensory	11.1 ± 4	11.1 ± 3.5	9.5 ± 5	0.848
Affective	0.7 ± 1.2	0.4 ± 0.7	0.1 ± 0.4	0.601
Evaluative	1.1 ± 1.6	0.9 ± 1.4	0.6 ± 1.2	0.871
Misc.	1.5 ± 1.1	1.6 ± 1.4	0.9 ± 1*,$	0.865

Abbreviations: PPI, present pain index; T-PRI, total pain rating index.

*Denotes significantly different from visit 1.

$Denotes significantly different from visit 2 (Wilcoxon $p < 0.05$).

cessation, participants become more attuned to when this offset occurs. There was also some systematic error in the McGill pain questionnaires whereby present pain intensity and total pain rating index were lower in the third visit compared with the first two visits. The lower total pain rating index appears to be driven by decreases in the miscellaneous component of the pain, but it is currently unclear why this was the case, particularly as the other components of the pain response did not change.

The small to moderate amount of variability observed in pain intensity measures (see Table 1) is most likely due to random variability associated with the injection protocol and factors intrinsic to the participant (i.e. biological and psychological variations). For the hypertonic saline protocol, all injections were manually administered using standardized procedures. Despite this, subtle variations in administration (e.g. needle placement, rate of infusion—although both of these were controlled for) may occur with this approach in comparison with the use of computer-controlled syringe pumps, which are suggested to provide a more standardized infusion (Graven-Nielsen & Arendt-Nielsen, 2003). However, a prior investigation has evidenced that infusion rates of 18 and 90 mL/h do not affect the area of pain and only modestly affect the temporal characteristics of the pain response, despite the five-fold difference in the infusion rate (Graven-Nielsen et al., 1997a). As a result, we speculate that any small variations in infusion rate (±0.1 mL/s) would produce a negligible difference in the pain response. Similarly, small variations within needle depth between days and during the 10 s infusion period could result in different sizes of the 'saline pool' (Graven-Nielsen, McArdle, et al., 1997), but the effect of needle depth on the saline pain response is yet to be investigated. Whilst computer-controlled infusion pumps may alleviate some of these concerns and allow for the gradual infusion of saline (but not standardized needle depth) to provide a desired pain response, this model is not feasible with experimental designs which require contractions of the injected muscle.

Alternatively, within-individual naturally occurring, biological and psychological variations are likely the main cause of the pain variability. Indeed, some injections were performed at different times of day with as much as a 6-h time difference. Diurnal variations in circulating hormone concentrations such as cortisol could influence the perception of pain (Aviram et al., 2015). In female participants, phases of the menstrual cycle can also change the response to experimental pain (Iacovides et al., 2015; Sherman & LeResche, 2006) due to fluctuations in progesterone, particularly in the luteal phase (Vincent et al., 2018). Unfortunately, we did not monitor this and therefore it is difficult to account for the extent to which this affected the reliability of the saline-evoked pain.

4.2 | Interindividual variability

Whilst intraindividual reliability of pain intensity variables displayed CVs of ~10%–25%, the variability between participants was much larger (CV ~30%–65%). A multitude of psychological, physiological, anatomical and genetic factors (which are beyond the scope of this

discussion) could contribute to the overall pain experience, particularly when all participants received the same dose (1 mL) of hypertonic saline. High levels of interindividual variability from the same nociceptive input can complicate the interpretation of outcomes of interest (Adamczyk et al., 2022), particularly when a number of 'low-responders' are present. For example, in our cohort, the lowest peak pain response observed was 22 and the greatest was 77 (Figure 2a). Some studies have omitted these 'low responder' participants (Canestri et al., 2021) whilst others have included them for analysis of dose–response effects (Farina et al., 2004; Martinez-Valdes et al., 2020; Norbury et al., 2022b).

4.3 | Methodological considerations

The sample size recruited for the study whilst justified, may still be underpowered as some of the ICCs reported were below the anticipated ICC of 0.85. Additionally, the results of the ICCs yielded large confidence intervals, which make some of the interpretations of the results limited and should be interpreted with caution.

As previously mentioned, sessions performed at different times of day and the menstrual cycle may significantly contribute to the test–retest reliability. Nevertheless, good MDCs and acceptable ICCs were still observed for most pain intensity measures, along with good to excellent Cronbach's alpha scores for pain quality measures. Whilst the reliability of pain hypertonic saline injections was investigated with 1 mL (5.85% NaCl) into the vastus lateralis, it is unclear whether this level of reliability applies to other muscles commonly used in hypertonic saline pain research (e.g. bicep brachii, tibialis anterior). Additionally, different hypertonic saline concentrations and infusion volumes used in other studies warrant consideration for reliability, but we contend that as long as a robust pain response is induced (i.e. similar in intensity to this study), the level of reliability observed should translate to other injection protocols.

4.4 | Perspectives and practical applications

The current findings suggest that a manual infusion of a hypertonic saline injection is sufficiently reliable across repeated visits. Therefore, the hypertonic saline model of experimental muscle pain can be used to assess changes in pain perception in response to an intervention or assess multiple responses to pain which require more than one experimental visit. Importantly, the MDC values of 11 and 18 points for mean and peak pain, respectively, are below the clinically important differences in pain outcomes of ~2/10 (i.e. 20/100) points (Farrar et al., 2000).

Future work could refine the infusion paradigm to improve the reliability of the pain response when the model is administered manually. This could be achieved through individualizing parameters such as needle depth or angle, volume infused and rate of infusion and improving the understanding of the tissue injected. It is acknowledged that computer-controlled syringes have previously been used, however, as previously mentioned this technique is not compatible with studies employing dynamic exercise and is only possible with specialist equipment. Other supplementary techniques such as ultrasound (combined with measures of pain intensity or quality; Henriksen et al., 2011) could be used to gain an insight into muscle characteristics and the effect of needle depth or angle, with the purpose to improve understanding of the saline volume distribution within the muscle and how this may relate to variation in the pain experience.

4.5 | Conclusion

In summary, we have shown that measures of pain intensity from intramuscular injection of 1 mL 5.85% hypertonic saline exhibit acceptable (ICC > 0.5) test–retest reliability but considerable interindividual variability. Furthermore, measures of pain quality also appear to be sufficiently reliable (Cronbach's $\alpha > 0.75$). Overall, this model of experimental muscle pain is appropriate for the study of muscle pain.

AUTHOR CONTRIBUTIONS

Samuel A. Smith involved in conception and design of the study, data collection, interpretation of results, writing of the manuscript and revising the manuscript critically. Ryan Norbury involved in data collection, interpretation of results, writing of the manuscript and revising the manuscript critically. Adam J. Hunt involved in conception and design of the study, data collection and interpretation of results. Alexis R. Mauger involved in conception and design of the study, data collection, interpretation of results, writing of the manuscript and revising the manuscript critically. All authors discussed the results and commented on the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest with this work.

ORCID

Samuel A. Smith https://orcid.org/0000-0002-0833-0878
Alexis R. Mauger https://orcid.org/0000-0001-6685-5800

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