Current Research in Physiology 3 (2020) 1–10

Contents lists available at ScienceDirect

Current Research in Physiology

journal homepage: www.editorialmanager.com/crphys/Default.aspx

A comparison of pain responses, hemodynamic reactivity and fibre type composition between Bergström and microbiopsy skeletal muscle biopsies

Jacob T. Bonafiglia [a,1], Hashim Islam [a,1], Nicholas Preobrazenski [a], Patrick Drouin [a], Andrew Ma [b], Austin Gerhart [a], Joe Quadrilatero [b], Michael E. Tschakovsky [a], Dean A. Tripp [c], Christopher G.R. Perry [d], Craig A. Simpson [e], Brendon J. Gurd [a,*]

[a] School of Kinesiology and Health Studies, Queen's University, Kingston, Ontario, K7L 3N6, Canada
[b] Department of Kinesiology, University of Waterloo, Waterloo, Ontario, N2L 3G5, Canada
[c] Departments of Psychology, Anesthesiology and Urology, Queen's University, Kingston, Ontario, K7L 3N9, Canada
[d] School of Kinesiology and Health Science, York University, Toronto, Ontario, M3J 1P3, Canada
[e] School of Medicine, Queen's University, Kingston, Ontario, K7L 3L4, Canada

ARTICLE INFO

Keywords:
Perceptions of pain
Heart rate
Mean arterial pressure
Skeletal muscle biopsies

ABSTRACT

This study tested the hypotheses that 1) skeletal muscle biopsies performed with the Bergström needle evoke larger perceptions of pain and greater hemodynamic reactivity compared to biopsies performed with the microbiopsy needle, and 2) both needles yield samples with similar fibre type compositions when samples are collected at similar skeletal muscle depths. Fourteen healthy (age: 21.6 ± 3.2 years; VO_2peak: 41.5 ± 5.8 mL/kg/min) males (n = 7) and females (n = 7) provided two resting skeletal muscle biopsies, one with each needle type, following a randomized crossover design. Participants completed the short-form McGill Pain Questionnaire and the Brief Pain Inventory before, during, and after the skeletal muscle biopsies. Hemodynamic reactivity was assessed by measuring heart rate (HR) and mean arterial pressure (MAP) at rest and during the biopsy procedures. Immunofluorescence analysis was used to assess fibre type composition in *vastus lateralis* samples. Compared to the microbiopsy needle, the Bergström needle elicited a larger perception of pain but similar hemodynamic reactivity during the biopsy. Both needles yielded skeletal muscle samples with similar fibre type composition and resulted in similar perceptions of pain and pain-related interference during the post-biopsy recovery period. Collectively, these findings suggest that studies should consider using the microbiopsy needle rather than the Bergström needle unless large amounts of muscle tissue or certain muscle fibre lengths are required. However, future work should determine whether our findings are generalizable to biopsies performed with different procedures and/or types of Bergström/microbiopsy needles.

1. Introduction

For nearly 70 years skeletal muscle biopsies have helped characterize various diseases and investigate physiological responses to exercise, diet, and many other homeostatic perturbations (Ekblom, 2017). The Bergström needle is used in the majority of skeletal muscle biopsies (Ekblom, 2017) as it yields large samples (~100–200 mg) and is believed to be minimally invasive (Hayot et al., 2005; Isner-Horobeti et al., 2014). Despite yielding smaller samples (~40–60 mg) (Hayot et al., 2005), the "microbiopsy" needle is gaining popularity under the assumption that its smaller diameter (~2 mm) is less painful than the Bergström needle (~3.5 mm) (Hayot et al., 2005; Hughes et al., 2015; Isner-Horobeti et al., 2014). However, to our knowledge, there is limited evidence comparing perceptions of pain between biopsies performed with Bergström and microbiopsy needles (Hayot et al., 2005).

Abbreviations: BPI, Brief pain inventory; BPI-6, Brief pain inventory question #6; BPI-9, Brief pain inventory question #9; HR, Heart rate; MAP, Mean arterial pressure; McGill-D, Descriptors from the McGill Pain Questionnaire; PCS, Pain catastrophizing scale; PPI, Present pain intensity; VAS, Visual analog scale; VO_2peak, Peak oxygen consumption.

* Corresponding author. School of Kinesiology and Health Studies, Queen's University, Kingston, Ontario, K7L 3N0036, Canada.
E-mail addresses: j.bonafiglia@queensu.ca (J.T. Bonafiglia), hashim.islam@queensu.ca (H. Islam), 12np22@queensu.ca (N. Preobrazenski), 11pjd3@queensu.ca (P. Drouin), andrew.ma.1@uwaterloo.ca (A. Ma), 16ag21@queensu.ca (A. Gerhart), jquadrilatero@queensu.ca (J. Quadrilatero), mt29@queensu.ca (M.E. Tschakovsky), dean.tripp@queensu.ca (D.A. Tripp), cperry@yorku.ca (C.G.R. Perry), simps1010@gmail.com (C.A. Simpson), gurdb@queensu.ca (B.J. Gurd).
[1] Authors contributed equally.

https://doi.org/10.1016/j.crphys.2020.05.001
Received 10 March 2020; Received in revised form 19 May 2020; Accepted 22 May 2020

When applying external pressure to skeletal muscle, larger pressure diameters augment perceptions of pain (Finocchietti, Nielsen, Mørch, Arendt-Nielsen, & Graven-Nielsen, 2011; Lautenbacher, Kunz, Strate, Nielsen, & Arendt-Nielsen, 2005). If biopsy needles produce a similar dose–response relationship, then it is reasonable to speculate that the Bergström needle's larger diameter may evoke larger perceptions of pain compared to the microbiopsy needle. Indeed, Hayot and colleagues (Hayot et al., 2005) reported larger pain responses during Bergström needle biopsies compared to biopsies performed with microbiopsy needles. Painful experiences also increase heart rate (HR) and mean arterial pressure (MAP) (Tousignant-Laflamme, Rainville, & Marchand, 2005); a response herein referred to as "hemodynamic reactivity" (Poitras, Slattery, Gurd, & Pyke, 2014). Therefore, we speculate that the Bergström needle elicits larger pain responses and causes greater hemodynamic reactivity compared to the microbiopsy needle.

Previous studies have also compared skeletal muscle morphological and molecular characteristics between samples obtained with Bergström and microbiopsy needles (Hayot et al., 2005; Isner-Horobeti et al., 2014; Hughes et al., 2015). For instance, Hughes et al. (Hughes et al., 2015) reported more type I fibres and less type IIX fibres in samples obtained with the Bergström needle compared to the microbiopsy needle. Because many molecular characteristics (e.g. mitochondrial content, substrate storage, etc.) are fibre type dependent (Schiaffino & Reggiani, 2011), this observation by Hughes et al. (Hughes et al., 2015) suggests that comparing whole-muscle Bergström and microbiopsy samples may lead to inaccurate conclusions. However, Hughes et al. (Hughes et al., 2015) obtained microbiopsy needle samples at shallower depths (needle insertion angle ~30°) compared to the Bergström needle (needle insertion angle ~90°; see Supplemental Figure 1 for visual representation). Given that fibre type composition varies by depth in the *vastus lateralis* (i.e. more type I fibres in deep muscle vs. superficial) (Dahmane, Djordjevič, Šimunič, & Valenčič, 2005; Lexell, Henriksson-larsén, & Sjöström, 1983), these authors speculated that sampling at similar depths would eliminate the difference in fibre type composition between Bergström and microbiopsy needle samples (Hughes et al., 2015).

The present study tested the hypotheses that 1) skeletal muscle biopsies performed with the Bergström needle elicits larger perceptions of pain and greater hemodynamic reactivity compared to the microbiopsy needle, and 2) the Bergström and microbiopsy needle yield samples with similar fibre type composition when samples are obtained at a similar depth of the *vastus lateralis*. Confirming our hypotheses would demonstrate that the microbiopsy leads to less perception of pain and smaller samples with similar fibre type compositions, and suggests that future studies should consider using the microbiopsy needle if large amounts of tissue or certain muscle fibre lengths are not required.

2. Methods

Fourteen healthy, young males (n = 7) and females (n = 7) volunteered to participate in the current study (participant characteristics presented in Table 1). Volunteers were enrolled in the study only if they were between 18 and 30 years of age, non-smokers, free of cardiovascular and metabolic diseases, and were recreational active (self-reported completing less than 3 h per week of moderate to vigorous physical activity). Participants attended a preliminary screening session during which they were briefed on the experimental protocol and its associated risks prior to providing written informed consent. All experimental procedures performed on human participants were approved by the Health Sciences Human Research Ethics Board at Queen's University.

Table 1
Participant characteristics.

	Males (n = 7)	Females (n = 7)
Age (years)	22.6 ± 3.3	20.2 ± 2.9
Height (cm) *	179.6 ± 6.8	165.1 ± 5.0
Body mass (kg)	81.1 ± 23.0	60.9 ± 2.6
BMI (kg/m^2)	25.0 ± 5.9	22.4 ± 1.7
VO_2peak (mL/kg/min)	43.6 ± 5.6	39.0 ± 5.5

All values mean ± standard deviation. BMI, body mass index, VO_2peak, peak oxygen uptake.
* Significant ($p < .05$) difference between males and females.

2.1. Experimental design

Participants attended the lab on four separate occasions, and the experimental design is illustrated in Fig. 1. During the first visit, participants performed an incremental step test on a Monark Ergomoedic 874 E stationary cycle ergometer (Monark Exercise, Vansbro, Sweden) to determine peak oxygen consumption (VO_2peak), as we have described previously (Edgett et al., 2013). The test protocol consisted of a 2-min warmup of load-less cycling at 80 revolutions per minute (RPM) followed by a step increase to 80 W for 1 min and subsequent increases of 24 W per minute until volitional fatigue (determined by the inability to maintain a cadence of 70 RPM). At least 72 h following the VO_2peak test, participants returned to the lab for their first skeletal muscle biopsy. Participants received both skeletal muscle biopsy needles following a within-subjects, randomized crossover design that followed experimental best practices, as described previously (Preobrazenski et al., 2019). Specifically, participants were stratified by sex and subsequently randomized in a 1:1 allocation to receive the biopsies in one of two orders: microbiopsy first and Bergström second or *vice versa*. In an attempt to reduce selection bias, N.P. created the random allocation sequence (generated using the random function on Microsoft Excel) and was not involved in screening/enrolling participants. Additionally, participant allocation was not revealed to J.T.B., H.I., or B.J.G. until the morning of the first biopsy visit. One week after the first skeletal muscle biopsy, participants returned to the lab for a third visit during which a second biopsy was performed using the other needle. We performed biopsies on separate visits to compare differences in activities of daily living between needles during the recovery period (e.g. changes in mood, physical activity, etc.; question #9 from the BPI). Finally, participants returned to the lab one week after the second biopsy to complete an exit survey to ascertain participants' overall perceptions and preferences of both needles.

2.2. Biopsy procedures

With the exception of the two needles requiring slightly different procedures (described below), the personnel and protocols were identical between both biopsy visits. Participants arrived at the lab after an overnight fast (~12 h) and ate a standardized breakfast (whole wheat bagel [190 kcal] with 2 tbsp of cream cheese [80 kcal] and an 300 mL bottle of orange juice [130 kcal]) ~30 min before the biopsy procedure. B.J.G. explained the biopsy procedures and its associated risks before cleaning the participants' mid-thigh with a disinfectant (Dexidin II; Laboratoire Atlas Inc., Canada) and injecting the skin with a local anesthetic (2% lidocaine with epinephrine; Teligent Inc., USA). For the microbiopsy needle (Medax Biofeather, Italy) procedure, a ~2 mm diameter cannula was then inserted vertically (~90° angle) ~4 cm into the *vastus lateralis*. The cannula served as a guide for the microbiopsy needle, which obtained a muscle sample at a depth of ~8 cm by making three separate cuts with the needle turning ~90° between each cut (Islam et al., 2019). We inserted the needle to the top of the cannula during each biopsy in an attempt to obtain samples at similar depths. Together, the guide and microbiopsy needle were inserted in the muscle for ~45 s. For the Bergström procedure, B.J.G. made a small incision in the skin and deep fascia prior to vertically inserting (~90° angle) the Bergström needle (~3.5 mm diameter) to a depth of ~8 cm. Unlike the microbiopsy needle, the Bergström technique is unable to control for needle insertion depth. As described previously (Edgett et al., 2013), we modified the Bergström technique (Bergström, 1975) with manual suction to obtain skeletal muscle samples. The Bergström needle was inserted in the

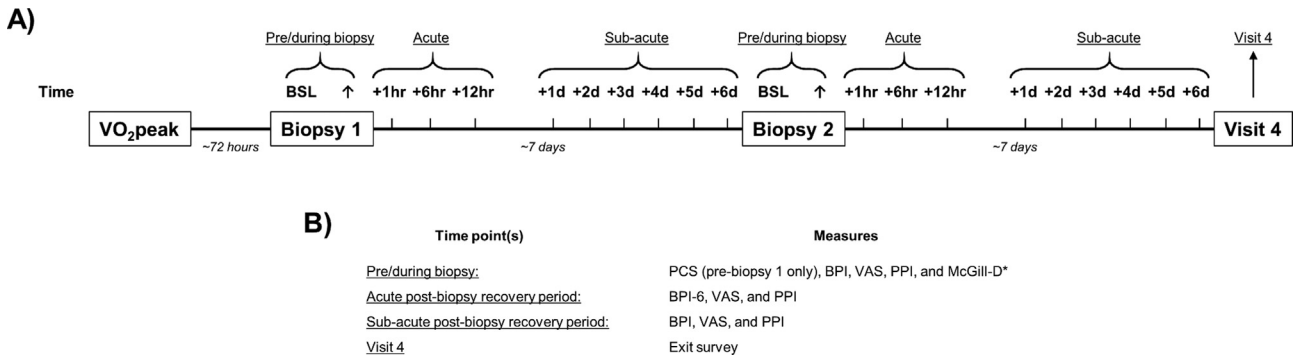

Fig. 1. Experimental design timeline (A) and list of measures at each time point (B). BSL, baseline; ↑, time of biopsy; VO_2peak, peak oxygen uptake test; PCS, pain catastrophizing scale; BPI, Brief Pain Inventory; BPI-6, only question #6 of Brief Pain Inventory; VAS, visual analog scale; PPI, present pain intensity; McGill-D, sensory and affective descriptors from the short form McGill Pain Questionnaire. * Measures collected only during biopsies (not collected pre-biopsy).

muscle for ~10 s. After a sample was obtained, B.J.G. covered the microbiopsy site with an adhesive bandage and closed the Bergström needle incision site with a suture prior to covering with an adhesive bandage. Given that the two needles require slightly different procedures, the participants could not be blinded to the biopsy needle. However, in an attempt to reduce the impact of performance and/or observer bias, the researchers provided equal verbal support during the biopsy procedures and did not share the above-mentioned hypotheses with participants (Boter, van Delden JJM, de Haan, & Rinkel, 2003). The location of the two biopsies were approximately one inch apart on the non-dominant leg (second biopsy sampled distally from the first biopsy site). We are not aware of any evidence suggesting that moving proximally vs. distally impacts pain responses, hemodynamic reactivity, or fibre type composition. Further, previous studies (Halkjaer-Kristensen & Ingemann-Hansen, 1981; Nederveen et al., 2020; Sahl et al., 2018; Simoneau, Lortie, Boulay, Thibault, & Bouchard, 1986) have reported similar fibre type composition between samples obtained from similar distances (2–3 cm apart) on the *vastus lateralis*. H.I. handled all skeletal muscle sample processing, which involved rapidly snap-freezing a portion of the sample in liquid nitrogen (~-80 °C) and embedding a second portion in optimal cutting temperature (O.C.T.) compound (Tissue-Tek, Sakura Finetek, U.S.A.) before being frozen in liquid nitrogen-cooled isopentane. All samples were subsequently stored at −80 °C until immunofluorescence (O.C.T. embedded tissue; described below) or weight analysis (tissue not embedded in O.C.T.).

2.3. Pain surveys

The present study used four pain surveys (all surveys included in supplemental material), and Fig. 1 depicts when each survey was completed. All surveys were described and administered by J.T.B. In an attempt to reduce observer bias, two other researchers (N.*Physiological and* A.G.) entered and coded all survey data so that outcome analysis was conducted in a blinded fashion.

2.3.1. Pre-biopsy surveys

Prior to the first skeletal muscle biopsy, participants completed the Pain Catastrophizing Scale (PCS), which is a validated and reliable 13-item survey to gauge the degree that an individual catastrophizes a painful stimulus (Osman et al., 1997; Sullivan, Bishop, & Pivik, 1995). In brief, the PCS asked participants to reflect on previous pain experiences and rate the degree to which they experienced a given thought or feeling from not at all (0) to all the time (4). A PCS score for each participant was calculated by summing scores from all 13 questions. PCS scores were used to determine whether biopsy-induced perceptions of pain were associated with participants' tendency to catastrophize painful experiences.

2.3.2. During-biopsy surveys

Three different measures were used to quantify perceptions of pain before (baseline) and during the skeletal muscle biopsies. First, participants completed the validated and reliable full Brief Pain Inventory (BPI) (Cleeland & Ryan, 1994; Jensen, 2003) at baseline and only question #6 (BPI-6) during the biopsies. BPI-6 asked participants to rate their pain from "no pain" (0) to "pain as bad as you can imagine" (10). Second, participants completed a visual analog scale (VAS), which asked participants to point to a spot on the scale somewhere between no pain (left boundary) to worst possible pain (right boundary), and J.T.B. drew a vertical dash at this point. VAS responses were determined by measuring the distance (cm) from the left boundary to the participant's vertical dash. Third, participants completed the Present Pain Intensity (PPI), a component of the validated and reliable McGill Pain Questionnaire (Jensen, 2003; Melzack, 1975), which asked participants to rate their pain across a 6-point scale ranging from no pain (0) to excruciating (5). Change scores (Δ) for BPI-6, VAS, and PPI were calculated for each participant by subtracting their baseline value from their during-biopsy-value. In addition to quantitative assessments of pain, the McGill Pain Questionnaire descriptors (Melzack, 1975), herein referred to as McGill-D, were used to qualitatively characterize perceptions of pain during the skeletal muscle biopsies. The McGill-D asked participants to provide a rating from none (0) to severe (3) across 11 sensory and 4 affective descriptors.

2.3.3. Post-biopsy surveys

To assess perceptions of pain following the skeletal muscle biopsies, participants completed the three quantitative pain measures (BPI [results for BPI-6], VAS, and PPI) during the acute (1–12 h) and sub-acute (1–6 days) post-biopsy recovery period. However, only PPI data was analyzed (see statistical analysis and results for more details). Additionally, in an attempt to gauge the impact of skeletal muscle biopsies on daily living, participants completed the BPI and results for question #9 were analyzed during the sub-acute (1–6 days) recovery period. BPI-9 asked participants to rate the degree of pain-related interference for seven different aspects of daily living (e.g. mood, sleep, walking ability; see supplemental material for full list) from "does not interfere" (0) to "completely interferes" (10). One week following the second biopsy, participants returned to the lab to complete the exit survey, which consisted of 5 questions gauging participants' overall perceptions and preferences of both needles.

2.4. Hemodynamic reactivity

We assessed hemodynamic reactivity by measuring HR and MAP before and during skeletal muscle biopsies (Fig. 1). With participants laying supine on the biopsy table (same position assumed during biopsies) and their hands maintained at heart level, a finger

photoplethysmography (Finometer MIDI, Finapres Medical Systems, The Netherlands) measured heart rate (HR) and mean arterial pressure (MAP) from the middle finger of the hand opposite the non-dominant leg. HR and MAP were obtained on a beat-by-beat basis. Participants lay supine for 7 min and resting HR and MAP were calculated as the average values over the last 2 min of this 7-min period. HR and MAP were recorded continuously during the biopsy procedure. During-biopsy HR and MAP values were calculated as the average HR and MAP during the time period that the biopsy needle was inserted in the leg. Biopsy-induced changes in HR and MAP (ΔHR and ΔMAP, respectively) were calculated by subtracting resting HR and MAP values from their respective during-biopsy values.

2.5. Fibre type composition

Fibre type composition was determined in O.C.T.-embedded skeletal muscle samples as we have previously described (Bloemberg & Quadrilatero, 2012; Scribbans et al., 2014). In brief, ten micrometre-thick cryosections were cut using a cryostat maintained at −20 °C and mounted onto positively charged microscope slides (SuperFrost Plus, VWR International, U.S.A.). Immunofluorescence analysis of myosin heavy chain (MHC) was performed using primary antibodies against MHCI (BA-F8), MHCIIA (SC-71), MHCIIX (6H1), and sarcolemmal dystrophin (MANDYS1; all primary antibodies from Developmental Studies Hybridoma Bank, U.S.A.) followed by isotype-specific fluorescent secondary antibodies (Life Technologies, Canada). Sections were then mounted with Prolong Gold Antifade Reagent (Life Technolgoies, Canada) and imaged the following day using an Axio Observer Z1 microscope (Carl Zeiss, Germany). This allowed us to identify type I (blue), type IIA (green), and type IIX/IIAX hybrid (red) fibres, and the dystrophin stain (red) helped discern adjacent fibres. All fibres within each cross-section were counted and composition of each fibre type (I, IIA, and IIX/IIAX) was expressed as a percentage of the total number of fibres. In an attempt to reduce observer bias, images were coded by N.P. so that J.T.B could conduct fibre type analysis in a blinded fashion.

2.6. Statistical analysis

Unpaired *t*-tests compared participant characteristics between female and male participants. As a first step to determine whether the order of needles impacted responses during skeletal muscle biopsies, we performed 2-way repeated-measures ANOVAs (between subject factor: order of needles; repeated-measures factor: needle) on baseline and during-biopsy BPI-6, VAS, PPI, HR, and MAP values (data collapsed across time and sex). This approach is similar to Wellek's method for testing for carryover effects in crossover designs (Wellek & Blettner, 2012), but modified by using a 2-way ANOVA rather than an unpaired *t*-test to account for 2 factors (needle and order of needles). Given that order of needles did not significantly impact any outcome (see results section for more details), order of needles was not included as a between-subject factor in our subsequent analyses.

Relationships between ΔBPI-6, ΔVAS, and ΔPPI were assessed using bivariate correlations (data collapsed across needle and sex). Because ΔBPI-6, ΔVAS, and ΔPPI values were highly correlated (see results section/Table 1 Fig. 2 for more details), all subsequent statistical analysis regarding quantitative perceptions of pain were performed on PPI values only. We chose PPI because it may be informative than BPI-6 and VAS given that each PPI value is tied to a qualitative descriptor (e.g. 0 = no pain, 1 = mild, 2 = moderate; see supplemental material for full list). Bivariate correlations examined the relationship between PCS scores and ΔPPI for both needles (data collapsed across sex).

3-way repeated measures ANOVAs compared mean pain responses (PPI) and hemodynamic reactivity (HR and MAP) over time and between needle and sex (repeated-measures factors: time and needle; between-subject factor: sex). Although we investigated sex-effects for our during-biopsy measures (2 time points), we believe our study was not sufficiently powered to detect possible sex-effects for post-biopsy measures as a possible sex-effect would likely not persist across the many time points included in the recovery period (n = 9 time points). Accordingly, post-biopsy PPI and BPI-9 values were collapsed across sex at each time point and analyzed using 2-way repeated measures ANOVAs (repeated-measures factors: time and needle). A 2-way repeated measures ANOVA (repeated measures factors: fibre type and needle) compared fibre type composition between samples obtained with the Bergström and microbiopsy needle. Bonferroni *post-hoc* tests compared composition (%) across fibre types. A paired *t*-test compared the weights of whole muscle tissue (non-O.C.T. embedded portions) collected using the Bergström and microbiopsy needle. Effect sizes for comparisons between two means were determined by calculating repeated measures Cohen's *d* values using G*Power (version 3.1) (Faul, Erdfelder, Lang, & Buchner, 2007), and values of .2, .5, or .8 were interpreted as small, medium, or large, respectively (Cohen, 1992). Significance was accepted at $p < .05$ except for the BPI-9 analysis, which used a significance threshold of $p < .007$ to account for BPI-9 containing seven sub-questions (Bonferroni correction: .05/7 = .007). Statistical analyses were conducted on SPSS version 25 (IBM, USA).

We did not perform statistical analyses on data from the McGill-D or the exit survey as our small sample size (n = 14) provided little statistical power for categorical analyses (Snapinn & Jiang, 2007). Additionally, the McGill-D was included to provide qualitative data only, and the exit survey was not validated (created by the authors) and was only included to provide exploratory information about participants' preferred needle.

3. Results

Table 1 presents participant characteristics, including average VO_2peak. There were no significant differences in participant characteristics between females and males except for height, which was significantly ($p < .01$) higher in males (Table 1). All pain survey analysis included 13 participants (M = 7, F = 6) as 1 female participant did not return completed surveys to the lab following the second biopsy visit and did not respond to subsequent communications. The 2-way ANOVAs did not reveal any significant (all $p > .22$) main or interaction effects involving order of needles for any pain (BPI-6, VAS and PPI) or cardiovascular (HR and MAP) outcome (data not shown). Accordingly, these non-significant effects provided justification to exclude order of needles as a factor in subsequent analysis.

3.1. Pain surveys: pre- and during-biopsies

ΔBPI-6, ΔVAS, and ΔPPI were highly correlated (all $p < .01$, $r > .64$; Fig. 2A–C). As mentioned above (*Statistical Analysis*), all subsequent statistical analysis regarding quantitative assessments of pain were performed on PPI values only. PCS scores did not significantly correlate with ΔPPI for biopsies performed with the Bergström or microbiopsy needle (both $p > .45$, $r < .25$; Fig. 2D).

For ease of viewing, Fig. 3A presents data from the 3-way ANOVAs as change scores (ΔPPI) in Fig. 3A. We observed a significant main effect of time ($p < .01$; $d = 2.52$) and a significant needle × time interaction effect ($p < .01$) whereby the Bergström needle increased PPI more than the microbiopsy needle ($d = .91$). The effect size for the interaction ($d = .91$) indicates a large effect of the Bergström needle evoking more pain than the microbiopsy needle. Additionally, we observed a significant main effect of sex ($p < .05$; $d = .31$), and a near-significant sex × time interaction effect ($p = .08$; $d = .85$) whereby the increase in PPI was trending to be greater in males than females (Fig. 3A). There were no other significant main or interaction effects for PPI scores.

Fig. 4 presents McGill-D responses during the skeletal muscle biopsies. The number of participants reporting a given response (0–3) for each McGill-D is presented in Fig. 4A. A total sum (Σ) was calculated for each McGill-D by adding responses across all participants (Fig. 4A). Accordingly, Σ reflects the magnitude that each McGill-D was endorsed

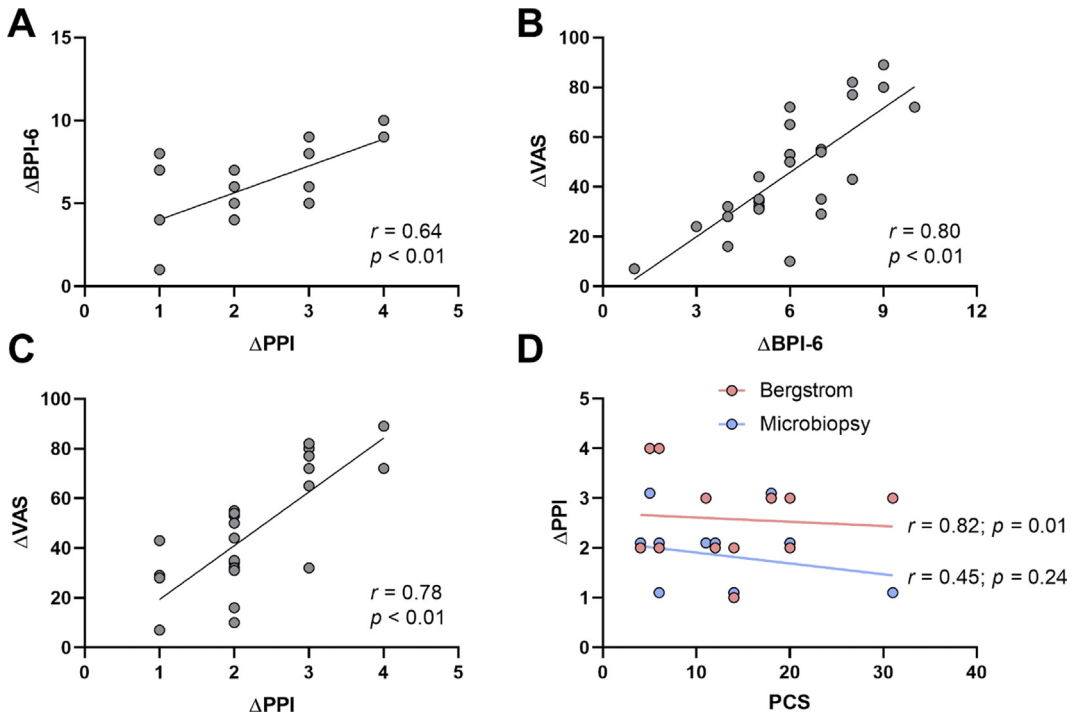

Fig. 2. Correlations for bivariate correlations between biopsy-induced pain ratings (A–C) and between pain catastrophizing scale (PCS) scores and biopsy-induced pain ratings (D). Pain ratings presented as change scores (during-biopsy pain score minus pre-biopsy pain score). As described in section 2.6, subsequent analysis (e.g. panel D) was performed on present pain intensity (ΔPPI) scores only because the three pain variables were highly related (panels A–C). Data in panels A–C collapsed across needle and sex, and data in panel D is collapsed across sex.

by participants during the skeletal muscle biopsies. The Σ for each McGill-D was then used to create a tag cloud (Fig. 4B) whereby the size of each McGill-D is reflective of Σ (i.e. the greater Σ the larger the font size).

3.2. Pain surveys: post-biopsy

Fig. 5 presents PPI scores during the acute (1–12 h) and sub-acute (1–6 days) post-biopsy recovery periods. We observed a significant main effect of time ($p < .01$), indicating that PPI scores decreased during the post-biopsy recovery period. However, post-biopsy PPI scores did not significantly differ by needle (main effect of needle: $p = .79$, $d = .08$; needle × time interaction: $p = .71$). For BPI-9 scores, we only observed significant ($p < .007$) main effects of time for general activity, mood, and walking ability indicating a decrease in pain-related interference over time for some but not all aspects of daily living (Supplemental Figure 2). Although we observed a significant ($p < .007$) needle × time interaction for walking ability, no other significant main or interaction effects were observed for BPI-9 scores (Supplemental Figure 2).

The results from the exit survey are presented in Fig. 6. In general, participants provided more favourable responses for the microbiopsy needle compared to the Bergström needle. For instance, although 9/13 participants stated that they would be willing to have another biopsy performed with the Bergström needle, all 13 participants answered "yes" to this question for the microbiopsy needle. Further, the majority of participants (10/13) preferred the microbiopsy needle over the Bergström needle.

3.3. Hemodynamic reactivity

Thirteen participants were included in the hemodynamic reactivity analysis (M = 7, F = 6). One set of female data was excluded from analysis due to inadequate pulse measurement integrity during measurements. For ease of viewing, data from the 3-way ANOVAs examining the impact of skeletal muscle biopsies on hemodynamic reactivity are presented as change scores (ΔHR and ΔMAP) in Fig. 3B–C, respectively. We observed a significant main effect of time ($p < .01$) for both HR ($d = 1.26$) and MAP ($d = 1.13$). However, there were no significant main effects of needle or needle × time interaction effects for HR or MAP (all $p > .28$; $d < .31$). Additionally, there were no significant main effects of sex for HR ($p = .30$; $d = .43$) or MAP ($p = .87$; $d = .08$) nor any interaction effect involving sex (all $p > .27$).

3.4. Fibre type composition

Thirteen participants were included in the fibre type composition analysis (M = 7, F = 6), as the microbiopsy did not yield enough tissue for O.C.T. embedding for one female participant. Fibre type composition results are presented in Fig. 7. The 2-way ANOVA revealed a main effect of fibre type ($p < .01$), and *post-hoc* tests revealed that there were less type IIX/hybrid fibres in muscle samples compared to type I and IIA ($p < .01$, $d > 2.0$), but no difference between type I and IIA ($p = .39$ and .12 and $d = .52$ and .78 for Bergström and microbiopsy, respectively). Importantly, there was no interaction (fibre type x needle) effect ($p = .84$), indicating that the two needles yielded skeletal muscle samples with similar fibre type composition. Additionally, weighing the whole muscle portions not embedded in O.C.T. revealed that the Bergström needle (85.62 ± 25.07 mg) yielded much more tissue ($p < .01$; $d = 2.46$) than the microbiopsy needle (26.23 ± 5.95 mg).

4. Discussion

The present study used a randomized crossover design to compare perceptions of pain and hemodynamic reactivity to skeletal muscle biopsies performed with the Bergström and microbiopsy needles. The main findings were 1) compared to the microbiopsy needle, the Bergström needle elicited larger PPI scores but similar hemodynamic reactivity during the skeletal muscle biopsy (Fig. 3), 2) both needles produced similar PPI scores during the post-biopsy recovery period (Fig. 5), 3) most participants preferred the microbiopsy needle over the Bergström needle (Fig. 6), and 4) despite the Bergström needle yielding larger skeletal

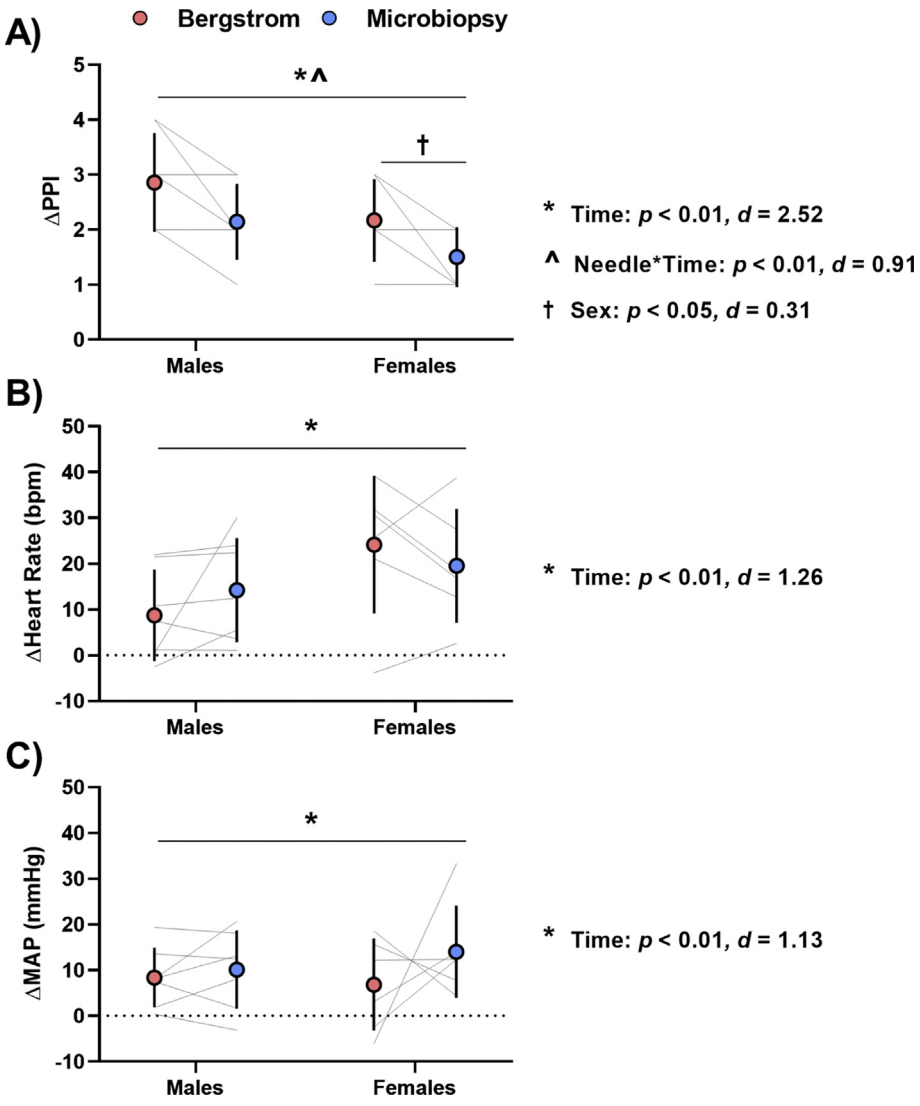

Fig. 3. Changes (Δ; during-biopsy value minus baseline value) in present pain intensity (PPI; A), heart rate (HR; B) and mean arterial pressure (MAP; C) during skeletal muscle biopsies performed with the Bergström and microbiopsy needles. Changes were calculated as during-biopsy value minus baseline value. Circles represent group averages, vertical lines represent standard deviations, and individual participant data are presented as the thin horizontal/angled grey lines (note two males and females have overlapping data in panel A; n = 7 males, n = 6 females). * Significant main effect of time, ^ significant needle × time interaction, † significant main effect of sex. All significance accepted at $p < .05$.

muscle samples, both needles yielded skeletal muscle samples with similar fibre type compositions (Fig. 7). Taken together, our findings suggest that studies not requiring large amounts of tissue (i.e. > ~30–40 mg) or certain muscle fibre lengths should consider using the microbiopsy needle. However, future work is needed to determine whether variations in biopsy procedures and/or types of Bergström/microbiopsy needles alter the finding that the Bergström needle is perceived as more painful and less favourable than the microbiopsy needle.

To our knowledge, little research has investigated pain responses during skeletal muscle biopsies in human participants (Hayot et al., 2005; Dengler et al., 2014), and we believe the present study is the first to qualitatively characterize perceptions of pain during biopsies. Interestingly, we found that "cramping", "aching", and "tender" were the three most heavily endorsed McGill descriptors for biopsies performed with the Bergström and microbiopsy needles (Fig. 4). Although the PCS is a validated survey that typically predicts pain scores (Sullivan et al., 1995), we did not observe significant correlations between PCS scores and biopsy-induced pain ratings (Fig. 2D). It is currently unclear whether these insignificant correlations suggest that the PCS does not predict perceptions of pain during skeletal muscle biopsies or whether they highlight insufficient statistical power owing to our small sample size. Nevertheless, our finding that the Bergström needle evoked a larger perception of pain than the microbiopsy needle (Fig. 3A) is consistent with previous work comparing pain responses during biopsies performed with both needles (Hayot et al., 2005) and other work demonstrating a dose–response relationship between pressure diameter and muscle pain response (Finocchietti et al., 2011; Lautenbacher et al., 2005). However, the Bergström needle did not elicit greater hemodynamic reactivity compared to the microbiopsy needle (Fig. 3B–C), and this finding opposes our hypothesis and the relationship between HR and pain intensity reported in previous work (Tousignant-Laflamme et al., 2005). The explanation for the apparent dissociation between perception of pain and hemodynamic reactivity in the present study is unclear, but may be attributable to our small sample size providing insufficient statistical power to detect needle-based differences in hemodynamic reactivity. Future studies with larger sample sizes should examine mechanisms associated with pain responses (e.g. activation of intramuscular mechanosensitive fibres and sympathetic activity (Graven-Nielsen & Mense, 2001; Tousignant-Laflamme et al., 2005)) to further interrogate the relationship between perceptions of pain and hemodynamic reactivity during skeletal muscle biopsies.

A large number of studies have investigated sex differences in pain responses, and the general consensus is that females are more sensitive to experimentally-induced pain compared to males (Fillingim, King, Ribeiro-Dasilva, Rahim-Williams, & Riley, 2009). However, we found that females reported lower perceptions of pain than males during skeletal muscle biopsies (Fig. 3A). Many mechanisms may explain sex

A)

Response	Number of participants per response: 0		1		2		3		Σ	
	B	M	B	M	B	M	B	M	B	M
Sensory McGill-Ds										
Throbbing	4	6	4	5	4	1	1	1	15	10
Shooting	3	4	7	5	3	4	0	0	13	16
Stabbing	4	5	6	4	2	3	1	1	13	12
Sharp	8	6	2	5	3	2	0	0	8	7
Cramping	3	5	1	3	5	3	4	2	23	17
Gnawing	3	5	6	5	4	3	0	0	14	12
Hot-Burning	11	12	2	1	0	0	0	0	2	2
Aching	2	5	4	4	5	3	2	1	20	16
Heavy	2	4	4	3	7	5	0	1	18	15
Tender	2	6	5	5	4	2	2	0	19	18
Slitting	11	10	1	3	1	0	0	0	3	3
Affective McGill-Ds										
Tiring-exhausting	8	9	3	3	1	1	1	0	8	7
Sickening	11	10	1	3	0	0	1	0	4	5
Fearful	7	10	2	0	2	2	2	1	12	10
Punishing-cruel	9	9	3	4	0	0	1	0	6	5

B)

Fig. 4. Qualitative assessments of perceptions of pain during the biopsy procedures. Panel A presents the number of participants reporting a given response (0–3) for each McGill-D and the total sum (Σ) of responses for each McGill-D. Σ reflects the magnitude that each McGill-D was endorsed by participants during the skeletal muscle biopsies. The Σ for each McGill-D was used to create a tag cloud (Fig. 3B) whereby the size of each McGill-D is reflective of Σ (i.e. the greater Σ the larger the font size). N = 13 (7M, 6F).

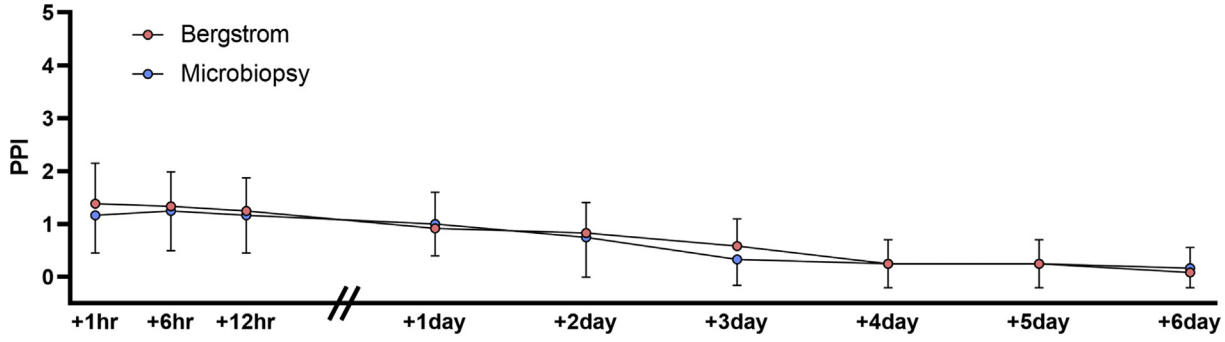

Fig. 5. Time course of present pain intensity (PPI) during the acute (1–12 h) and sub-acute (1–6 days) post-biopsy recovery period. Circles represent group means and error bars represent standard deviations. N = 13 (7M, 6F).

differences in pain responses (Fillingim et al., 2009), and one possible factor is the sex of experimenters. Some studies have found that participants report lower perceptions of pain when experimenters are of the opposite sex (reviewed in (Daniali & Flaten, 2019; Fillingim et al., 2009; Racine et al., 2012)). Accordingly, female participants may have reported lower perceptions of pain because 3 male researchers were present during the skeletal muscle biopsy procedures. Future studies including both male and female experimenters are needed to further explore the possible interaction between experimenter and participant sex on perceptions of pain during skeletal muscle biopsies.

Future work is also needed to determine whether our findings are generalizable to biopsies performed using different procedures and/or needle types. For instance, we made 3 cuts with the microbiopsy needle (~45 s total) while others studies made 5 cuts in an attempt to increase muscle sample yield (Hughes et al., 2015). It remains unknown whether participants respond less favourably if more cuts are made and the microbiopsy needle is inserted for more than ~45 s. Additionally, other microbiopsy needles may differ in sharpness or not involve a guiding cannula (e.g. one manufactured by Cook Medical, USA), and it is unclear whether our findings are generalizable to different types of microbiopsy needles. Thus, while our findings confirmed our hypothesis that the larger-diameter Bergström needle evoked greater perceptions of pain than the microbiopsy needle, future studies should test this hypothesis using different biopsy procedures and/or needle types.

The present findings point to several important practical implications for future studies performing skeletal muscle biopsies on human participants. First, mean PPI scores following the muscle biopsies were low for both needles (range ~0–1.5 out of 5 maximum) suggesting that pain induced by the biopsies did not persist during the post-biopsy recovery period (Fig. 5). Further, the relatively flat lines for mean BPI-9 scores indicated that biopsies performed with either needle resulted in little to no pain-related interference across several aspects of daily living (Supplemental Figure 2). Although we did not observe biopsy-related complications in the present study, Tarnopolsky et al. (Tarnopolsky, Pearce, Smith, & Lach, 2011) reported remarkably few complications (e.g. arterial bleeding, hematomas, and local skin infections) following

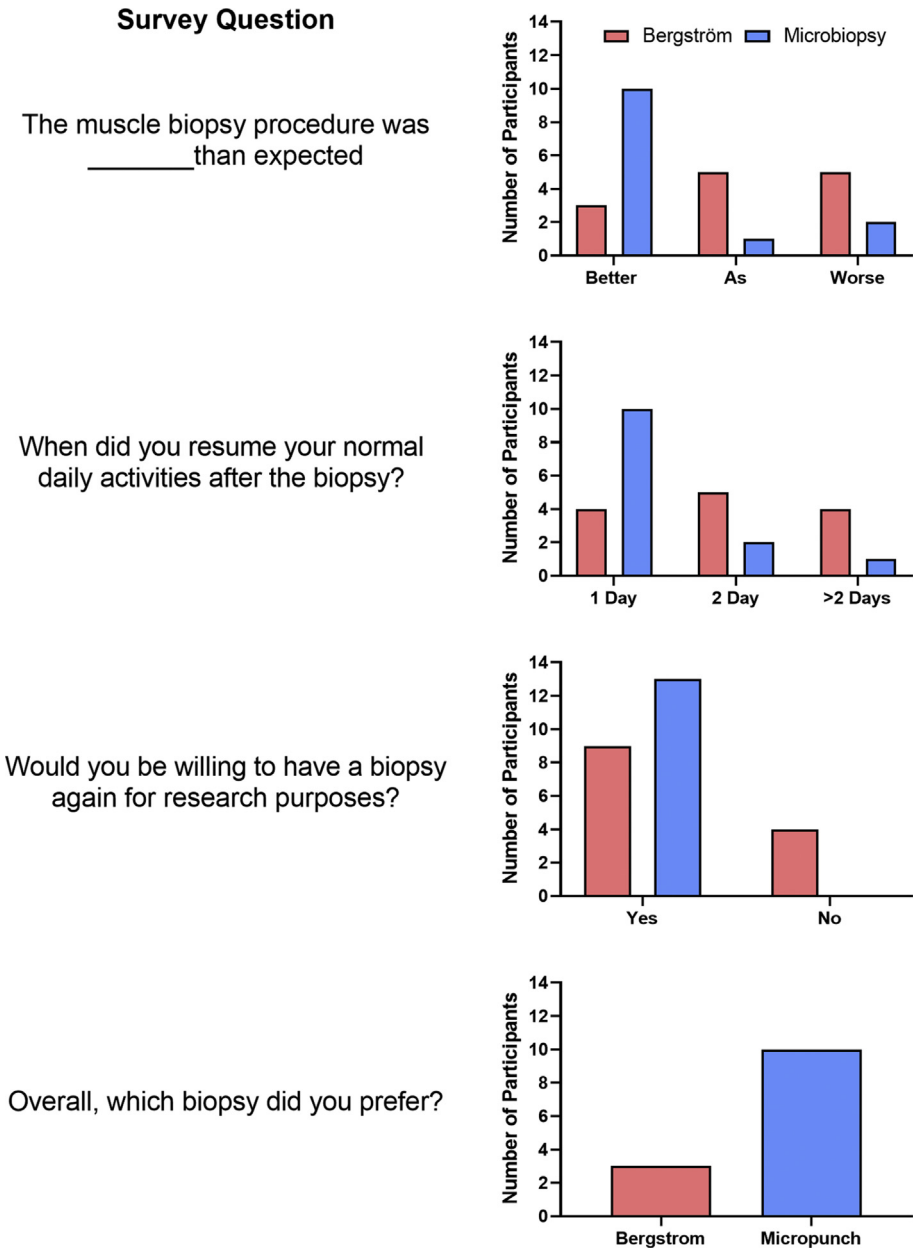

Fig. 6. Data from the exit survey that gauged participants' overall perceptions and preferences of the Bergström and microbiopsy needle procedures. N = 13 (7M, 6F).

~14 000 Bergström needle skeletal muscle biopsies. Collectively, these findings suggest that skeletal muscle biopsies elicit moderate but transient painful experiences and are safe procedures that do not negatively impact various aspects of daily living. Second, our exit survey found that the majority of participants preferred the microbiopsy needle over the Bergström needle (Fig. 6). Moreover, 4 participants said they were unwilling to have another Bergström needle biopsy whereas all 13 participants were willing to have another biopsy performed with the microbiopsy needle (Fig. 6). Although it appears the microbiopsy needle is perceived more favourably than the Bergström needle, the findings from our exit survey should be interpreted with caution as the validity and reliability of this survey are unknown.

In addition to measuring pain responses and hemodynamic reactivity, we found that the Bergström and microbiopsy needles yielded skeletal muscle samples with similar fibre type compositions (Fig. 7). This apparent similarity is consistent with previous studies comparing fibre type composition between two biopsies performed with the same type of needle (Sahl et al., 2018; Nederveen et al., 2020; Simoneau et al., 1986; Halkjaer-Kristensen & Ingemann-Hansen, 1981). However, repeated biopsies do not always yield samples with similar fibre type compositions (Boman, Burén, Antti, & Svensson, 2015), and we recently reported poor reliability in fibre type composition between separate portions of the same biopsy sample (Islam et al., 2019). Although the discrepancies between these findings are currently unclear, our findings support the hypothesis that differences in fibre type compositions between the Bergström and microbiopsy needles are attributable to obtaining samples at different muscle depths (Hughes et al., 2015) (Supplemental Figure 1). Given that many molecular characteristics are fibre type dependent (e.g. mitochondrial content, substrate storage, etc.) (Schiaffino & Reggiani, 2011), our findings suggest that molecular comparisons can be made between samples obtained with the Bergström and microbiopsy needle as long as samples are obtained at similar muscle depths. However, we did not measure insertion depths during muscle sampling, and we cannot be certain that samples were collected at an identical relative position within the *vastus lateralis*. For instance, it is possible that sex-based differences in thigh adiposity (Maden-Wilkinson, McPhee, Rittweger, Jones,

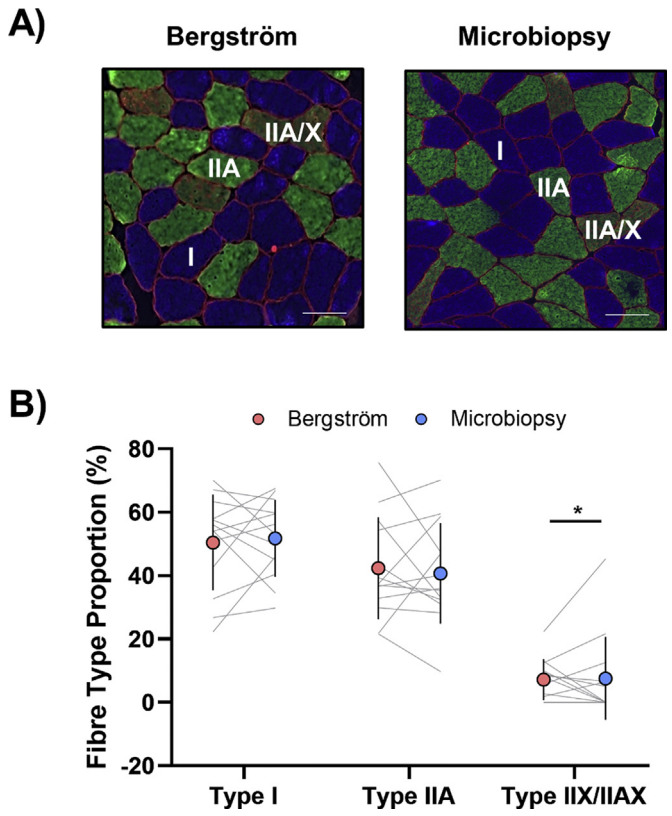

Fig. 7. Representative images from a single participant (A) and fibre type composition results (B) of skeletal muscle samples collected using the Bergström and microbiopsy needles. In panel A, blue fibres are type I, green are type IIA, and red/green are IIX/IIAX (labelled as "IIA/X"), and scale bars represent 100 μm. In panel B, circles represent group averages, vertical lines represent standard deviations, and individual participant data are presented as the thin horizontal/angled grey lines. N = 13 (7M, 6F). * Significantly ($p < .05$) different than type I and type IIA fibres.

& Degens, 2014; Nevill, Stewart, Olds, & Holder, 2006) resulted in different needle insertion depths between male and female participants. Future work should consider measuring muscle sampling depths to confirm the finding that the Bergström and microbiopsy needles yield samples with fibre type composition when collected at the same relative position within the muscle.

5. Conclusion

We found that skeletal muscle biopsies performed with the Bergström needle are perceived as more painful than biopsies performed with the microbiopsy needle, and both needles elicited similar hemodynamic reactivity and yielded samples with similar fibre type compositions. These findings suggest that future studies should consider using the microbiopsy needle if large amounts of tissue (i.e. > 30–40 mg) or certain muscle fibre lengths are not required. However, future studies are needed to determine whether our findings are generalizable to biopsies performed with different procedures and/or types of Bergström/microbiopsy needles. Future work can also explore whether differences in needle characteristics (e.g. level of sharpness) impact perceptions of pain or hemodynamic reactivity.

Author contribution

Conceptualization: J.T.B.; H.I.; N.P.; D.A.T.; C.G.R.P.; B.J.G.
Methodology: All authors
Formal analysis: J.T.B.; H.I.; N.P.; A.G.; B.J.G.
Investigation: J.T.B.; H.I.; N.P.; P.D.; A.M.; A. G.; J.Q.; B.J.G.
Resources: J.Q.; M.E.T.; D.A.T.; C.G.R.P; C.A.S.; B.J.G.
Data curation: N.P.; P.D.
Writing – original draft: J.T.B.; H.I.; B.J.G.
Writing – review and editing: All authors
Approval of manuscript: All authors
Funding acquisition: B.J.G.

Funding

This project was supported by a discovery grant by the Natural Sciences and Engineering Research Council of Canada (NSERC) awarded to Brendon J. Gurd (grant number: 402 635). Jacob T. Bonafiglia was supported by a NSERC Vanier Canada Graduate Scholarship, and Hashim Islam was supported by a NSERC Post-Graduate Scholarship (Doctoral). The author's declare no competing interests or conflicts of interest.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crphys.2020.05.001.

References

Bergström, J., 1975. Percutaneous needle biopsy of skeletal muscle in physiological and clinical research [Internet] Scand J Clin Lab Investig 35, 609–616. Available from: http://www.tandfonline.com/doi/full/10.1080/00365517509095787.

Bloemberg, D., Quadrilatero, J., 2012. Rapid determination of myosin heavy chain expression in rat, mouse, and human skeletal muscle using multicolor immunofluorescence analysis. Plos One 7.

Boman, N., Burén, J., Antti, H., Svensson, M.B., 2015. Gene expression and fiber type variations in repeated vastus lateralis biopsies [Internet] Muscle & Nerve 52, 812–817. https://doi.org/10.1002/mus.24616. Available from:

Boter, H., van Delden JJM, de Haan, R.J., Rinkel, G.J.E., 2003. Modified informed consent procedure: Consent to postponed information. BMJ Br Med J 327, 284–285.

Cleeland, C.S., Ryan, K.M., 1994. Pain assessment: Global use of the brief pain inventory. Ann. Acad. Med. Singapore 23 (2), 129–138.

Cohen, J., 1992. A power primer [Internet] Psychological Bulletin 112, 155–159. Available from: http://www.nature.com/articles/141613a0.

Dahmane, R., Djordjevič, S., Šimunič, B., Valenčič, V., 2005. Spatial fiber type distribution in normal human muscle [Internet] J Biomech 38, 2451–2459. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0021929004005147.

Daniali, H., Flaten, M.A., 2019. A qualitative systematic review of effects of provider characteristics and nonverbal behavior on pain, and placebo and nocebo effects [Internet] Front Psychiatry 10. Available from: https://www.frontiersin.org/article/10.3389/fpsyt.2019.00242/full.

Dengler, J., Linke, P., Gdynia, H.J., Wolf, S., Ludolph, A.C., Vajkoczy, P., et al., 2014. Differences in pain perception during open muscle biopsy and bergstroem needle muscle biopsy. J Pain Res 7, 645–650.

Edgett, B.A., Foster, W.S., Hankinson, P.B., Simpson, C.A., Little, J.P., Graham, R.B., et al., 2013. Dissociation of increases in PGC-1α and its regulators from exercise intensity and muscle activation following acute exercise [Internet] Plos One 8, e71623. Available from: http://dx.plos.org/10.1371/journal.pone.0071623.

Ekblom, B., 2017. The muscle biopsy technique. Historical and methodological considerations. Scandinavian Journal of Medicine & Science in Sports 27, 458–461. https://doi.org/10.1111/sms.12808. Available from:

Faul, F., Erdfelder, E., Lang, A.-G., Buchner, A.G., 2007. * power 3 : A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior Research Methods 39, 175–191.

Fillingim, R.B., King, C.D., Ribeiro-Dasilva, M.C., Rahim-Williams, B., Riley, J.L., 2009. Sex, gender, and pain: A review of recent clinical and experimental findings [Internet]. Elsevier Ltd The Journal of Pain: Official Journal of the American Pain Society 10, 447–485. https://doi.org/10.1016/j.jpain.2008.12.001. Available from:

Finocchietti, S., Nielsen, M., Mørch, C.D., Arendt-Nielsen, L., Graven-Nielsen, T., 2011. Pressure-induced muscle pain and tissue biomechanics: A computational and experimental study [Internet]. European Federation of International Association for the Study of Pain Chapters European Journal of Pain: EJP 15, 36–44. https://doi.org/10.1016/j.ejpain.2010.05.010. Available from:

Graven-Nielsen, T., Mense, S., 2001. The peripheral apparatus of muscle pain: Evidence from animal and human studies [Internet] The Clinical Journal of Pain 17, 2–10. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00002508-200103000-00002.

Halkjaer-Kristensen, J., Ingemann-Hansen, T., 1981. Variations in single fibre areas and fibre composition in needle biopsies from the human quadriceps muscle [Internet] Scandinavian Journal of Clinical and Laboratory Investigation 41, 391–395. Available from: http://www.tandfonline.com/doi/full/10.3109/00365518109092061.

Hayot, M., Michaud, A., Koechlin, C., Caron, M.-A., LeBlanc, P., Prefaut, C., et al., 2005. Skeletal muscle microbiopsy: A validation study of a minimally invasive technique [Internet] The European Respiratory Journal 25, 431–440. Available from: http://erj.ersjournals.com/cgi/doi/10.1183/09031936.05.00053404.

Hughes, M.C., Ramos, S.V., Turnbull, P.C., Nejatbakhsh, A., Baechler, B.L., Tahmasebi, H., et al., 2015. Mitochondrial bioenergetics and fiber type assessments in microbiopsy vs. Bergstrom percutaneous sampling of human skeletal muscle. Front Physiol 6, 1–10.

Islam, H., Edgett, B.A., Bonafiglia, J.T., Shulman, T., Ma, A., Quadrilatero, J., et al., 2019. Repeatability of exercise-induced changes in mRNA expression and technical considerations for qPCR analysis in human skeletal muscle [Internet] Experimental Physiology 104, 407–420. https://doi.org/10.1113/EP087401. Available from:

Isner-Horobeti, M.E., Charton, A., Daussin, F., Geny, B., Dufour, S.P., Richard, R., 2014. Microbiopsies versus Bergström needle for skeletal muscle sampling: Impact on maximal mitochondrial respiration rate [Internet] European Journal of Applied Physiology 114, 885–889. Available from: http://link.springer.com/10.1007/s00421-014-2819-8.

Jensen, M.P., 2003. The validity and reliability of pain measures in adults with cancer [Internet] The Journal of Pain: Official Journal of the American Pain Society 4, 2–21. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1526590002650011.

Lautenbacher, S., Kunz, M., Strate, P., Nielsen, J., Arendt-Nielsen, L., 2005. Age effects on pain thresholds, temporal summation and spatial summation of heat and pressure pain [Internet] Pain 115, 410–418. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00006396-200506000-00023.

Lexell, J., Henriksson-larsén, K., Sjöström, M., 1983. Distribution of different fibre types in human skeletal muscles 2. A study of cross-sections of whole m. vastus lateralis [Internet] Acta Physiol Scand 117, 115–122. https://doi.org/10.1111/j.1748-1716.1983.tb07185.x. Available from:

Maden-Wilkinson, T.M., McPhee, J.S., Rittweger, J., Jones, D.A., Degens, H., 2014. Thigh muscle volume in relation to age, sex and femur volume [Internet] Age (Omaha) 36, 383–393. Available from: http://link.springer.com/10.1007/s11357-013-9571-6.

Melzack, R., 1975. The McGill Pain Questionnaire: Major properties and scoring methods [Internet] Pain 1, 277–299. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00006396-197509000-00006.

Nederveen, J.P., Ibrahim, G., Fortino, S.A., Snijders, T., Kumbhare, D., Parise, G., 2020. Variability in skeletal muscle fibre characteristics during repeated muscle biopsy sampling in human vastus lateralis [Internet] Appl Physiol Nutr Metab 45, 368–375. Available from: http://www.nrcresearchpress.com/doi/10.1139/apnm-2019-0263.

Nevill, A.M., Stewart, A.D., Olds, T., Holder, R., 2006. Relationship between adiposity and body size reveals limitations of BMI [Internet] American Journal of Physical Anthropology 129, 151–156. https://doi.org/10.1002/ajpa.20262. Available from:

Osman, A., Barrios, F.X., Kopper, B.A., Hauptmann, W., Jones, J., O'Neill, E., 1997. Factor structure, reliability, and validity of the pain catastrophizing scale. J Behav Med 20, 589–605.

Poitras, V.J., Slattery, D.J., Gurd, B.J., Pyke, K.E., 2014. Evidence that meal fat content does not impact hemodynamic reactivity to or recovery from repeated mental stress tasks [Internet] Appl Physiol Nutr Metab 39, 1314–1321. Available from: http://www.nrcresearchpress.com/doi/10.1139/apnm-2014-0111.

Preobrazenski, N., Islam, H., Drouin, P.J., Bonafiglia, J.T., Tschakovsky, M.E., Gurd, B.J., 2020. A novel gravity-induced blood flow restriction model augments acc phosphorylation and PGC-1α mRNA in human skeletal muscle following aerobic exercise: A randomized crossover study [Internet] Appl Physiol Nutr Metab 45 (6), 641–649. apnm-2019-0641. Available from: http://jpaus.jaranboston.org/?eid.=957714.

Racine, M., Tousignant-Laflamme, Y., Kloda, L.A., Dion, D., Dupuis, G., Choinière, M., 2012. A systematic literature review of 10 years of research on sex/gender and pain perception – Part 2: Do biopsychosocial factors alter pain sensitivity differently in women and men? [Internet] Pain 153, 619–635. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00006396-201203000-00017.

Sahl, R.E., Morville, T., Kraunsøe, R., Dela, F., Helge, J.W., Larsen, S., 2018. Variation in mitochondrial respiratory capacity and myosin heavy chain composition in repeated muscle biopsies. Analytical Biochemistry 556, 119–124.

Schiaffino, S., Reggiani, C., 2011. Fiber types in mammalian skeletal muscles [Internet] Physiological Reviews 91, 1447–1531. Available from: https://www.physiology.org/doi/10.1152/physrev.00031.2010.

Scribbans, T.D., Edgett, B.A., Vorobej, K., Mitchell, A.S., Joanisse, S.D., Matusiak, J.B.L., et al., 2014. Fibre-specific responses to endurance and low volume high intensity interval training: Striking similarities in acute and chronic adaptation. Plos One 9.

Simoneau, J.-A., Lortie, G., Boulay, M.R., Thibault, M.-C., Bouchard, C., 1986. Repeatability of fibre type and enzyme activity measurements in human skeletal muscle [Internet] Clin Physiol 6, 347–356. https://doi.org/10.1111/j.1475-097X.1986.tb00240.x. Available from:

Snapinn, S.M., Jiang, Q., 2007. Responder analyses and the assessment of a clinically relevant treatment effect [Internet] [Trials Electronic Resource] 8, 31. Available from: http://trialsjournal.biomedcentral.com/articles/10.1186/1745-6215-8-31.

Sullivan, M.J.L., Bishop, S.R., Pivik, J., 1995. The pain catastrophizing scale: Development and validation [Internet] Psychological Assessment 7, 524–532. https://doi.org/10.1037/1040-3590.7.4.524. Available from:

Tarnopolsky, M.A., Pearce, E., Smith, K., Lach, B., 2011. Suction-modified Bergström muscle biopsy technique: Experience with 13,500 procedures [Internet] Muscle & Nerve 43, 716–725. https://doi.org/10.1002/mus.21945. Available from:

Tousignant-Laflamme, Y., Rainville, P., Marchand, S., 2005. Establishing a link between heart rate and pain in healthy subjects: A gender effect [Internet] The Journal of Pain: Official Journal of the American Pain Society 6, 341–347. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1526590005003688.

Wellek, S., Blettner, M., 2012. On the proper use of the crossover design in clinical trials. [Deutsches Ärzteblatt International Electronic Resource] 109, 276–281.