SUCTION-MODIFIED BERGSTRÖM MUSCLE BIOPSY TECHNIQUE: EXPERIENCE WITH 13,500 PROCEDURES

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ABSTRACT: *Introduction:* Bergström needle muscle biopsies have been used by exercise physiologists for over 35 years but have been less accepted by neuromuscular clinicians due to size concerns. *Methods:* We retrospectively reviewed over 13,500 muscle Bergström needle biopsies done over a 21-year period to determine sampling success, patient/subject experience, and complications. We compared sample yield between two different needles (Bergström vs. UCH), with and without suction modifications. *Results:* Needle biopsies adequate for histology and enzymology were obtainable from the vastus lateralis, deltoid, biceps brachii, soleus, and medial gastrocnemius muscles, with a success rate of >99.9% and a minor complication rate of 0.15%. Approximately 450 muscle fibers were submitted for histologic assessment; suction modification and use of the Bergström vs. UCH needle were associated with larger sample size ($P < 0.05$). *Conclusions:* The suction-modified Bergström needle muscle biopsy technique is safe and provides an adequate sample size for histologic, ultrastructural, DNA, and enzyme analysis.

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The development of a needle muscle biopsy technique by Bergström in the 1960s provided a viable alternative to the more invasive open biopsy technique.[1] Universal acceptance of the needle biopsy by exercise physiologists and muscle metabolism researchers followed[2–6]; however, neuromuscular clinicians and neuropathologists have traditionally been reluctant to use the method due to concerns about small sample sizes that were inadequate for diagnosis.

Advocates of the open biopsy procedure have claimed larger sample size and direct visualization of the muscle as important factors favoring the technique. Limitations of the open biopsy method include: the need for longer anesthesia/conscious sedation in children; a higher risk of infection (due to a longer incision); the need for multiple suture removal; and a longer and more prominent surgical scar (Fig. 1).[7] Importantly, the open method often requires the involvement of a non-neuromuscular clinician (usually an orthopedic or plastic surgeon) and prior booking (often of operating room time), both of which add to inconvenience and cost. By relative contrast, the use of a needle biopsy procedure has advantages when compared with the open technique, such as: ease of performance in an outpatient neuromuscular clinic (adults) or with conscious sedation (young children); less local anesthetic is required; less time and paramedical support is needed (reducing cost); and a there is a much smaller scar (4–5 mm; Fig. 1).

In order to diagnose myopathies, it is important that the sample size is adequate for the neuropathologist, and this has been the critical issue in the universal acceptance of the needle methods in neuromuscular diagnosis. We are completely in agreement with the latter contention when suctionless needle techniques are used; however, we and others[8–11] have developed suction techniques to increase sample size. The purpose of this study was to describe our clinical experience with over 13,500 muscle biopsy procedures completed by the same neuromuscular physician (M.T.) between 1988 and 2009. Furthermore, we sought to compare sample yield obtained with the two most commonly used muscle biopsy needles [Bergström[1] vs. University College Hospital (UCH)[12]], with and without suction modification.

METHODS

The first aspect of our study was a retrospective review of the muscle biopsy experience by one neuromuscular clinician (M.T.) at McMaster University from 1988 to 2009. The total number of biopsies was calculated from the number of biopsies taken for research studies and diagnosis during the aforementioned time period. The number of biopsies was calculated as an independent incision for a biopsy. For example, some of the research studies required that up to seven samples be taken at different time-points through separate incisions in the same individual, and this was counted as seven biopsies, given that there were seven incisions, and seven samples were obtained from a different region of the muscle. The Neuromuscular and Neurometabolic Clinic at McMaster University Medical Center recorded every clinical

Abbreviations: ANOVA, analysis of variance; COX, cytochrome *c* oxidase; CPEO, chronic progressive external ophthalmoplegia; EMG, electromyography; ERK, extracellular signal–regulated kinase; INR, international normalized ratio; MnSOD, manganese superoxide dismutase; NADH, nicotinamide adenine dinucleotide dehydrogenase; RT-PCR, reverse transcription–polymerse chain reaction; SDH, succinate dehydrogenase; UCH, University College Hospital; VDAC, voltage-dependent anion channel

Key words: Bergström, enzymology, histology, needle biopsy, neuromuscular diagnosis

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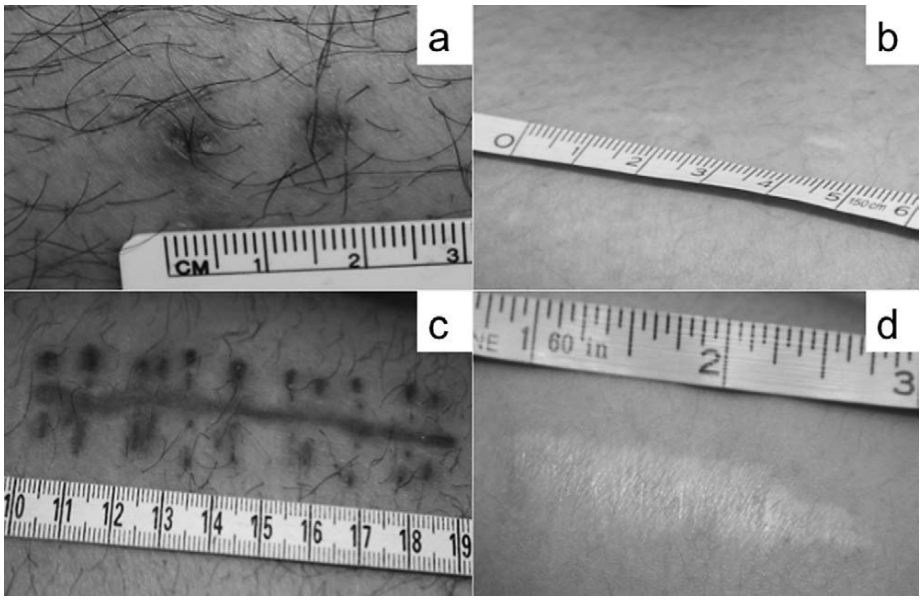

FIGURE 1. Examples of the scars from open and needle muscle biopsy techniques. Early **(a)** (<14 days) and later **(b)** (> 6 months) scars from a Bergström 5-mm needle. Early **(c)** (<1 month) and later **(d)** (>20 years) scars from open biopsy techniques.

biopsy for both pediatric ($N = 288$) and adult ($N = 1242$) patients over the time-course. A subgroup of patients ($N = 153$) were analyzed over a 12-month period (2009) to determine the proportion of biopsies that were: normal; insufficient for interpretation; specific for a diagnosis; non-specific/non-diagnostic changes not contributing to the final diagnosis; or non-specific changes that contributed to a subsequent diagnosis. We also verified the sample count using the number of O-rings that were purchased over the time-course (one O-ring is used per biopsy, see later).

Patients and research subjects were instructed to page the doctor and to report any complications following the procedure and to keep detailed records. The duration of discomfort and the subjective perception of the procedure were obtained from patients when they returned to discuss the results of the biopsy and by research subjects who returned for repeat biopsies or other testing procedures. We also looked at hematoxylin-and-eosin–stained histologic sections taken for diagnostic purposes from seven adult and three pediatric patient biopsies over a 2-week period in 2009, and these were analyzed in a blinded manner by the same student on 2 separate days for total fiber number. Permission for the study was obtained from the research ethics board at Hamilton Health Sciences.

Procedure. We have used the same 5-mm type of Bergström muscle biopsy needle over the 21-year period, and all have been purchased from the same manufacturer (Stille, Stockholm, Sweden). There are five main modifications that are completed on every needle by the Department of Biomedical Engineering (Fig. 2): (1) the top of the outer needle is dyed (D); (2) the end (B) is beveled [to allow for a snug-fit of the O-ring (A)]; (3) a modified screw-like bolt (E) is created with an inner tap (to screw onto the needle in 1) and an outer knurling (to grip for a tight closure); (4) the top of the inner trochar (B) is drilled out and polished in a beveled fashion to exactly receive; and (5) the suction adapter (F) is joined via silicone tubing to a sterile 60-ml syringe (G). All needles come with a plunger (C) for removal of pieces that may enter the inner trochar during the biopsy. A picture of the needle with our modifications is shown in Figure 2.

After obtaining informed consent, the area for biopsy is sterilized using chlorhexidine with a minimum of three expanding concentric circles. The specific site for biopsy is usually the vastus lateralis just anterior to the lateral fascia, between 25% and 50% of the distance from the lateral joint line to the greater trochanter. For suspected inflammatory myopathies, needle electromyography is used to determine the muscle that is most affected (right vs. left and vastus lateralis vs. deltoid), and the biopsy is taken from the most affected muscle. It is important to note that the electromyogram (EMG) and the biopsy are taken from the same muscle, but the sites are separated by at least 3 cm in the deltoid and 5 cm in the vastus lateralis. For suspected myopathy, the muscle for biopsy is one that is clinically affected while avoiding those where there is minimal or no palpable voluntary contraction.

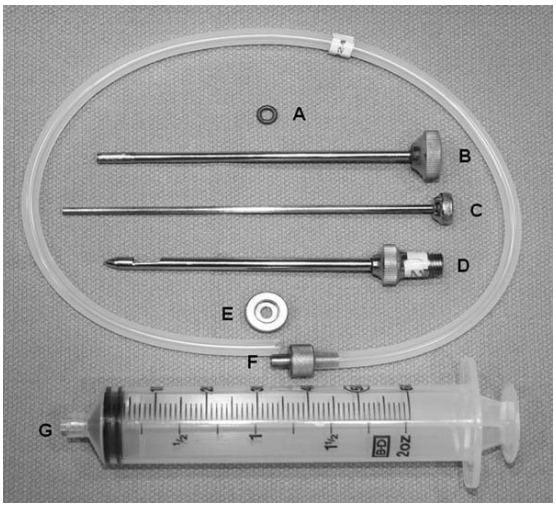

FIGURE 2. Bergström muscle biopsy needle and adaptations. The needle parts from the factory include the outer cannula, D, the inner trochar, B, and the plunger, C. The custom part, E, slides up the trochar, B, and the O-ring, A, slides on with a small amount of sterile petroleum jelly. The trochar is slid into D, and the nut, E, is screwed onto the outer cannula, D. The metal part of F is inserted with 'snug-fit' into the end of B, and the other end is attached to the 60-ml syringe.

The specific steps used in taking a biopsy are shown in Figure 3. After the site is chosen, the skin and subcutaneous tissue are infiltrated with ∽1 ml of 2% lidocaine (50% with and 50% without epinephrine) using a 26G 0.5-in. needle (Fig. 3a). After ∽1 min, further local anesthesia is implemented using 3–4 ml of lidocaine and a 22G 1.5-in. needle. The latter is used to "feel" the outside of the fascia, and the lidocaine is "salt and peppered" in the intended direction of the biopsy. It is essential that the operator be experienced enough to perceive the very distinctive change in resistance that is apparent when the fascia is touched so that the muscle is not infiltrated with the lidocaine mixture.

After about 2 min, a 4–5-mm stab incision is made through the skin, subcutaneous tissue (dermis and fat), and fascia (Fig. 3b). The needle is advanced gently in the direction of the incision, and the end of the needle is used to gently feel the small incision in the fascia. If the fascial defect is not apparent with the biopsy needle end, the scalpel is used to re-do the incision by advancing a bit further into the muscle. Once the biopsy needle enters the fascia, there is a bit more resistance as the needle is gently, but firmly advanced (sometimes using a twisting motion) into the muscle and at least 1 cm beyond the fascia, and the direction of the needle is advanced cephalad (Fig. 3c). The subject will usually feel a deep pressure sensation at this point, and the muscle will occasionally twitch. The inner trochar is pulled back by ∽1 cm such that the cutting port is completely open, and an assistant is instructed to pull suction with the 60-ml syringe (∽20 ml each clip). The needle is

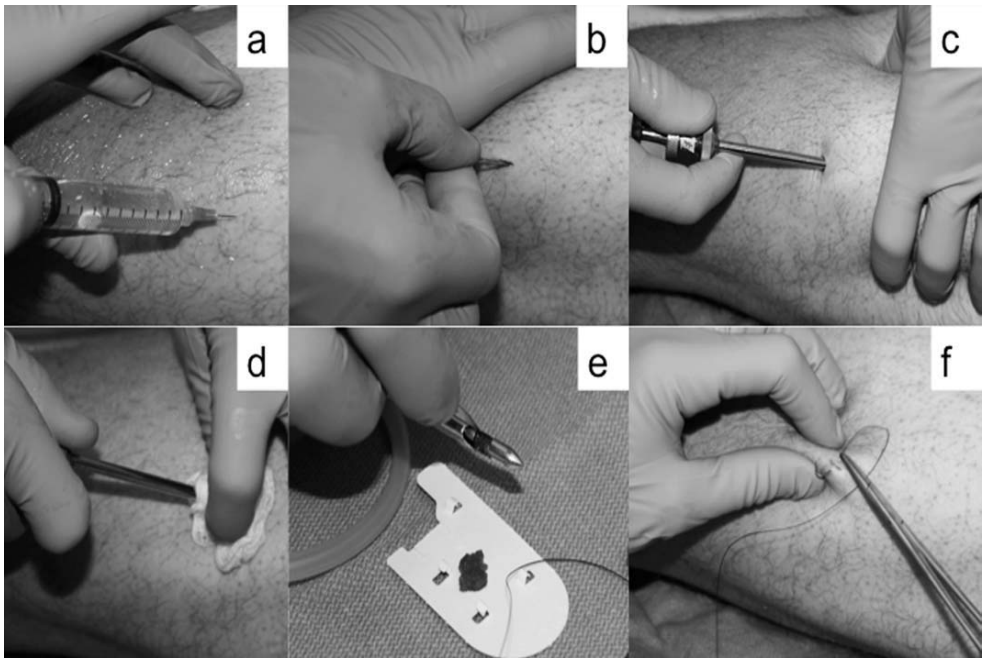

FIGURE 3. Bergström muscle biopsy technique. The skin and subcutaneous tissue are infiltrated with 2% lidocaine **(a)**, and a 4–5-mm stab incision is made to 'nick' the fascia **(b)**. The needle is inserted into the muscle, the trochar is pulled open, suction is applied, and the needle is closed **(c)**. The needle is removed with a twisting motion and counterpressure **(d)** and then inspected and divided into appropriate pieces **(e)**. A 3-0 silk suture is used for closure **(f)**.

firmly closed while maintaining the distance of the end of the needle at the same position within the muscle (Fig. 3c). The needle is than rotated 90° to the right, and another sample is taken. This is followed by a 180° rotation to the left, and the biopsy is retaken with coordinated communication with the suction assistant. The reason for the "three clips in one biopsy" method is that we usually need five separate pieces of muscle (minimum of 150–200 mg), and subjects find it more uncomfortable to go back in for three separate insertions as compared to taking them quickly with one needle insertion (clip–rotate–clip–rotate–clip).

The needle is removed using counterpressure with the opposite hand and a twisting motion (Fig. 3d), and the sample is inspected for adequacy (Fig. 3e, see later for amounts needed). The incision is closed using a single 3-0 silk suture (Fig. 3f), which is removed in 6 days. Some operators will use a tape closure such as a Steri-Strip or "butterfly," but we have found that the scar is less with the silk suture, because the risk of the wound opening up is much narrower than with the tape-type closures. Pressure should be held on the biopsy site for 10–15 minutes to reduce any bleeding, often using a Tensor-type bandage and an ice pack. Patients/subjects are given instructions to clean the area once or twice per day (and any time the area may have become contaminated) with alcohol and apply a new bandage. They are instructed not to sit in a bath/pool/hot tub for 6 days until the suture is removed (most patients and subjects remove the suture themselves or have a friend/relative/spouse do so). They are given an instruction sheet and clinic and pager numbers and instructed to report any unexpected issues. Patients are allowed to take ibuprofen or acetaminophen (if no other contraindications) as needed if there is discomfort not alleviated by icing. Research subjects are not allowed to take these medications if a subsequent timed biopsy is planned, for they can alter muscle protein synthesis.[13] We tell patients not to stop aspirin or other antiplatelet agents and ask the prescribing doctor if it is safe to hold warfarin for 5 days before the procedure. In seven cases, patients on warfarin were at high risk for blood clots and needed a muscle biopsy (myositis). All were completed with international normalized ratios (INRs) of 1.7–2.5 with no complications (we apply pressure for an extra 10 minutes and have them wait for 30 minutes after the procedure and re-inspect). The resulting scar from the needle biopsy is very small and often difficult to visualize after a few months, as compared with the scars from an open biopsy (Fig. 1).

For clinical specimens, the sample is placed on a sterile piece of cardboard (the back of the suture, Fig. 3e), and cut into pieces for: (1) electron microscopy (a longitudinal piece of pristine-appearing muscle—$\backsim$10–15 mg placed immediately into chilled 2% glutaraldehyde); (2) light microscopy [a round or square piece ($\backsim$30 mg) of pristine-appearing muscle placed onto tissue that has been pre-moistened with several drops of sterile 0.9% saline and placed into a sterile culture tube]; (3) protein and/or enzyme analysis (50–75 mg placed into a sterile 1.5-ml microcentrifuge tube); (4) mitochondrial DNA evaluation (50–60 mg for mtDNA mutation analysis and/or deletions and/or depletion), which can be used for other tests if needed; and (5) an extra piece (15–60 mg) for back-up and unexpected testing required after the initial pathology evaluation. Consequently, a biopsy procedure that yields a sample size of 150–200 mg can be used for electron microscopy,[14] light microscopy,[15] mitochondrial enzymes,[16] Western blotting,[17] reverse transcription–polymerase chain reaction (RT-PCR) for specific mRNA species and/or mitochondrial DNA depletion,[16] long-range PCR for mitochondrial deletions,[18] and global mRNA abundance using microarray technology[19] on the same sample.

The pathology samples are transported on wet ice to the pathology laboratory in the sterile culture tube on wet ice and are embedded and frozen in optimal cutting temperature medium. Biopsy specimens are cut at 7 μm and used for the following stains and histochemical methods in all cases: hematoxylin and eosin; modified Gomori trichrome; van Gieson method for elastin containing connective tissue; periodic acid–Schiff reaction; oil red O; myosin adenosine triphosphatase at pH 4.3, 4.6, and 10; nicotinamide adenine dinucleotide dehydrogenase (NADH); succinic dehydrogenase (SDH); cytochrome *c* oxidase (COX); alkaline and acid phosphatases; non-specific esterase; myophosphorylase; phosphofructokinase (in selected cases); and adenylate deaminase. Immunohistochemistry for the components of the sarcolemma has been carried out in the majority of childhood cases and selected adult patients using antibodies to the following antigens: dystrophin (central, carboxyl, and amino domains); alpha-, beta-, delta-, and gamma-sarcoglycans; beta-dystroglycan (all from Novocastra); dysferlin (ID Laboratories); caveolin-3 (BD Biosciences); and merosin (Sigma). For subtyping of inflammatory cells, monoclonal antibodies are applied for detection of the following cluster of differentiation antigens: CD3, CD4, CD8, CD20, CD68, and CD45.

Visualization of the antigen/antibody reaction is achieved using the streptavidin–biotin detection system (Histostain Plus; Invitrogen) or supersensitive polymer detection kit (Invitrogen), and with diaminobenzidine (Sigma) as the chromogen. The

dilution of primary antibodies is established empirically within the recommended range by the distributor/producer and each testing is accompanied by normal control muscle tissue. Small samples from each biopsy are also fixed in 2.4% glutaraldehyde and routinely processed for Epon embedding, thick sectioning, and subsequent electron-microscopic examination (Models 1230 or 1200EX; JEOL, Ltd., Tokyo, Japan).

Other samples are either placed into liquid nitrogen (with a 22G needle hole at top to prevent bursting) or packed in dry ice, and placed into a freezer at −80°C for subsequent analysis.[20] Research samples are processed similarly, but RNA analysis requires that the sample be rapidly plunged into liquid nitrogen and then stored at −80°C for up to 12 months.

Suction Modification and Sample Yield. In order to determine whether suction modification alters sample yield, we randomly chose one of our custom-modified 5-mm Bergström needles and purchased a 5-mm UCH needle (Dynamedical Corp., London, Ontario, Canada). We purchased a 1-kg round roast from a local butcher and performed four sets of 10 meat biopsies at a separate site with each of the needles, with and without the suction modification, in random order. For the UHC needle, we attached the silicone tubing to the side-port and used a 60-ml syringe; we used the same silicone tubing and syringe for the Bergström needle. One operator (M.T.) completed the meat biopsy with one experienced technician operating the suction (E.P.), and only a single "clip" was performed for each meat biopsy. To determine whether there were differences between non-suction and suction modification and between the Bergström and UHC needles, we completed a one-way analysis of variance (ANOVA) with the Tukey post hoc test to detect pairwise differences. $P < 0.05$ was considered significant. Values in the table and figures are expressed as mean (standard deviation).

RESULTS

Sample Adequacy/Complications. Samples sufficient for histology and electron-microscopic analysis were obtainable in 286 of 288 (99.3%) children, 1 of whom was a 3-week premature infant (−21 days). In pediatric cases, we were also able to get another piece of muscle suitable for protein/enzyme testing in 280 of 288 (97.2%). In adult research subjects, we obtained at least 1 sample in all lean and obese men and women from ages 18–89 years with sufficient samples for all analyses required in the study (usually three or four pieces at ∽50 mg each) in >99% of subjects. In adult patients, we were unable to obtain sufficient samples for histology or ultrastructural analysis in only 4 subjects, and all were later shown by magnetic resonance spectroscopy to have complete replacement of the quadriceps muscle with fat (2 with advanced limb-girdle muscular dystrophy and 2 with inclusion-body myositis). Overall, at least one sample of muscle >50 mg [i.e., enough for light and electron microscopy (the minimum needed for clinical diagnosis), or for any combination of the testing methods just described for research (usually Western blotting and enzymes)] was obtained in 99.9% of all patients/subjects (13,900 of 13,914).

Table 1. Biopsy complication rates.

	Adult (>18 y), n = 13,626		Pediatric (<18 y), n = 288	
Complication description	Male	Female	Male	Female
Arterial bleed	1	0	1	0
Ecchymosis/hematoma	0	2	0	0
Local skin infections (n = 8)	0	0	0	0
Occlusive dressing	3	0	0	0
Stitch left in >10 days	2	0	0	0
Unexplained	1	1	0	1
Localized numbness	3	2	0	0
Localized pain >3 days	3	2	0	0

Overall, the complication rate for all biopsies was 1 in 579 (1 in 619 adult patients, 1 in 144 pediatric patients; Table 1). The most common complication in both groups was local skin infection, and most had an identifiable cause. Most people feel a dull ache at the site of the biopsy starting in the evening after the biopsy and a slightly worse ache upon awakening the next morning. Pain that was perceptible and bothersome for >3 days was reported in 3 men and 2 women (1 in 2783), and the same frequency was also seen for localized numbness just distal to the biopsy site. The numbness was ∽4 cm^2 in area, non-dysesthetic, and disappeared within 3 months. It likely represented local trauma to a small branch of the lateral femoral cutaneous nerve. An intramuscular arterial hemorrhage was seen in 1 adult research subject and 1 pediatric patient, which resolved with 20 minutes of direct compression with no long-term consequences. Finally, 2 adult female patients developed an ecchymosis (likely slow venous bleeding) that was maximally evident at ∽4 days after the procedure. One of the women was later found to have a bleeding diathesis due to an α2-antiplasmin deficiency.

Examples of Pathology and Western Blotting. Examples of histology (Fig. 4a–d) and ultrastructure (Fig. 4e–h) from clinical (diagnostic) samples that were "definitive for the diagnosis" are shown for

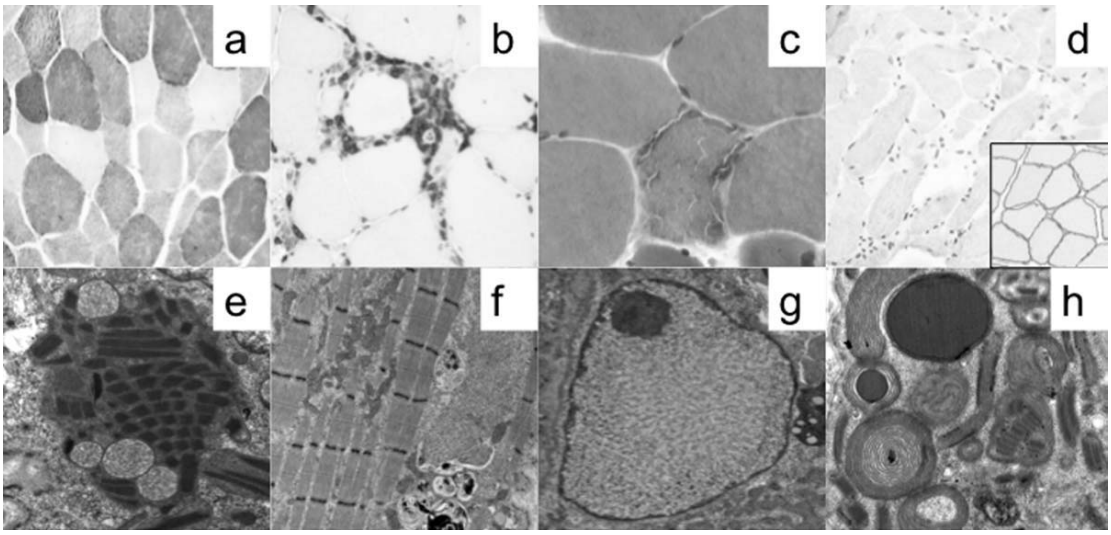

FIGURE 4. Examples of pathology from clinical samples taken with the Bergström needle. Upper panel histology: **(a)** COX-negative fibers in a patient with chronic progressive external ophthalmoplegia (CPEO); **(b)** endomysial infiltration with $CD8^+$ cells in a patient with overlap myositis due to mixed connective tissue disease; **(c)** ragged red fiber with modified Gomori trichrome staining in a patient with MELAS (mitochondrial encephalopathy with lactic acidosis and strokelike symptoms) syndrome; **(d)** immunohistochemistry staining with anti-merosin antibody in a boy with merosin-negative congenital muscular dystrophy (inset: positive control). Lower panel ultrastructure: **(e)** paracrystalline inclusions in a patient with a single large mtDNA deletion; **(f)** intracytoplasmic filamentous inclusion in a 76-year-old patient with inclusion-body myopathy; **(g)** filamentous nuclear inclusions in a patient with oculopharyngeal muscular dystrophy; and **(h)** pleomorphic mitochondria in a CPEO case with with COX deficiency.

illustrative purposes. Further details on the electron-microscopic sectioning and staining and the histologic techniques have been described in detail in several of our earlier reports.[20–23] Of the 153 patient samples sent for pathologic evaluation from the clinic over a 12-month period in 2009 we found: 12 samples with "normal muscle"; 2 samples insufficient for interpretation (fat and connective tissue in end-stage muscle); 54 that were specific for a diagnosis; 57 that showed non-specific/non-diagnostic changes that did not directly contribute to the final diagnosis; and 28 that showed non-specific changes (i.e., "dystrophic change," "neurogenic change," "consistent with mitochondrial disease") that led to a subsequent diagnosis (i.e., calpain-3 deficiency, spinal muscular atrophy type 2, and novel mtDNA point mutation, respectively). Of the 54 biopsies that were specific for a diagnosis, the following were found: mitochondrial myopathies [chronic progressive external ophthalmoplegia (CPEO) with COX-negative fibers ($N = 8$)]; mitochondrial cytopathies with ragged red fibers and/or COX-negative fibers and/or paracrystalline inclusions [myoclonus epilepsy associated with ragged red fibers, MELAS, and other novel mtDNA mutations ($N = 13$)]; inflammatory myopathies [inclusion-body myositis ($N = 5$), dermatomyositis ($N = 1$), and polymyositis/overlap myositis ($N = 4$)]; mini-core/multi-core/central core myopathy ($N = 6$); dystrophinopathy ($N = 4$); dysferlinopathy ($N = 2$); sarcoglycanopathy ($N = 1$); nemaline rod congenital myopathy ($N = 2$); McArdle disease ($N = 3$); phosphofructokinase deficiency ($N = 1$); merosin deficiency with congenital muscular dystrophy ($N = 1$); oculopharyngeal muscular dystrophy ($N = 1$); and statin-induced myonecrosis ($N = 2$). Figure 5 shows an example of Western blot determination of calpain-3 protein content in 2 patients with novel mutations in the *CAPN3* gene (vs. control), and extracellular signal–regulated kinase (ERK) 1/2;, voltage-dependent anion channel (VDAC), and manganese superoxide dismutase (MnSOD) in healthy men (research subjects).

Sample Yield Experiments. Analysis of 10 consecutive diagnostic biopsies revealed that the sample sent for histology contained an average of 425 muscle fibers in cross-section (range = 288–623). The UCH (26.9 mg) and Bergström (34.5 mg) needles were comparable in the beef sample yield with no suction (P = not statistically significant), and there were very significant increases in sample yield ($P < 0.01$) for both the UCH (+270%) and Bergström (+360%) needles (Fig. 6) with suction. The sample yield [mean (SD) from the Bergström needle [125.8 (38.1) mg] was greater than that of the UCH needle [73.2 (17.1) mg] ($P < 0.001$).

DISCUSSION

These findings provide the largest data set on muscle biopsy experience yet reported by nearly

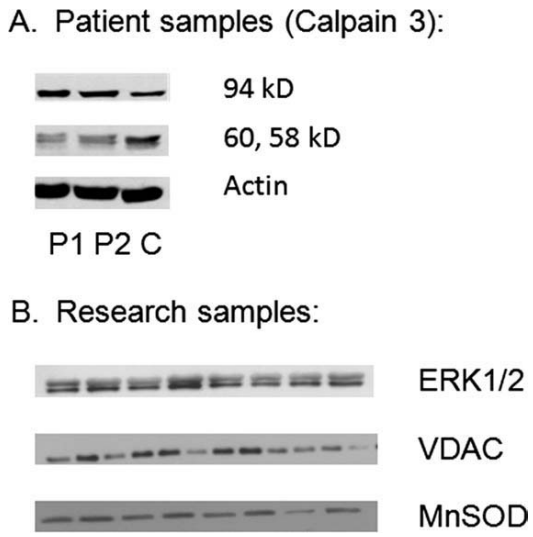

FIGURE 5. Examples of Western blot results for different proteins. **(A)** Calpain-3 was determined using a murine monoclonal antibody (Calp3c/12A2; Hybridoma Bank, Iowa City, Iowa) with the main band at 94 kDa and the autolyzed lower-molecular-weight doublets below (60, 58 kDa). It is less abundant for the 2 patients (actin is a loading control). P1 and P2 are patients with limb-girdle muscular dystrophy and mutations in the *CAPN3* gene as compared with a control patient (C) with no neuromuscular disease (after extensive work-up). **(B)** Examples of Western blotting of common proteins [ERK = extracellular signal–regulated kinase; VDAC = voltage-dependent anion channel (both from Cell Signaling, Danvers, Massachusetts); MnSOD = manganese superoxide dismutase (Abcam, Inc., Cambridge, Massachusetts)] in young men.

tenfold. The largest experience previously reported comprised a total of 1301 research biopsies,[24] and just over 900 for clinical diagnostic biopsies.[25] A major concern regarding the use of a needle muscle biopsy is that the sample size is not adequate for neuromuscular pathologic diagnosis. Our findings support this contention in that a non–suction-modified Bergström needle does not yield consistent sample sizes sufficient for comprehensive neuromuscular diagnosis (i.e., light and electron microscopy and samples for biochemical and/or molecular methods). Other investigators have found that needle biopsy methods without suction yield sample sizes of <25 mg.[10,26] In contrast, our data clearly show that the addition of suction modification to the UCH and Bergström needles clearly improved the sample yield by 270% and 360%, respectively. Similar increases in yield with the addition of suction have been observed in other studies.[8,10,11] In addition to the larger samples sizes that are possible with multiple clips, which we and others have seen,[10] there are 6-mm Bergström needles available that can further increase sample size.[11] Another benefit of the needle method is that in cases such as inflammatory myopathy, where the disease may be "patchy," it is possible to easily re-angle the needle into a new area of muscle to increase the sampling "field." The latter advantage of the needle biopsy has been discussed by other investigators in relation to inflammatory myopathy biopsies.[7,27–29] It is also our practice to section through the entire block of tissue when an inflammatory myopathy is suspected and the initial sections do not show the characteristic features of an inflammatory myopathy (Fig. 4).

Consistent with the view that the suction-modified Bergström needle provides sufficient samples for diagnostic purposes is the fact that we found a specific diagnosis from the biopsy alone in 35% of patients, and in another 12% of patients the biopsy contributed to the diagnosis directly (i.e., 47% of the time the biopsy led to the diagnosis). Because we do not do open muscle biopsies, the two methods could not be compared directly for diagnostic yield; however, other investigators have reported that the open biopsy "confirmed the diagnosis" in 49% of cases.[29] It is important to note also that a normal or "non-specific" result on a muscle biopsy is also helpful in "ruling out" diagnoses. Furthermore, in cases where the changes in the muscle biopsy were "non-specific" and "non-contributory" to the diagnosis, the biopsy was often helpful as a source of tissue for the definitive test. The most common example of the latter is in children with mitochondrial cytopathies, where the histopathologic changes are often subtle or lacking. We frequently use the tissue in such cases for definitive molecular (i.e., mtDNA sequencing, mtDNA

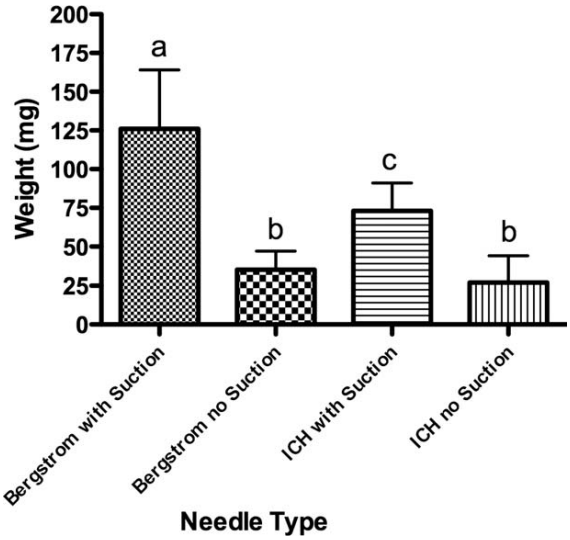

FIGURE 6. Sample yield from different needle types with and without suction. Average sample yield from 10 samples using a single clip in round steak with two needle types with or without suction. Values with different letters are significantly different from each other.

deletions and depletion) or enzyme (i.e., complex I deficiency) analysis. Consequently, the designation of "non-specific and non-contributory" refers only to the non-contribution of the pathology to the final diagnosis and not the valuable contribution of the biopsy *per se* as a source of material for the accurate diagnosis. Our data on diagnoses made from the biopsy samples in 2009 contains a somewhat higher-than-expected frequency of metabolic myopathy cases due to a referral bias at our clinic; however, the various diagnoses made (muscular dystrophy, neurogenic atrophy, mitochondrial myopathy, inflammatory myopathy, etc.) are representative of cases found in most tertiary-care centers.

This study has directly compared two different needles for sample yield. The higher overall yield from the Bergström needle vs. the UCH is due to the effect of the screw top and the O-ring adapter on the modified Bergström needle, which ensures an air-tight seal between the inner trocar and the outer cylindrical casing. There is an audible air leak that one can hear and feel when using the UCH with the side-port suction adaptation. Several other groups have also shown a variety of suction adaptations to the Bergström needle, yet none have presented a completely airtight sealing mechanism[8–11] and/or they described a suction unit where the sample can enter the tube or unit.[2,11]

Another advantage of a needle biopsy technique is that the procedure can be done in an outpatient setting with minimal set-up. The latter confers many advantages, such as the ability to immediately biopsy a muscle when a consultation is suggestive of inflammatory myopathy and the treatment can begin immediately. Furthermore, the neuromuscular clinician who completes the consultation can identify affected muscles using EMG and immediately biopsy the muscle (vastus lateralis, deltoid, biceps brachii, medial gastrocnemius) without having to arrange for surgical consultation and procedure room time. An obvious advantage to the needle biopsy is that a small scar remains (Fig. 3). The latter is important in case of the need to rebiopsy a patient, and we have found little reluctance for repeat needle biopsy in contrast to a repeat open biopsy. From a research perspective, the needle biopsy is also far more acceptable to participants and has the advantage of allowing for time-course studies where up to three separate muscle biopsies from each leg can be obtained safely.[5,30] From a scientific perspective, we have also reported that the biopsy data obtained in one leg from healthy older adults is essentially identical to that in the contralateral leg.[31] Other studies have shown that, when separate incisions are made (as is always our practice), a prior biopsy does not influence subsequent biopsy data.[32] The latter data are important for research experiments where repeated biopsies are required and/or an intervention is performed on one leg with the other as a control.

The complication rate that we have seen in both patients and research subjects is far less than 1%, and is similar to previous reports.[24,25] The most frequently reported complication was local skin infection (0.06%), and in the majority of these cases there was a subject error that contributed to the complication (occlusive dressing, stitch left in too long). The latter have not been seen in the past 3 years now that we give explicit written instructions regarding these issues. Furthermore, skin infections were not seen in any of the adult patients and in only 1 child who was immunocompromised. The 2 (1 adult research subject and 1 pediatric patient) arterial hemorrhage cases were identified by the pulsatile nature of the blood. This was resolved with 20 minutes of compression, and no hematoma developed.

Overall, we conclude that the Bergström muscle biopsy technique with suction yields adequate sample size for both clinical evaluation and research studies, is cost- and time-efficient, has a minimal complication rate, and offers a less invasive procedure when compared with the open biopsy method. Importantly, we have also shown that the suction-modified Bergström needle muscle biopsy technique is safe and can be performed in the neuromuscular clinic as part of a consultation or follow-up in all age groups.

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