Video Article

Human *Vastus Lateralis* Skeletal Muscle Biopsy Using the Weil-Blakesley Conchotome

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Abstract

Percutaneous muscle biopsy using the Weil-Blakesley conchotome is well established in both clinical and research practice. It is a safe, effective and well tolerated technique. The Weil-Blakesley conchotome has a sharp biting tip with a 4 - 6 mm wide hollow. It is inserted through a 5 - 10 mm skin incision and can be maneuvered for controlled tissue penetration. The tip is opened and closed within the tissue and then rotated through 90 -180° to cut the muscle. The amount of muscle obtained following repeated sampling can vary from 20 mg to 290 mg which can be processed for both histology and molecular studies. The wound needs to be kept dry and vigorous physical activity kept to a minimum for approximately 72 hr although normal levels of activity can restart immediately following the procedure. This procedure is safe and effective when close attention is paid to the selection of subjects, full asepsis and post procedure care. Both right and left *vastus lateralis* are suitable for biopsy dependent on participant preference.

Video Link

The video component of this article can be found at https://www.jove.com/video/53075/

Introduction

Obtaining skeletal muscle for the diagnosis of a myopathy and other neuromuscular degenerative diseases relies on safe and efficient methods which are acceptable and not incapacitating to patients ¹. Historically, methods of obtaining muscle tissue for analysis included open biopsies performed under general anesthesia or from post mortem. These techniques allowed direct visualization of the muscle and permitted a larger piece of muscle to be biopsied. Given the potential disadvantages these techniques have to the patient in terms of hospital stay and recovery, percutaneous 'semi-open' muscle biopsy methods were introduced as alternatives. This technique was first performed by Duchenne (1806-1875), who used a self-constructed needle with a trocar and was able to obtain a muscle sample from a living subject without general anesthesia ². Since then, various forms of biopsy needles have been used ^{3,4,5}. However, the biopsy needle developed by Bergstrom in 1962 has been the most commonly used in clinical practice; both in children and adults, as well as in research ^{6,7,8,9,10}. It possesses a sharp trocar, a cutting cannula and a pushing rod to expel the tissue post biopsy. Muscle yields obtained from this procedure have been reported to range from 25 -293 mg after repeated sampling ^{11,9}.

Henriksson introduced the Weil-Blakesley conchotome in 1979 as an alternative semi-open muscle biopsy method (**Figure 1**) ¹². The instrument is a single structure unlike the Bergstrom construct and is designed like a forcep with a sharp biting tip. The tip size can range from 4 mm to 6 mm in width. When the two edges of the biting tip oppose, a hollow is formed that ensures capture of the muscle. The conchotome is inserted through a 5 - 10 mm skin incision and avoids the need for a trocar to penetrate the muscle and the overlying fascia as a scalpel can be used to make a track down to the muscle.

This method allows controlled tissue penetration with a high degree of maneuverability ^{13,14}. It is especially useful for sites where undue pressure could potentially damage neurovascular or underlying bony structures *e.g.*, at the site of the tibialis anterior ^{15,14}. Muscle yields using the conchotome can be variable and in our practice 20 - 200 mg of muscle has been obtained after repeated sampling. One potential advantage of the conchotome over the Bergstrom needle, in addition to the high degree of maneuverability it offers, is that the biting tip does not need to be sharpened or replaced as often as the trocar of the Bergstrom construct ¹⁶.

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The overall goal of muscle biopsy using the Weil Blakesley conchotome is to obtain sufficient amounts of muscle tissue to enable histochemistry and molecular analysis for both diagnostic and research study. It is a simple and safe procedure that can be learned easily. With this technique, muscle suitable for biopsy includes biceps, triceps, deltoid, gastrocnemius, tibialis anterior, soleus and the sacrospinals ^{17,15,7,18,14}. The outermost part of the *vastus lateralis* is the most common site used for biopsy as it avoids the main neurovascular structures within the thigh. It is identified approximately two thirds down an imaginary line joining the anterior superior iliac spine and the patella. Muscle morphology data, for example myofibre area, myofibre proportions, capillary density derived from *vastus lateralis* sampling are widely available in the literature allowing comparison between studies ^{11,19}.

Protocol

Ethics statement: A description of the muscle biopsy procedure we follow within our institution now follows. The muscle biopsy procedure is used in the Hertfordshire Sarcopenia Study which has been approved by the Hertfordshire Research Committee number 07/Q0204/68. All participants gave written informed consent ²⁰.

NOTE: A single operator can perform the procedure, aided by an assistant who can engage the participant in conversation in order to reduce anxiety and ensure that they are comfortable at all times. The procedure room should be equipped with a sink, a height adjustable couch, clean surfaces and a procedure trolley.

1. Prepare the Participant (Figure 2)

- Ask the participant to lay comfortably, supine on a couch. Expose the preferred thigh from the groin crease. Spread a disposable absorbent sheet under the exposed thigh. Ensure that the leg remains straight and relaxed throughout the procedure but the thigh is tensed momentarily to outline the vastus lateralis.
- 2. Mark the procedure site approximately two-thirds down an imaginary line from the anterior superior iliac spine to the patella.

2. Prepare the Skin (Figure 2)

- 1. Shave the skin to approximately 4 cm in diameter surrounding the proposed biopsy site with a sterile shaving blade and clean the skin with an alcohol swab.
 - NOTE: This is to prevent any subsequent hair entrapment in the wound during the healing process. This step is optional according to the operator's preference.
- 2. Infiltrate the skin and overlying fascia with local anesthetic. Use a 25 G needle to raise a subcutaneous bleb of 2% lidocaine then infiltrate deeper into the subcutaneous tissue with a 23 G needle. Aim to penetrate to at least half to one inch depending on the subcutaneous tissue content at the biopsy site. This depth should be sufficient to ensure the overlying fascia is anesthetized. Allow 2 5 min for the anesthetic to action. Confirm anesthesia by probing the skin gently with the needle or scalpel blade tip.

3. Prepare a Sterile Field (Figure 2)

- 1. Wash hands with soap and maintain clean hands until a sterile gown is worn. Cover hands with sterile gloves before creating a sterile field on the procedure trolley.
- 2. Sterilize the biopsy site with 2% chlorhexidine/isopropyl alcohol solution or an iodine based skin disinfectant. Apply a sterile drape with an adhesive aperture to expose the biopsy site but to also maintain a sterile field.

4. Perform the Procedure (Figure 2)

- 1. With a size 11 scalpel, make a 5 10 mm incision on the skin and down and through the overlying fascia. Insert the closed biting tip of the conchotome through the incision at a right angle to the long axis of the femur, to a depth averaging half to one inch. If required use the free hand to hold and support the thigh surrounding the biopsy site while the tip of the conchotome is opened and closed within the muscle.
- 2. Rotate the conchotome through 90 180° to cut the muscle. Withdraw the conchotome and open the tip onto sterile gauze dampened by sterile normal saline. Repeat the procedure within the single wound site, if necessary, to obtain sufficient muscle tissue. Transfer the gauze into a container placed on ice. Transfer the container to a preparation laboratory to process the muscle according to the local institutional protocol.

5. Post Procedure (Figure 2)

- 1. Apply direct pressure to the wound for up to 5 min. Close the wound with steri-strips by placing them parallel to the wound as opposed to right angles to avoid potentially removing scar tissue and reopening the wound if the steri-strips are removed inadvertently. Place a sterile absorbent dressing on the steri-strips and tie a two-layer bandage for compression and secure with tape.
- 2. Explain the wound dressing method to the participant as they will have to perform a dressing change 3 4 days after the procedure. Ensure that the participant removes the compression bandage before going to bed later that evening.

6. Post Biopsy Advice

1. Inform the participant that it is common to experience some thigh stiffness which can be relieved by gentle exercise (e.g., walking). Use simple analgesia such as acetaminophen. Warn the participant about transient numbness around the biopsy site that may persist for up to two weeks as well as the potential complication of wound infection.

7. Instructions for the Subject Post Procedure

- 1. Ask the participant to avoid vigorous activity for 72 hr (such as climbing, running, heavy lifting). Ask the participant to avoid immersion in water for 48 72 hr. However, instruct the participant to wrap cling film or similar around the biopsy site when having a shower to keep the dressing dry.
- 2. Ask the participant to change the dressing after 3 4 days and remove the steri-strips after one week.

 NOTE: The participant should be given written instructions for post biopsy care, spare dressings and emergency contact details of the team responsible for performing the procedure.

Representative Results

The procedure described above is safe and acceptable in both clinical and research settings. When the biopsy is performed as part of the diagnostic process, the muscle should be chosen according to patient's symptoms and signs of muscular weakness. The biopsy site should be free from previous injuries, contractures or instrumentation ^{21,1}. In research, standardized conditions such as fasting or exclusion of patients with diabetes may be required ¹⁹. Anticoagulants such as warfarin need to be stopped and clotting time checked before the procedure. Simple aspirin at a dose of 75mg can be continued as our experience suggests it does not significantly increase the risk of excess bleeding ²². Furthermore, consideration should be given to participants taking drugs that impair wound healing. Essentially, the risks of stopping medication should be weighed against the benefits of the procedure in each case. During participant selection, a detailed history of potential allergies to local anesthetic, iodine/chlorhexidine and wound dressings should be obtained and alternatives used as required. Participants should be warned about the rare but potential occurrence of allergic reactions which can manifest as increased redness of the skin, local swelling or blistering. In these circumstances removal of the dressing(s) and treatment with anti-histamines/steroids may be necessary.

The procedure takes approximately 15 - 20 min. It is essential to pay close attention to asepsis in order to minimize the risk of wound infection. During the procedure participants may experience some discomfort. Occurrence of any sharp pain may necessitate further infiltration of local anesthetic. After the procedure, thigh stiffness is commonly experienced and can be relieved by gentle exercise (e.g., walking). In the Hertfordshire Sarcopenia Study (HSS), the pain scores measured with the pain visual analogue scale (VAS) (scale from 0 mm - 'no pain' to 100 mm - 'pain as bad as can be') were typically low with a median of 7 mm during the procedure and 4 mm one day after the procedure suggesting the subjects in this research study found the procedure minimally painful ²² (Materials Table). The rate of wound complications reported in the literature ranges from 1% to 3% and mainly include wound hematoma formation ^{13,9,14}. One study described a serious a subfascial hematoma and a subsequent deep vein thrombosis ¹³. In the HSS the rate of wound complication (described as a hard lump underneath the scar for 3 weeks) was 1% ²². Participants may experience numbness around the small incision site given need to incise through the skin and subcutaneous tissue. In our experience this numbness has been transient and has fully resolved within one or two weeks.

With respect to representative histological results, we previously studied the association between developmental influences and muscle morphology 19 . In this study mean Type I myofibre area (SD) in 48 lower birthweight (\leq 3.18 kg) men was 4903 μ m² (1354 μ m²) and 4644 μ m² (1022 μ m²) in 47 higher birthweight (\geq 3.63 kg) men, whilst the mean type II myofibre area was 4046 μ m² (1166 μ m²) and 3859 μ m² (1127 μ m²) in lower and higher birth weight men respectively.



Figure 1. Image of Weil-Blakesley Conchotome. The Weil-Blakesley conchotome with a 6 mm biting tip is used in this study. Please click here to view a larger version of this figure.



Figure 2. Muscle Biopsy Technique Using the Weil-Blakesley Conchotome. (A) The leg was exposed from the groin crease. The biopsy area over the *vastus lateralis* was shaved of hair, marked, infiltrated with lidocaine and cleaned with antiseptic. The biopsy area was isolated. The skin and overlying fascia were then punctured with a size 11 scalpel. (B) The conchotome was inserted into the track made by the scalpel and rotated through 90° to excise the muscle. C&D. The 5 - 10 mm wound was closed with steri-strips and dressed (Previously published ²²). Please click here to view a larger version of this figure.



Figure 3. Steri-strip Application. The steri- strips are applied parallel to the small incision. This method is associated with a good rate of healing; typically within one week. Please click here to view a larger version of this figure.



Figure 4. Muscle Biopsies of the Vastus Lateralis. Please click here to view a larger version of this figure.

Visual Analogue Scale Pain Score (mm)	Median (Interquartile Range [IQR])
During the procedure	7 (1 - 34)
One day post procedure	4 (0 - 16)
Seven days post procedure	1 (0 - 4)
Daily Activity Resumed	N (%)
After one day	60 (65%)
After two days	26 (28%)
More than two days	7 (7%)

Table 1. Pain Visual Analogue Scale (VAS) Score and Resumption of Activity in 93 Research Participants after Conchotome Muscle Biopsy in the Hertfordshire Sarcopenia Study. The pain scale ranged from 0 mm - 'no pain' to a maximum of 100 mm - 'pain as bad as can be'. The majority of participants resumed usual daily activity one day post procedure.

Discussion

To ensure participant safety and perform the muscle biopsy efficiently, it is vital to pay attention to critical steps within the protocol.

The participants must be selected after carefully considering the exclusion criteria to avoid complications such as bleeding and poor wound healing. Strict asepsis throughout the procedure is essential. Application of the correct technique will ensure participant comfort, minimize unwanted trauma to participant's tissues and allow a sufficient sample yield for analysis. Occasionally, it may be difficult to obtain a muscle sample, especially if the participant has excessive subcutaneous fatty tissue. Excess bleeding may occur even after careful selection of participants in which case a decision to proceed with procedure should be made. Direct pressure to the wound site for up to 5 min may be necessary to encourage hemostasis and minimize bruising. Another method to minimize bleeding is to use lidocaine with epinephrine which will cause a local vasoconstriction. However, epinephrine may interfere with subsequent analyses and caution must be used. It is the author's preference not to use epinephrine during this procedure.

Multiple analyses can be performed in muscle tissue given the advances in microscopy, image analysis, histochemical and molecular analytical methods. These include assessing muscle morphology *e.g.*, fiber composition, fiber cross sectional area, vascularity, single fiber contractile activity, analysis of RNA, protein as well as enzyme activity ^{23,24,25,19,26,27}. Though used routinely used in the clinical diagnostic process of myopathy, muscle biopsy is increasingly performed in research. For example, we have previously shown that developmental influences on

muscle morphology may explain the associations between lower birth weight and sarcopenia¹⁹. Potential limitations of this technique, similar to other semi-open techniques, include being limited to one site where the morphology *i.e.*, muscle fiber type may not be representative of the whole muscle group.

Muscle biopsy with the Weil Blakesley conchotome is a feasible and acceptable procedure. This procedure provides an alternative method to the Bergstrom and other needle biopsy methods. It is easy to master and gives a good sample yield for analysis. The complication rate is low and discomfort kept to a minimum when the procedure is performed using a standardized technique as described above, strict aseptic conditions maintained and comprehensive advice on post biopsy care is given.

Disclosures

None declared.

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