Increase in percutaneous muscle biopsy yield with a suction-enhancement technique

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HENNESSEY, JAMES V., JOSEPH A. CHROMIAK, SHIRLEY DELLAVENTURA, JENNIFER GUERTIN, AND DAVID B. MACLEAN. Increase in percutaneous muscle biopsy yield with a suction-enhancement technique. J. Appl. Physiol. 82(6):1739-1742, 1997.—The percutaneous muscle biopsy technique is used in clinical practice and biomedical research. We developed a new enhanced-suction technique [suction-enhancing nipples (SEN)] and compared it with techniques currently in practice by assessing biopsy yields on anesthetized pigs. We applied the enhanced-suction technique to human subjects participating in a clinical trial. In the pig, there was a mean 91% (1.9-fold) increase in the size of the samples obtained with the 4-mm needle when SEN was used and a mean 507% (fivefold) increase in sample size when the SEN was applied to the 6-mm needles. Nine passes of the 6-mm needle with SEN obtained from five consecutive human subjects yielded a mean individual sample size of 109.4 mg or 219.4 mg per needle pass when using the double-sample technique. Adequate tissue samples for histomorphometric and other analyses were obtained in all samples obtained. The percutaneous muscle biopsy performed with enhanced suction using inexpensive, readily available nipples enhances tissue yield two- to fivefold.

A PERCUTANEOUS MUSCLE BIOPSY technique described by Bergström (1) is recognized as an essential tool in clinical practice and biomedical research (6). This technique, which also has been referred to as a punch biopsy (13) or semiopen biopsy (9), is useful for biochemical, histochemical, and histomorphometric analyses (3, 4, 10, 11, 14) and achieves results similar to those obtained with open muscle biopsy procedures (7, 12). The addition of suction through the cutting trocar was described in 1982 as a means of enhancing the size of the tissue samples obtained with each insertion of the needle (6). The resultant increase in tissue yield made it possible to apply a wider variety of analytic assays to each sample, without increasing discomfort to the patient (6). In the process of standardizing the percutaneous biopsy technique, as most recently described (12), for application in a human protocol, we noted difficulty in obtaining an adequate connection between syringe and cutting cannula that is necessary for optimal suction generation with both 4- and 6-mm Bergström needles. We instituted several modifications to the technique to ensure reliable connections for both the 4- and 6-mm cutting cannulas, including a gasket system, to further enhance suction. In our hands, these modifications objectively enhanced sample size up to sixfold, compared with specimens obtained without suction enhancement in our animal model, and resulted in abundant material for our investigations in all human subjects sampled.

METHODS

Equipment for Performing Suction-Enhanced Needle Biopsies of the Musculature

Biopsy needles. The needles used were 4- and 6-mm Bergström cutting trocars (Stille, Stockholm, Sweden).

Cutting cannula adapters. Cutting cannula adapters were B-D InterLink vial access cannula (no. 303367; Becton Dickinson, Franklin Lakes, NJ) (4 mm) and Argyle female luer lock connector (no. 8888–275008; Sherwood Medical, St. Louis, MO) (6 mm).

Suction-enhancing nipples (SEN). We used infant nipple, Ross Laboratories, Columbus OH (4 mm) and cross-cut nipple, Mead Johnson no. 4288–13 (a division of Bristol-Meyers Squibb, Evansville, IN) (6 mm).

Lubricant. For 6-mm cutting cannula, we used Surgilube, Fougera (a division of Altana, Melville, NY).

We also used a 10-ml syringe, scalpel no. 11, sterile supplies towels, fenestrated drape, petri dish, 4 x 4-in. sterile dressings (6), 2 x 2-in. dressings (2), iodophor solution, 10 ml 1% Novocain without epinephrine, 10 ml sterile saline, 1/4-in. Steristrips, and foam or elastic tape (as compressive dressing).

Technique

Human procedure. From a presterilized tray containing most of the equipment outlined above, a small sterile field was prepared on a Mayo stand. A 2 x 2-in. sterile dressing was placed in a petri dish and soaked with 5 ml of saline; the dish was covered and placed on crushed ice in preparation for receiving the sample.

The Bergström needle and all related supplies were gathered before beginning the procedure (Fig. 1). Next, the needle was assembled to ensure proper alignment and sliding action. With the clearing rod fully inserted through the cutting cannula, the blunt end was eased through the opening of a nipple from the outer approach so as to accomplish a positioning of the nipple on the cannula shaft as illustrated in Fig. 2. The cannula-nipple assembly was placed on the trocar so that the nipple slides firmly over the open end of the trocar (Fig. 2) creating a tight seal. The nipple was held in place using the thumb and third finger of the nondominant hand. At this point, the cutting cannula was fully inserted into the trocar to ensure that the thickness of the nipple material being utilized allowed full closure of the cutting chamber. The use of a sterile surgical lubricant further maintains both the seal and
the integrity of the nipple opening (by avoiding tearing), especially when working with the 6-mm needle. The lubricant diminishes friction when the trocar is moved within the nipple and prevents tearing of the nipple opening. The male end of an appropriately sized adapter was inserted into the proximal end of the cutting cannula and attached to a syringe via connecting tubing (Fig. 2), creating a closed system with which to generate suction. The syringe plunger was withdrawn to the 3-ml mark before muscle insertion to create a buffer of air for use in removing the sample at a later time (see below).

The patient assumed a comfortable reclining position with both legs outstretched. The vastus lateralis biopsy site, at a point 25 cm proximal from the tuberositas tibiae and 5 cm lateral from the midline of the femoral course, was prepared with iodophor paint. Skin and subcutaneous tissue were infiltrated with 10 ml of 1% Novocain, ensuring that the fæsðæ lata was well infiltrated. After ensuring adequate local anesthesia, a stab wound was made in the lateral thigh at the biopsy site directly through the overlying skin, subcutaneous fat, and fæsðæ lata. The width of a no. 11 blade is sufficient for insertion of the 4-mm trocar directly through the stab wound pathway into the muscle to be biopsied. When the 6-mm trocar was utilized, the stab wound was extended laterally 2–3 mm to accommodate the larger needle. Once the trocar has been inserted through the fæsðæ (generally the sensation of overcoming resistance is obvious), it was advanced to ensure that the cutting chamber opening (window) would lie fully within the muscle. It is very important to ensure that the whole needle window is within the muscle when suction is
applied, since interruption of the fragile vessels in the fat and connective tissue may lead to excessive bleeding and/or pain. While the nipple atop the trocar was held as described above, the cutting trocar was withdrawn 2.5 cm. An assistant immediately applied suction by fully withdrawing the plunger of a 10-ml syringe, thus drawing muscle tissue into the cutting chamber as the cutting trocar was simultaneously advanced, sliding off the sample as the assistant released the suction. To maximize the amount of muscle sample obtained per insertion, the trocar was rotated 90° in a clockwise fashion, and the procedure was repeated before withdrawal of the trocar from the thigh (double-sample technique).

Animal experiments. To directly compare tissue yields obtained with our modified technique with those obtained by using the conventional technique described by Evans et al. (6) and Mubarak et al. (12), we obtained multiple biopsies on a pig with both techniques. An anesthetized pig was sampled, following an unrelated laparoscopic procedure that had been approved by the animal use committee before planned death of the animal. Multiple needle biopsies were obtained on the lateral thigh musculature in a 5-cm area midway between the proximal and distal femur. A total of 25 samples, through three separate stab wounds on each thigh, were obtained with both needle sizes using a 10-ml syringe with and without SEN. All samples were weighed immediately in the procedure room, and results were recorded. Statistical analysis of data was performed by using t-tests for parametric data and the Mann-Whitney rank sum test for nonparametric data. A P value of < 0.05 was accepted as statistically significant.

Human sampling. Based on our experience with SEN in animal sampling (see below), we used SEN in the conduct of a clinical trial evaluating the effects of growth hormone on muscle. Consecutive samples obtained on a subset of these subjects were weighed to quantify the sample weights of the suction enhanced technique obtained with a 6-mm needle.

RESULTS

Animal Studies

The results of the sampling experiment carried out to test the effectiveness of the SEN are outlined in Fig. 3. There was a 91% (1.9-fold) increase in the size of the samples obtained with the 4-mm needle when a SEN was used and a 507% (fivefold) increase in tissue yield when 6-mm needles were used.

Table 1. Biopsy sample sizes using a suction-enhanced 6-mm Bergström in five patients

<table>
<thead>
<tr>
<th>Subject</th>
<th>Pass No. 1</th>
<th>Pass No. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample 1</td>
<td>Sample 2</td>
</tr>
<tr>
<td>1 (M)</td>
<td>90.3</td>
<td>82.1</td>
</tr>
<tr>
<td>2 (F)</td>
<td>103.1</td>
<td>123.6</td>
</tr>
<tr>
<td>3 (M)</td>
<td>157.4</td>
<td>114.4</td>
</tr>
<tr>
<td>4 (M)</td>
<td>114.0</td>
<td>125.3</td>
</tr>
<tr>
<td>5 (M)</td>
<td>113.1</td>
<td>80.3</td>
</tr>
</tbody>
</table>

Values are means ± SD in mg muscle wet wt. Mean wet wt per sample: 109.4 ± 27.6 mg; mean wet wt per pass: 218.8 ± 33.9 mg. M, male; F, female.

Human Studies

Both the growth hormone research and biopsy-technique procedures were approved by the Rhode Island Hospital Institutional Review Board, and all human subjects provided written informed consent documentation. Forty-six subjects (28 men, 18 women), recruited as part of a project on the use of human growth hormone in the frail elderly, underwent 83 quadriceps muscle biopsies. In subjects undergoing repeat biopsies (n = 37), the second tissue procedure was performed 6 mo after the first. The subjects’ mean age was 71.3 ± 4.5 yr at the time of their biopsies. Most subjects were studied with the 6-mm sampling needle (eight biopsies were performed with the 4-mm needle on five subjects), as initial review of sample sizes with the 4-mm needles indicated that larger volumes of tissue would be required for the array of analyses planned for protocol evaluation. Each procedure required ~15 min from initial positioning, shaving, local anesthesia, incision, sampling, and postprocedural closure and occlusion.

Only two subjects (2.4%) complained of discomfort after the procedure that limited their usual activities. One of these individuals was a 72-yr-old man whose initial sampling was observed to have been accompanied by an immediate return of blood into the suction tubing and to contain both muscle and fat tissue in one cohesive piece. He reported pain and swelling in the affected leg for 24 h after the procedure, but a large ecchymosis was also evident, which resolved slowly over the next week. In most of the other biopsies, there was no evidence of superficial fat or facial tissue included in the samples, which reinforces the importance of operator experience and adequate penetration into the muscle bed. In this and the other case, symptoms resolved within 5 days after the biopsy. Most subjects reported no change in their daily routine after the procedure, and many participated in recreational sports such as bowling and golf without reporting a compromise in performance. Although nine subjects did not participate further in the study for a variety of reasons, none dropped out because of the initial muscle biopsy experience.

In most cases, the first samples were not weighed before dividing or freezing. Although a small amount of blood was observed with each sample obtained, the volume of blood did not confound the collection of the samples, which were quickly and easily separated and
removed from the window of the needle with smooth forceps. Wet weights were measured on tissue obtained on nine passes of the 6-mm needle with SEN from five consecutive subjects (4 men, 1 woman) and are outlined in Table 1. The mean weight of muscle fragments was 109.4 mg or 218.8 mg per needle pass when using the double-sample technique. Sample sizes ranged from 77.7 to 175 mg per piece and from 172.4 to 271.8 mg per pass. In all cases, adequate tissue samples were obtained for histomorphometric and other analysis.

**DISCUSSION**

The application of percutaneous muscle biopsies has been a mainstay of research in exercise physiology for nearly two decades (2, 5, 8, 14). More recently, clinical application of the technique for the diagnosis and classification of neuromuscular disorders has been enthusiastically endorsed (12). In the course of our research, we had the opportunity to implement the needle biopsy technique and have modified the procedure to optimize sample size. This has allowed the completion of multiple assays on samples obtained at one procedure and minimized the need for repeated sampling.

In 1962, Bergström (1) described a technique for obtaining skeletal muscle samples with a biopsy needle apparently known to Duchenne in the 1850s. As reported by Evans et al. (6), sample sizes have been enhanced by the addition of suction through the cutting cannula applied through a catheter connector by rapidly evacuating a 30-ml disposable syringe. With this modification, sample sizes of 65.5 and 78.5 mg wet wt were obtained with a 4- and 5-mm Bergström needle, respectively (6). The study of Evans et al. differed from our present work in that a 30-ml syringe without SEN was used. These inexpensive, readily available nipples enhance the suction by blocking the passage of air between the cutting and outer trocar and, thus, increasing the amount and duration of negative pressure that can be developed. The mean wet weight of the muscle samples obtained in our clinical work with the 6-mm needles was ~40% greater than reported for a 5-mm Bergström needle (6). In our clinical trial, tissue yield was enhanced further by using a double-sampling technique. Reference has been made to a double-chop technique (14), which is simple to perform but has not been described in detail. In our experience, we found that after closing the cutting chamber to slice off the first sample the trocar could be rotated 90° clockwise and the sampling procedure repeated before removing the trocar from the muscle. In this way, we were able to obtain an average 218.8 mg of muscle tissue per needle pass.

In a recent publication, Mubarak et al. (12) noted that the addition of suction via a syringe to the Bergström needle enhanced the yield per needle insertion by 60–95%. Although we obtained nearly double the tissue yield using SEN with a 4-mm needle compared with no suction enhancement, the fivefold increase in weight of muscle sample was more dramatic with a 6-mm Bergström needle. In their report, Mubarak et al. reviewed their experience with the Bergström needle technique on 379 children and adults. Utilizing essentially the same procedures as our protocol except for the SEN, they noted that the size and quality of the muscle samples obtained were sufficient to confirm accurate diagnosis in 98.4% of the patients biopsied. However, it was noted that five to six passes were necessary to obtain sufficient tissue to perform the necessary assays. Most recently, Kristiansen et al. (11a) have reported muscle tissue yields similar to ours in a study of six healthy male subjects. Unfortunately, a direct comparison of tissue yield between the methods in these last two studies and that reported here is not possible as the first report did not specify sample weights (12) and details of the muscle biopsy procedure were not included in the second (11a). However, it appears that the enhancement of sample size with our method may improve the success rate of the procedure when used clinically and decrease trauma to the patient, as an adequate sample should be obtained with fewer needle passes.

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