

Video Article

A Technique for Subcutaneous Abdominal Adipose Tissue Biopsy via a Non-diathermy Method

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Abstract

Adipose tissue biopsies offer tissue samples that, upon analysis, may provide insightful overviews of mechanisms relating to metabolism and disease. To obtain subcutaneous adipose tissue biopsies in the abdominal area, researchers and physicians use either a surgical or a needle-based technique. However, surgical subcutaneous fat biopsies can offer tissue samples that may provide a more comprehensive overview of the complexities of biological indices in white adipose tissue. Usually, a surgical adipose tissue biopsy includes a diathermy treatment for cauterizing blood vessels to prevent excessive bleeding. Nevertheless, side effects, such as flash fires and skin lesions in the tissue, have been reported after diathermy. Therefore, we aimed to standardize a surgical abdominal adipose tissue biopsy performed under local anesthesia using a non-diathermy method. We conducted 115 subcutaneous adipose tissue biopsies in healthy men using a non-diathermy abdominal surgical biopsy method. Our results showed three cases of excessive post-operation bleeding out of 115 operations (2.61%). In conclusion, our standardized subcutaneous abdominal adipose tissue surgical biopsy using a non-diathermy method can be safely applied to healthy men at the bedside, with minimal side effects.

Video Link

The video component of this article can be found at <https://www.jove.com/video/55593/>

Introduction

Adipose tissue biopsies offer tissue samples that, upon analysis, may provide an insightful overview of mechanisms relating to metabolism and disease. For instance, they are used for breast cancer tumor identification¹, fatty acid composition examination², and research on important metabolic and hormonal disease pathways³. To obtain subcutaneous adipose tissue biopsies in the abdominal area, researchers and physicians use either a surgical or a needle-based technique. However, surgical subcutaneous fat biopsies can offer tissue samples that may provide a more comprehensive overview of complex biological mechanisms [e.g., messenger ribonucleic acid (mRNA) expression, histological analysis, etc.]⁴. They have previously been used in healthy adults⁴ and can be safely performed at the bedside⁵.

The needle-based biopsy technique requires intense liposuction that may destroy the integrity of the tissue, and it usually retrieves a small amount of tissue (100-500 mg)⁶. These tissue samples may not be appropriate for complex research studies. Furthermore, compared to the needle-based technique, surgical subcutaneous fat biopsies can offer tissue samples that may draw a more complete picture of several inflammatory indices (e.g., B- and T-cell signaling); cytoskeleton regulation; cellular pathways; metabolic pathways of lipids, carbohydrates and amino acids; and oxidative phosphorylation pathways⁴. Also, the needle-based technique includes a higher risk for blood cell contamination than the surgical one⁴, while providing little information about the fibrotic regions of the tissue^{4,7,8}.

Usually, a surgical adipose tissue biopsy includes a diathermy treatment for cauterizing blood vessels to prevent excessive bleeding⁹. A diathermy is also used to burn and destroy tissues affected by neoplasms and warts or tissues that are infected by disease⁹. This technique is particularly applicable in neurosurgery and eye surgery⁹.

The excessive use of diathermy may lead to the formation of necrotic tissue, causing infections and delaying the healing process¹⁰. Indeed, side effects such as flash fires and skin lesions in the tissue have been reported following diathermy⁹. Additionally, concerns have been raised about the toxicity of surgical smoke released by diathermy into the surgery environment, which may be inhaled by the participant/patient and the operating staff⁹. Indeed, the use of diathermy may cause air toxicity in the operating room equal to that produced by smoking six cigarettes¹¹ and, although operating rooms typically have good ventilation systems, the contamination can last for at least 20 min¹². However, in patients undertaking a tonsillectomy surgery, the diathermy method has shown less, but non-significant, post-operative pain¹³ and bleeding¹⁴ than the

classic ligation hemostasis method. Overall, evidence regarding differences in the post-operative pain and bleeding rates after adipose tissue biopsies in healthy individuals barely exists. Given the lack of evidence and the side effects of diathermy, standardization of a non-diathermy method for adipose tissue biopsy is warranted. Accordingly, the aim of this study was to standardize a surgical technique for subcutaneous abdominal adipose tissue biopsy, performed under local anesthesia using a non-diathermy method in healthy men. Moreover, we outline the procedures for tissue collection to determine mRNA expression and protein concentration and to perform histological analyses.

Protocol

The study conformed to the standards set by the Declaration of Helsinki and was approved by the University of Thessaly, Department of Exercise Science, Bioethics Committee.

1. Preparation of the Surgery Equipment and Consumables

1. Position a disinfected surgical field on a 4-wheel surgical Mayo stand.
2. Position the following consumables and surgical equipment on a 4-wheel surgical Mayo stand:
 - One pair of straight operating scissors (15 cm)
 - One scalpel (No. 11)
 - One scalpel handle
 - One pair of curved scissors (14 cm)
 - One mosquito forceps
 - One pair of Kocher tweezers
 - One surgical tweezer
 - Povidone-iodine (on a sterile gauze)
 - One suture (4.0)
 - One pair of straight scissors (11 cm)
 - One needle holder (15 cm)
 - Five sterile gauzes
 - One adhesive sterile gauze
 - One 10-mL syringe with a disinfected needle
 - 10mL of 2% xylocaine (no adrenaline)
 - One pair of disinfected surgical gloves
3. Position the following materials on a separate bench:
 - Two microcentrifuge tubes
 - One tube containing 5 mL of 10% formalin
 - One small container containing 50-100 mL of liquid nitrogen at -190 °C.

2. Preparation of the Participants

1. Have participants follow an 8 h fast¹⁵ prior to the biopsy procedure.
2. Have participants refrain from physical exercise, excessive stressors, alcohol, and active and passive smoking 72 h prior the biopsy^{16,17}.

3. Performing the Subcutaneous Adipose Tissue Biopsy

1. Position the participant on the surgical bed in a supine position.
2. Disinfect the region of the incision using povidone-iodine.
3. Inject 10 mL of 2% xylocaine (no adrenaline) slowly and steadily¹⁸ into the selected region to serve as local anesthesia.
4. Position a sterile surgical field over the area to cover the body of the participant, excluding the region of the incision.
5. At least 3-5 min afterwards, apply pressure with a pair of tweezers to the area of local anesthesia and ask the participant if he feels any pain¹⁹ to ensure that local anesthesia has been achieved.
6. Attach the No. 11 scalpel to the scalpel handle.
7. Create an incision of 2-2.5 cm in length, 3-5 cm to the left or right of the navel.
8. Remove the subcutaneous tissue with a pair of straight 15 cm operating scissors.
9. Once the adipose tissue is revealed, capture it with a pair of tweezers and a pair of curved 14 cm scissors.
10. Cut and remove approximately 1 g of adipose tissue.
11. Following tissue removal, position sterile gauzes over the incision to achieve hemostasis.
12. When hemostasis is confirmed, capture the 4.0 suture with a 14 cm needle and the skin with a pair of surgical tweezers until the subcutaneous tissue is visible.
13. Insert the suture-needle into the skin, moving towards the subcutaneous tissue while the skin and subcutaneous are closely aligned.
14. With the suture-needle, follow a continuous zig-zag technique into the subcutaneous tissue until driven externally off the skin. Continue until the skin incision is closed.
15. Externally tie the suture.
16. Clean the area of incision with saline and cover it with an adhesive sterile gauze.

4. Deposition of the Tissue

1. Separate the collected adipose tissue into three pieces: 150, 350, and 500 mg.

2. Position the 150 and 350 mg pieces of sample tissue in microcentrifuge tubes.
3. Immerse the two microcentrifuge tubes in the small container with the liquid nitrogen at -190 °C until final deposition in a freezer at -80 °C.
NOTE: In advance, use a sterile needle to puncture a small hole in the microcentrifuge tubes to avoid cup failure during the immersion of the microcentrifuge tubes in the liquid nitrogen at -190 °C.
4. Immerse the 500 mg piece of sample tissue-to be used for histological analysis-in a tube containing 5 mL of 10% formalin.
NOTE: Use the mosquito forceps only in cases of excessive bleeding during the surgery. In such cases, capture the vessel with the mosquito forceps to ligate and close until hemostasis is achieved.

Representative Results

The characteristics of the participants are provided in **Table 1**. The stitches in the area of the incision fell off within the next 8-12 days. We completed a total of 115 subcutaneous adipose tissue biopsies using the non-diathermy abdominal surgical biopsy method in healthy adult men (age: 35.2 ± 6.9 years; body mass index: 27.3 ± 4.4; and body fat percentage: 27.0 ± 9.0%). Post-biopsy excessive bleeding was observed in three cases (prevalence: 2.61%) two days after the surgery. The three individuals who experienced excessive bleeding were older than their counterparts who experienced no side effects [$t_{(113)} = -2.115$, $p = 0.037$], and no other differences (*i.e.*, body mass index and body fat percentage) were observed ($p > 0.05$) between the two groups. Finally, three months after each operation, none of our participants reported any side effects (including skin wounds).

	Overall	No side effects	Excessive bleeding
Participants	115	112	3
Age	35.15±6.90	34.93±6.77	43.33±7.57
Body mass index	27.33±4.36	27.35±4.38	26.70±3.99
Body fat %	27.04±9.04	26.97±9.03	29.90±10.94

Table 1: Characteristics of the participants.

Discussion

The aim of this study was to standardize a surgical technique for subcutaneous abdominal adipose tissue biopsy, performed under local anesthesia with a non-diathermy method in healthy men. Diathermy is commonly used in the operating room; however, possible side effects (*i.e.*, flash fires and environmental toxicity)⁹ and delays in healing¹⁰ may occur. These unwanted effects of diathermy can generate health risks for both the operating staff and the patients/participants. Therefore, a surgical non-diathermy method for subcutaneous adipose tissue biopsy is warranted.

We observed excessive bleeding in three of our participants two days after the surgery. These three individuals reported pain in the area of the incision. To treat these cases, the area of the skin incision was reopened, any excess blood was removed, and the area was cleaned with saline and closed using the same technique described above. No complications were reported after that, and normal healing was observed in the subsequent days. Our results have shown minimal side effects after the non-diathermy method: three cases of excessive post-operation bleeding out of 115 operations (2.61%). Previous evidence from needle-based adipose tissue biopsies showed minor bleeding in one out of 10 participants 12 h after the surgery¹⁵, while no significant bleeding occurred in healthy obese individuals after a side-cutting adipose tissue biopsy needle technique⁶. Based on our results, we anticipate that a non-diathermy surgical adipose tissue biopsy method may eliminate the risk for skin lesion, flash fires, and toxicity released in the operating environment, which are caused by diathermy during surgical adipose tissue biopsy.

Previous reports have indicated that aging is positively associated with abnormalities in hemostasis²⁰. Interestingly, the three individuals who experienced excessive bleeding in the current study were significantly older (by eight years, on average) than those who did not experience any side effects. Therefore, it may be noted that older individuals have a higher risk for side effects when the non-diathermy surgical adipose tissue biopsy method is applied. However, we believe that the prevalence of such side effects is too low (*i.e.*, 2.61%) to cause any significant concerns. It should be noted that our participants filled in advance a medical history questionnaire to determine their health status. No previous chronic disease conditions were reported by the participants, including the three participants who showed excessive post-operation bleeding. Similarly, no previous chronic medication treatment was reported by both the participants who showed and those who did not show any excessive bleeding. Therefore, the post-operation excessive bleeding we observed in the current study cannot be attributed to previous chronic disease conditions and/or previous chronic medication treatment. In this light, the prevalence of this side effect may be different if the technique is applied to clinical populations.

A critical step during the proposed surgical adipose tissue biopsy is to ensure local anesthesia before the incision, which prevents any pain and discomfort in the participant. Furthermore, a pre-screening of allergic history to the anesthetic must be done to prevent allergic complications during the anesthetic injection. Also, it is crucial to ensure hemostasis before the incision is closed to prevent post-operation bleeding, while it is important to keep in contact with the participants for several days after the procedure is performed until complete healing is achieved to discover any potential abnormalities and to prevent infection and/or cases of post-operation bleeding.

Limitations of the proposed biopsy technique include the discomfort that area of the incision may cause to the participants, particularly relating to their ability to comfortably sit down and take a shower/bath, until complete healing is achieved. The participants should be instructed to avoid washing the area of the incision to prevent infection, as well as to replace the adhesive sterile gauze over the incision area every day until complete healing is achieved. This daily procedure may cause discomfort. Finally, it is important to note that our purpose was to introduce and standardize a new technique. In this light, we did not use a control group undertaking needle-based subcutaneous fat biopsies in order to quantify the differences between this technique and the newly-introduced non-diathermy method.

As previously suggested^{4,7,8}, the proposed surgical biopsy technique may offer tissue samples with higher integrity than the needle-based technique. Indeed, using this technique, we were able to perform mRNA and protein analyses on the obtained tissue and, in addition, to form

paraffin cubes for use in histological analyses. Also, we suggest avoiding diathermy in surgical subcutaneous adipose tissue biopsies, given that hemostasis and normal healing can be achieved with our technique.

In conclusion, our standardized subcutaneous abdominal adipose tissue surgical biopsy using a non-diathermy method can be safely performed in healthy men at the bedside, with minimal side effects.

Disclosures

The authors declare that they have no competing financial interests.

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