

## Evaluation of the donor site after liposuction with a syringe in rabbits<sup>1</sup>

### Avaliação da zona doadora após lipoaspiração com seringa em coelhos

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#### ABSTRACT

**PURPOSE:** To evaluate the donor site of adipocytes as well as histopathological alterations secondary to liposuction.

**METHODS:** All animals underwent liposuction with a syringe on the right side of the back. While the left side of the back was used as control and did not undergo intervention. The 10 rabbits were divided into two groups A and B according the postoperative day which were submitted to euthanasia: 90 and 120 days. All adipose tissue from the donor site was analyzed and compared with the control macroscopic and light microscopy. Tissues were weighed and analyzed searching for histological changes and late inflammatory response to trauma such as fibrosis, fat necrosis and inflammation and macrophage infiltration.

**RESULTS:** There was wide variation in adipose tissue volume between the experimental and the control on macroscopic analysis. The presence of histopathological changes was found in two samples at 90 days.

**CONCLUSIONS:** There was a relationship between the presence of fibrosis with the weight and number of days after liposuction surgery in rabbits. The study show macroscopic difference between control and experiment sides in all rabbits.

**Key words:** Adipose Tissue. Lipectomy. Back. Rabbits.

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#### RESUMO

**OBJETIVO:** Avaliar a zona doadora de adipócitos bem como as alterações histopatológicas secundárias a lipoaspiração.

**MÉTODOS:** Todos os animais foram submetidos à lipoaspiração com seringa no lado direito do dorso. Enquanto o lado esquerdo do dorso foi usado como controle, não sofrendo intervenção. Os 10 coelhos foram divididos em dois grupos A e B conforme o dia pós-operatório (DPO) os quais foram submetidos à eutanásia: 90 DPO e 120 DPO. Todo tecido adiposo da zona doadora foi analisado e comparados com o controle macroscopicamente e à microscopia óptica. Os tecidos foram pesados e foram analisadas as variações histológicas em busca de uma resposta inflamatória tardia ao trauma como fibrose, inflamação, necrose gordurosa e infiltrado macrofágico.

**RESULTADOS:** Foi observada grande variação de volume de tecido adiposo entre o experimento e o controle do dorso a macroscópica e a presença de alteração histopatológica em duas amostras aos 90 dias.

**CONCLUSÕES:** Houve relação entre a presença de fibrose com o peso e numero de dias pós-operatório na lipoaspiração de coelhos. O estudo mostrou diferença entre os lados experimento e controle em todos os coelhos.

**Descritores:** Tecido Adiposo. Lipectomia. Dorso. Coelhos.

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## Introduction

The longevity of the correction, the rate of graft survival depends on the withdrawal and transfer technique<sup>1</sup>. After the introduction of liposuction in 1977, many plastic surgeons interested began using this technique for the treatment of bodily deformities. By introducing the liposuction as a new technique for collecting fat and use it for fat grafting, was supposed to open a new era for the method<sup>2</sup>.

The essential philosophy had changed from free transfer of adipose tissue resected intact cell transplantation for fat-free, but the main limitations of the technique were still present<sup>3</sup>. Many experimental and clinical studies have sought to develop less traumatic methods to collect, purify, re-injecting fat, and increase the survival rate<sup>1</sup>.

Due to unpredictable resorption rate, fat autograft has the disadvantage of requiring a donor area and subjecting it to some local morbidity<sup>4</sup>. Despite the disadvantage, interest in fat grafting is large due to the nature autologous graft biocompatibility and absence of allergic reactions<sup>4</sup>. The availability of adipose tissue is associated with the peculiarities of each patient and indication of the donor site should therefore also be individualized.

## Methods

The study protocol was analyzed and approved by the Ethic Commission on Animal Research of Bahiana School of Medicine and Public Health (EBMSP). The use of laboratory animals followed the ethical code for animal experimentation of the Council for International Organization of Medical Sciences.

It was used 10 rabbits (*Oryctologus cuniculus*), male, of the New Zealand race between two and three years, weighing 3.0 to 4.0kg, from the EBMSP. The animals were introduced to adapt to conditions 15 days before the surgery being done with standard ration food, two times a day, coupled with adequate fluid supplementation.

For the anesthetic technique, food and water was removed for eight to 12 hours before anesthesia. The anesthetic protocol adopted consisted of premedication with ketamine 40mg/kg and xylazine 9mg/kg via intramuscular<sup>5</sup>.

### Operative technique

Procedures were performed at the Nucleous of Experimental Surgery of EBMSP. The rabbit was positioned in prone on the operating table suitable for rabbits. Trichotomy was performed in the dorsal region above the scapulas.

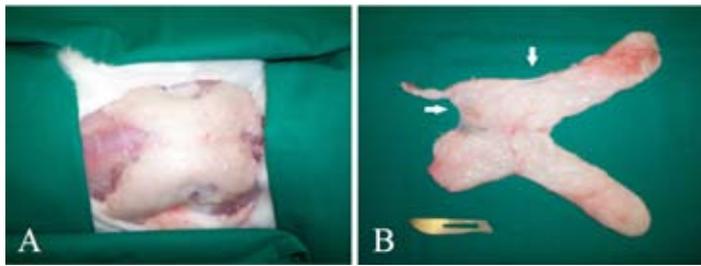
The act consisted of half a centimeter paramedian incision to the right of the animal's back to 2cm height of the shoulder blades. After direct visualization of the fatty capsule, an incision was made with a cold scalpel to insert the liposuction cannula with a syringe. It was chosen using a liposuction cannula of 03 mm together in a 10 ml syringe. After insertion of the cannula, a liposuction was made with a syringe with a maximum pressure of 5 ml and 8 ml of adipose tissue aspirated (Figure 1). Closure of the wound was made using nylon 4.0, with two simple points and occlusive dressing.



FIGURE 1 – Liposuction with syringe. Donor site.

All the 10 rabbits underwent liposuction with a syringe. Each animal was considered the control and also the experiment group at the same time. The right side of the back of the rabbit considered as the experiment side was conducted with liposuction with a syringe. The left side of the back of each control did not undergo intervention. The Animals were divided into two groups of five animals. Each group corresponded to a specified period of post operative that they were sacrificed (Group 1: 90 days; Group 2: 120 days).

All rabbits were euthanized pharmacology, which consisted of the application of prior anesthesia, as mentioned above, associated with a dose of 2 ml of potassium chloride intravascularly. Euthanasia occurred as follows: at 90 and 120 days. The fatty tissue of the animal's back was all removed (Figure 2).



**FIGURE 2** – Resection of the donor site. **A.** Anatomic delimitation of adipose tissue. **B.** The control side (proximal) and the experiment side (distal). Decrease in adipose tissue (arrow).

*Histological evaluation*

Adipose tissue was analyzed macroscopically and then fixed in 10% formalin and sent to the histopathology laboratory. In the laboratory, the sections of the surgical specimens were submitted to routine histological processing and microscopic examination. The histopathological evaluation was observed or not the development of inflammation, necrosis, fibrosis, macrophage infiltration and collagen. Microscopic findings of each slide were quantified using a graduated scale from 0 to 3. Inflammation was graded as absent (0), mild (1), moderate (2), severe (3). Fibrosis and fatty degeneration were graded as absent (0), less than 20% of the donor site to a cross-section (1), between 20% and 50% of the donor (2), more than 50% of the donor (3).

**Results**

The results showed differences between control and experimental side in all samples during the macroscopic evaluation. It was described lower fat volume at macroscopic inspection of all experiment side of the rabbit’s back. There were no complications during surgery. The postoperative recovery was early without the need of additional analgesia.

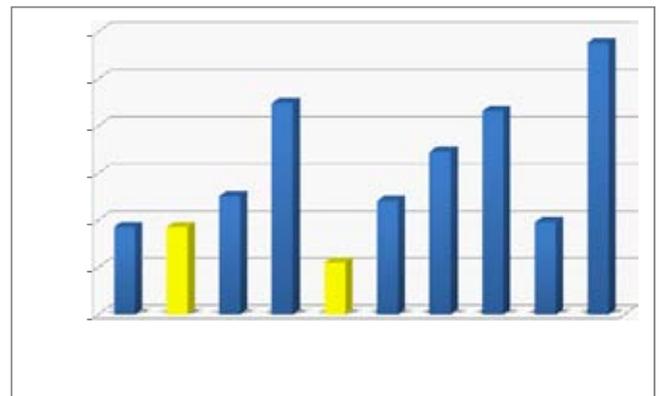
Variation was observed between fat volume of the back and weight of the rabbit (Table 1). The animal with the greater volume of the back was not necessarily the heaviest animal (T Student test,  $p>0.05$ ).

**TABLE 1** – Comparison of weights.

Rabbit number	Weight of tissue resected (g)	Weight of the rabbit (g)	Microscopy alteration
01	37.0624	3700	-
02	37.0330	2600	+
03	50.2141	2700	-
04	89.6575	3234	-
05	21.9627	2912	+
06	48.1914	3400	-
07	69.0963	3200	-
08	86.2752	3100	-
09	39.0745	3400	-
10	115.2851	2910	-

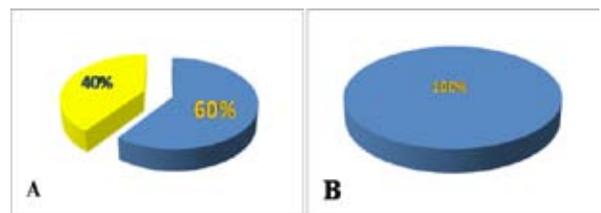
T Student test: NS: Non significant differences between the weight of the animal and histopathological change event ( $p=0.095$ ).

Relationship was observed between the animal’s weight and histopathological change event with no statistical significance (Figure 3). The event of histological change was related with the volume resected of the back, however with  $p>0.05$  (T Student test).

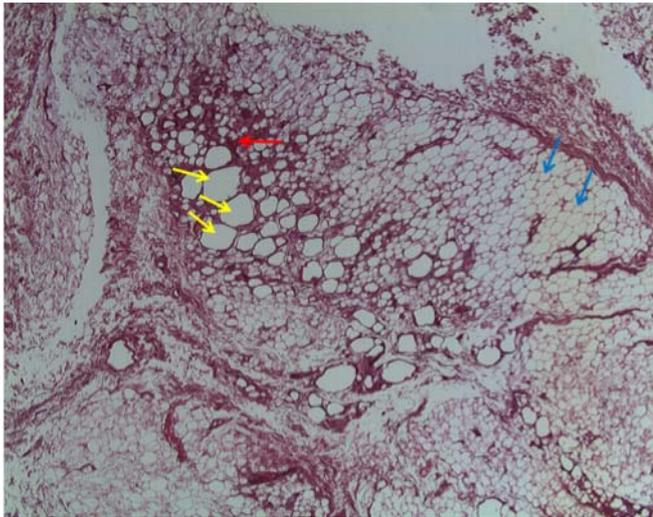


**FIGURE 3** – Weight in grams of the fat resected from the dorsum. The number two and five rabbit showed histopathological alteration in microscopy (yellow).

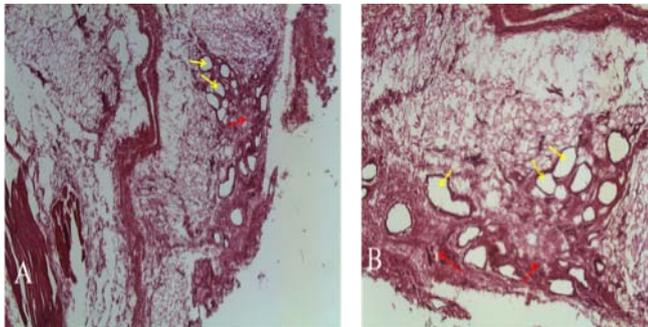
Histopathological change was found in two samples from animals sacrificed at 90 days (Figure 4). Fibrosis was observed in both animals with changes during the microscopic study (Figures 5 to 7).



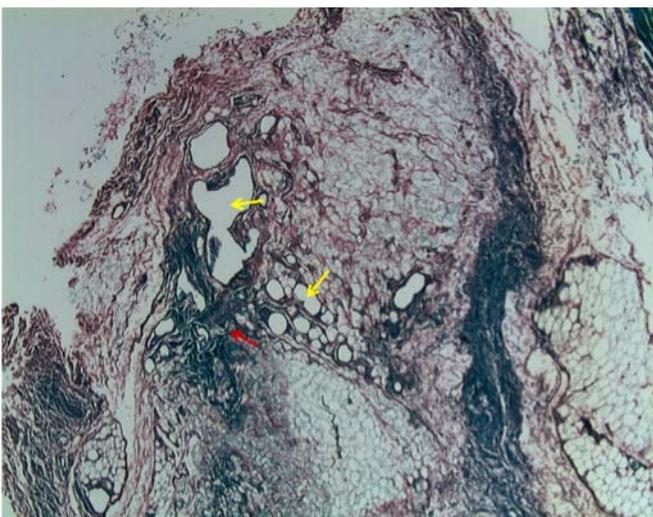
**FIGURE 4** – Relation between the event of histopathological alteration and the groups. (Chi-square test,  $p=0,114$ ) **A.** 90 days **B.** 120 days. Histopathological alteration (yellow); without alteration (blue).



**FIGURE 5** – Photomicrograph of adipose tissue after liposuction. Presence of fatty degeneration and necrosis in all rabbits with microscopic change; Chi-square test,  $p=0.007$ . Microcystis (yellow arrow), fibrosis (red arrow), without adipose tissue alterations (blue arrow). Hematoxylin eosin 2x.



**FIGURE 6** – Photomicrograph of adipose tissue after liposuction rabbit 5. Relation between inflammation and pathological changes; Chi-square test,  $p=0.035$ . Hematoxylin eosin, microcystis (yellow arrow), fibrosis (red arrow) A. 2x B. 4x.



**FIGURE 7** – Photomicrograph of rabbit 5. Greater presence of fibroses, inflammatory tissue and macrophage infiltration in the animal with the lower weight (Chi-square test,  $p<0.05$ ). Fibrosis (red arrow), microcysts (yellow arrow) Picrosirius red 2x.

## Discussion

Studies involving experimental animals usually remove autologous fat from the groin, often removed by dissection and subsequent processing, or even liposuction<sup>4,5</sup>. Our study used the fat of the animal's back with great ease due to the use of syringe liposuction technique. This technique was facilitated because of well-defined amount of tissue, allowing the palpation of the underlying tissue and the proper orientation of the tip of the cannula, the same way the aspiration is performed in humans. The study indicated that the removal of fat with minimal trauma is important to reduce the degree of injury of the donor and increase the survival of adipocytes.

Chajchir *et al.*<sup>6</sup> claims not find a difference between the donor areas in relation to the survival time of adipose tissue grafts removed for body and face, reporting equivalence between the grafts from areas such as abdomen, trochanter region, inner thigh, hip, knee, groin and buttocks in humans<sup>6</sup>. However, in a study in humans comparing tissue taken from the gluteal-femoral region, abdomen, breast and face suggested an advantage for the gluteal-femoral region in relation to the other for having the largest and most adipocyte lipogenic activity<sup>7</sup>. Rochrich *et al.*<sup>8</sup> showed quantitatively that the viability of human adipocytes did not differentiate according to anatomical regions<sup>8</sup>.

The choice of the donor site must be very careful evaluating outcomes following tissue accommodation<sup>6</sup>. When the area already has some degree of laxity, graft removal should be more careful to prevent disruption of the overlying skin. The choice of the back is also because of the benefit that the animal can't manipulate the area where the fat was removed, with no suture dehiscence on the back. Our study did not aim to remove all the fat volume of the animal's back, however, was observed decrease in anatomic volume in all animals with both 90 and 120 days compared to control. Because it is a clear plan in subcutaneous tissue of animals was not observed changes in the skin as well as retrace or signs of fibrosis.

For some authors there are certain areas in which should be avoided or should not have liposuction in humans because of the characteristics of the region, which has associated less volume of subcutaneous fat such as lower buttock and knee<sup>2</sup>. The choice of the donor should be careful because there are different recommendations in the literature<sup>9,10</sup>.

The technique chosen to obtain adipose tissue directly influences the outcome<sup>4</sup>. With introduction of liposuction in 1977, grow the number of plastic surgeons interested in using this technique for the treatment of bodily deformities. Large

volumes of anesthetic solutions associated with vasoconstrictor facilitate the removal of adipose tissue with less damage of the donor site, providing anesthesia during surgery, low blood loss and postoperative analgesia<sup>9</sup>.

The research project chose the use of liposuction technique without infusion of solutions, this method is quite common in surgical practice. It was decided to assess the effect of the cannula into the tissue and the grafted material could be aspirated without changing the initial composition, in the presence of various substances.

It is observed that many authors empirically alter the concentration of lidocaine trying to reduce the risk of complications due to the toxicity associated with the use of lidocaine. The choice of each solution can directly affect the final result for the survival of the adipose tissue in the receptor site<sup>11,12</sup>.

Liposuction by the dry technique is related to the increase in bleeding complications<sup>8</sup>. There was no such complication in our experimental model. The surgical procedure proved to be mild in severity, with low morbidity and a good postoperative recovery. The study sought to preserve the tissue aspirated for subsequent grafting did not use any anesthetic and vasoconstrictor. The aspiration of all rabbits revealed no major bleeding or bruising.

In the present study was chosen using a 3 mm cannula to decrease tissue injury. The larger is the diameter of the cannula higher the viability of adipocytes<sup>8</sup>. Between cannulas of 2.5 or 3 mm or 18-gauge needles is described no significant alteration of the integrity of fat cells<sup>13</sup>.

In our study, the choice was for the use of 10 ml syringe for aspiration, using this option was better when compared with the use of vacuum suction device<sup>4</sup>. According to the author, the vacuum pressure interferes significantly in the destruction of adipocytes and the level of absorption, and at 700 mmHg the lesion reaches 15% increase in absorption after grafting, however pressures of 250, 300, 500 mmHg damages the cell less than 2%<sup>13</sup>. According to Nguyen *et al.*<sup>4</sup> suction an 1 ATM can destroy 90% of adipocytes (cells broken and irregular shape). The author noted that the use of aspiration with 10 cc syringe and G14 intracath the fat cells preservation was 95%.

Our aspiration was performed with 10 ml syringe causing 5ml negative pressure. The smaller size of the syringe will result in less damage to the adipocyte during the liposuction<sup>10,14</sup>. Another author after aspiration with a 10 ml syringe under a maximum pressure of 5 ml, obtained a suspension of fat with anesthetic with minimal presence of oil<sup>9</sup>. These data agrees with our findings.

With regard to the histopathological evaluation findings there were no changes in adipose tissue of the donor in a period

as longer as 120 days. However there are two events at 90 days of reaction to trauma healing. Rabbit 02 showed evidence of fibrosis of low intensity (1) and presence of fatty tissue necrosis graded as 2, unchecked inflammatory tissue and macrophage infiltration. Rabbit 05 showed presence of moderate amount of fibrosis (2), low fat necrosis (1) the presence of inflammation and macrophage infiltration graded as (1).

About the weight of adipose tissue resected from the animal's back, there was a large variation, values ranging from 21.9627 to 115.2851 grams. The two events of histologic changes occurred in animals that had lower weight of fat taken from the back. 21.9627 and 37.0330. These data could be interpreted that the donor site with a smaller volume would receive more trauma at the time of liposuction leaving a scar with fibrous tissue. It can be compared to clinical practice that excessive removal of fat, or very close to the epithelial layer can result in skin injury and stiffness due to fibrosis of the donor site. However there is no data to prove the permanence of this evidence histologic with more than 90 days.

## Conclusions

The study show macroscopic difference between control and experiment sides in all rabbits. There was a relationship between the presence of fibrosis with the weight and number of days after liposuction surgery in rabbits.

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