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ORIGINAL ARTICLE BASIC SCIENCE/EXPERIMENTAL

Effect of Decantation Time on Viability and Apoptosis in Adipocytes After Liposuction

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Abstract

Background The effect of decantation time on viability and apoptosis in adipocytes has not been described. The objective of the study was to describe viability and apoptosis in adipocytes up to 2 h after harvesting.

Methods Twenty patients who underwent esthetic liposuction from the abdomen were included. The lipoaspirate was obtained from the infra-umbilical area with the tumescent technique. Liposuction was performed with a 60-ml syringe and a 3-ml cannula. Lipoaspirates were centrifuged at 50 g for 5 min at 0, 60 and 120 min after harvesting. One gram of fat was digested with 0.1% type 1 collagenase and incubated at 37 degrees for 30 min. Adipocytes were counted on 10 random microscopic fields. Apoptosis was determined by TUNEL assay. A fluorescence microscope was used to visualize the staining nuclei and cells.

Results Regarding viability, immediately after harvesting, 57.6 ± 18.9% of the cells were viable, whereas 60 min after liposuction the viability decreased to 51.62 ± 8.8% and 120 min after liposuction the percentage of viable cells was 46.8 ± 16.9%. The percentage of apoptotic cells at time 0 was 38.2 ± 8.0%, whereas it was 51.24 ± 8.1% at 60 min and 62.9 ± 16.1% at 120 min after collection.

Conclusions Apoptosis and mortality of adipocytes after liposuction increase directly proportional to the time of decantation. Lipoinjection should be performed as soon as possible after harvesting.

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Keywords Adipocytes · Apoptosis · Viability · Decantation · Fat grafting

Introduction

Liposuction and autologous fat grafting are some of the most commonly performed surgeries for esthetic and reconstructive purposes due to their positive outcomes [1–3]. However, we are still struggling to achieve predictable outcomes and high take rates of fat grafts [4]. The reported loss in volume after fat grafting usually ranges from 40 to 50% [5, 6]. However, losses of up to 90% of infiltrated fat grafts have been reported [7]. It has also been reported that the take of fat grafts is influenced by the choice of the donor site; methods of harvesting, handling and preparing the graft; and characteristics of the donor site [8–10]. It is evident that the more viable the cells inserted are, the better the chances of retaining the maximum volume after fat injection.

After liposuction, collected fat is usually decanted for variable times prior to processing, and subsequent injection for grafting. Some surgeons prefer to wash the fat, to

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remove blood, oil and the infiltrated solution. The best-known protocol for fat grafting is the Coleman technique [11]. This technique has been widely adopted because it leads to more reproducible outcomes, though some volume loss still occurs after grafting [4] in addition to cellular damage [12]. Consequently, some surgeons prefer to decant the lipoaspirate to prevent damage to adipose cells.

Washing not only increases the vascularity and viability of grafted samples, but also produces more fibrosis and cysts [13]. Condé-Green et al. [14] demonstrated that decanted adipocytes preserved their morphology, whereas washed and centrifuged samples retained a very small number of intact cells. They also found that decanted and centrifuged adipocytes showed a trend toward less viability compared to centrifuged and cell-supplemented cells at 12 weeks post-injection, although no statistical difference was achieved [13]. Other studies have found more pre-adipocytes and cell proliferation in decanted adipose tissue than in centrifuged samples [15].

Once removed from their vascular bed, adipocytes are deprived of blood and oxygen, thereby reducing their viability. We could not find information on the rate at which adipocytes die while left in decantation prior to injection. Rohrich et al. studied the viability of adipocytes from different body areas after storage for up to 3 h using centrifuged and non-centrifuged samples. They found similar results with both samples, but the percentage of viable cells was not reported as this was not the purpose of their study [16].

Furthermore, it remains unknown whether apoptosis is initiated in viable cells during decantation. This programmed cell death might further reduce the rate of cell survival. We hypothesized that apoptosis is present and increases over time in adipocytes during decantation. The purpose of the study was to determine the viability and rate of apoptosis of adipocytes during decantation following liposuction. We investigated the percentage of viable and apoptotic cells during the first 2 h after liposuction.

Patients and Methods

In this descriptive, prospective and observational study, 20 consecutive patients who underwent primary liposuction from the abdomen for esthetic reasons were included. We excluded smoker patients, patients with history of any chronic disease, with a history of any surgery in the abdomen or evidence of infection at the moment of the surgery. Exclusion criteria included samples not processed according to the protocol. All patients consented with the processing of their samples.

Lipoaspiration

The lipoaspirate was obtained from the infra-umbilical area using the tumescent technique. Briefly, modified Klein's solution [1000 cc of Hartmann's solution (PISA, Guadalajara, Mexico added to 1 mg of epinephrine (PISA, Guadalajara, Mexico)] was infused through a suprapubic incision, using a 12-G infiltration cannula (Byron, Mentor, Santa Barbara, USA). To ensure vasoconstriction, there was a waiting period of 20 min after infiltration. Liposuction was performed with a 60-ml syringe and a 3-ml Mercedes cannula (Byron, Mentor, Santa Barbara, USA) under low negative pressure until 30 ml of the aspirate was obtained.

Five ml of fat were poured into 20 ml-conical centrifugal tubes (Corning Inc, NY, USA) and decanted for 0, 60 and 120 min for further processing.

Cellular Viability Assay

Lipoaspirates were centrifuged at 50 × g for 5 min to eliminate oil and blood, immediately after harvesting, at 1 h and 2 h after liposuction. Then, 1 g of the fat fraction was digested with 0.1% type 1 collagenase (US Biological, Salem, MA, USA.) and incubated at 37 °C for 30 min. Digestion was stopped, and the cell suspension was diluted in the ratio of 15:1 with 0.4% trypan blue. Using a Neubauer chamber, adipocytes were counted in 10 random microscopic fields at 40× magnification.

Apoptosis Assessment

After harvesting, 10 ml of the lipoaspirate was fixed with freshly prepared 4% paraformaldehyde (Sigma-Aldrich, St. Louis, USA.) in phosphate-buffered saline at 0, 60 and 120 min after harvesting for standard histological processing. Adipose tissue apoptosis was determined using the TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) assay. The TUNEL assay was performed for each sample following the manufacturer's instructions (TACS TdT in situ fluorescein apoptosis detection Kit; TREVIGEN, Gaithersburg, MD, USA.). The stained nuclei and cells were visualized under a fluorescence microscope. Adipocyte apoptosis in adipose tissue sections was quantified by counting the number of positive nuclei in 10 random microscopic fields at 20x magnification.

Results

Twenty female patients with a mean age of 40 (range 22–44) years were included (Table 1). None of the patients were excluded. All samples were processed for

Table 1 Patient demographics

Characteristics	No. of patients (n = 20)
Gender (n, %)	
Male	0 (0%)
Female	20 (100%)
Age (years)	
Mean	40
Range	20–44
Ethnicity (n, %)	
Hispanic	20 (100%)

determining viability and rate of apoptosis. Immediately after harvesting (0 h), 57.6 ± 18.9% of the cells were viable. Viability decreased to 51.62 ± 8.8% at 60 min after liposuction and further decreased to 46.8 ± 16.9% at 120 min after liposuction (Fig. 1).

The percentage of apoptotic cells was 38.2 ± 8.0% immediately after harvesting (0 h), 51.24 ± 8.1% at 60 min and 62.9 ± 16.1% at 120 min after harvesting (Figs. 2, 3).

Discussion

Although fat grafting for esthetic and reconstructive purposes has been proven to be effective and long-lasting [17], its outcome is still far from ideal. The first report on free fat grafting was published by Neuber in 1893 when he treated a scar using an autologous fat graft from the upper arm of a patient [18]. In 1982, Illouz was the first to describe autografting using fat obtained from liposuction [19]. He stated that adipocytes are highly fragile cells with very short lives. Once removed from the body, they cannot tolerate excessive manipulation, refrigeration or major trauma associated with harvesting [19]. Neuber also reported that grafts larger than an almond led to poor outcomes [18]. One of the main drawbacks of fat grafting is the unpredictable outcome due to variable resorption.

As fat preparation is one of the variables inherent in fat grafting, it plays an important role in adipocyte survival. The most commonly used methods for adipocytes preparation are decantation, concentration, centrifugation and washing with different solutions. The literature contains contradictory results regarding the superiority of these techniques. Coleman used centrifugation to remove undesirable elements from the lipoaspirate to improve the survival of grafted fat [20]. Boschert et al. [21] reported that centrifugation can stratify the percentage of adipocytes within the different layers of the lipoaspirate. Conversely, some studies have reported that centrifugation, at least at high speeds, adversely affects the viability of adipocytes [12, 13, 22, 23]. Rohrich et al. [16] reported similar viability in centrifuged and non-centrifuged adipocytes.

Condé-Green et al. [13, 14] studied the effects of different adipocyte preparation methods on viability and cell architecture. In the first test, they employed three different techniques to process the adipose tissue: decantation, washing and centrifugation. They observed a more preserved cell architecture after decantation than after the other two techniques [14]. They did not mention the decantation time before performing analyses, making it difficult to assess the effect of decantation on viability. Furthermore, they stated that cells were sent for processing less than 24 h after collection. Therefore, we cannot be certain that the time interval between harvesting and performing the analysis was standardized in all samples.

In a further study, the same group compared the outcomes (i.e., volume retention and histologic analysis) up to 12 weeks after injecting adipocytes prepared using decantation, washing and centrifugation. They found that decanted cells retained less volume than washed, centrifuged and cell-supplemented adipocytes, but their results were highly variable [13]. As we previously mentioned, no statistical difference was achieved. Although these results seem to favor washing over decantation and centrifugation, no actual cell viability studies were conducted prior to grafting. Thus, the grafting techniques used and the local conditions of the recipient site may affect the results.

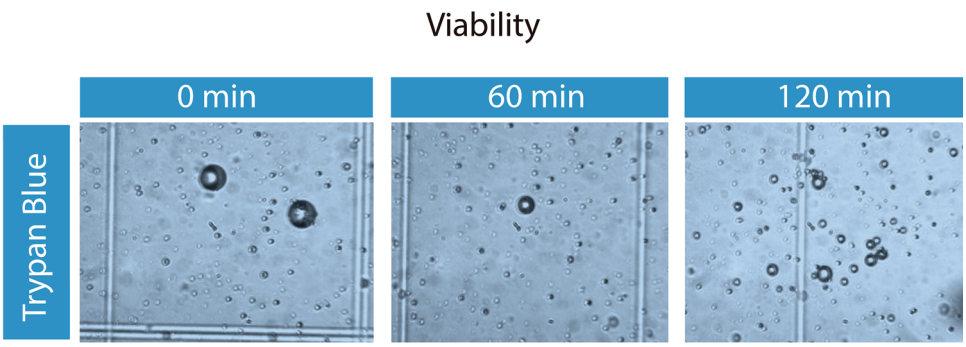

Fig. 1 Trypan blue staining of adipocytes from the lipoaspirate at 0, 60 and 120 min after harvesting. The adipocytes were diluted in the ratio of 15:1 with 0.4% trypan blue and then counted in a Neubauer chamber at 100× magnification

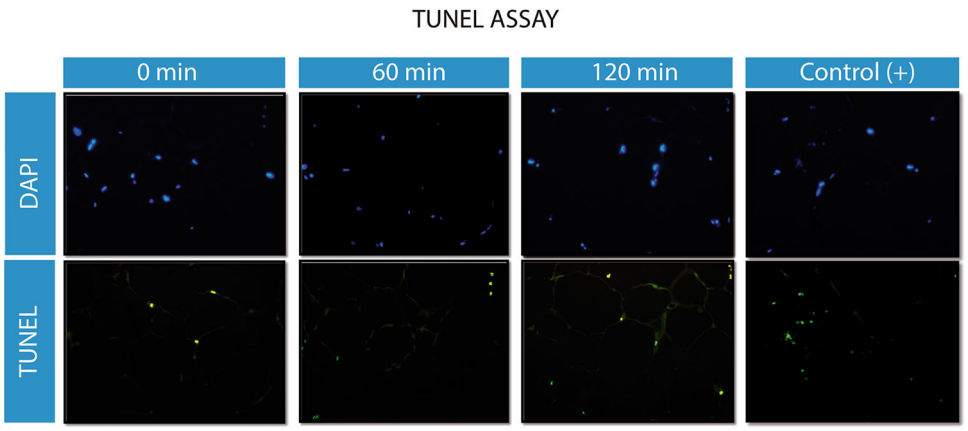

Fig. 2 TUNEL staining of adipose tissue sections from the lipoaspirate at 0, 60 and 120 min after harvesting. Green staining indicates apoptotic cells. The nuclear binding dye DAPI was used to determine the total number of cells per × 40 × field

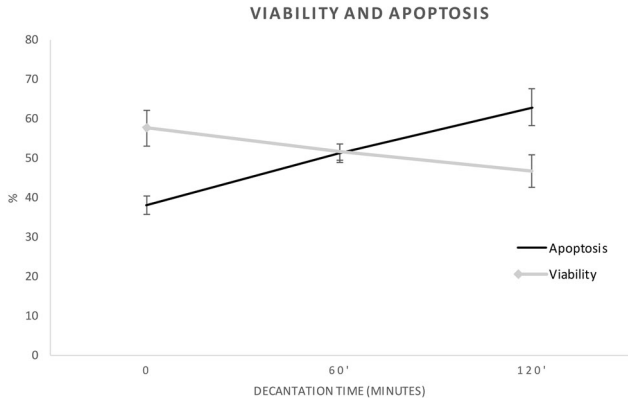

Fig. 3 Viability and apoptosis

Hoareau et al. [22] examined the viability of adipocytes after centrifugation at different speeds and decantation. In their study, they decanted the lipoaspirate for 4 min before injecting the adipocytes into mice. Prior to injection, they assessed viability by measuring the amount of oil obtained after centrifugation, but no measurement was conducted on the decanted samples. No actual study on viability was conducted on the decanted fat. The authors found that the volume of injected fat was similar with all preparation methods, but more oil was observed both macroscopically and microscopically in decanted adipocytes than in centrifuged adipocytes.

Ferraro et al. [12] demonstrated that decantation does not damage cells, as they observed an intact cell architecture. They also assessed viability and the rate of apoptosis in decanted cells, which were used as controls. They found that approximately 72% of the cells were viable and that apoptosis was present in 17% of the cells centrifuged at low speeds [12]. The variability of their results was not described. They did not observe apoptosis in decanted cells, and viability in these cells (decanted) was not reported. No information about the decantation time was provided.

The difference in the rate of apoptosis reported in the above study (no apoptosis) is very different from the nearly 40% immediately after harvesting (0 h) observed in our study. This may be due to the use of different methods for extraction. Unfortunately, the authors did not identify the device used for liposuction, which limits the potential for comparisons. However, we speculate that liposuction with a syringe leads to a higher number of apoptotic adipocytes.

The method selected to assess viability in our study was trypan blue staining, which is a long-standing and widely used method that is based on the fact that only cells with intact membranes can effectively exclude the dye [24]. However, this technique has its limitations when analyzing a large number of samples [25]. The use of trypan blue staining has been questioned for assessing the viability of adipose tissue [26]. However, we chose this method for its simplicity and the fact that our laboratory personnel have significant experience in performing the technique. We believe that the results obtained for viability are valid.

We decided to use 60-ml syringes to obtain fat, for speeding up the process and reducing cell manipulation as we could fill the tubes directly from the syringe. The available literature has shown that the negative pressure in the syringe depends on the amount of fat aspirated and not on the caliber of the syringe [27]. Additionally, low pressures have been shown to lead to better preservation of adipocytes [28]. However, we observed high mortality and apoptosis in the adipocytes immediately after harvesting. It may be easier to pull the plunger beyond the desired amount in clinical settings, leading to higher pressures. Low-pressure suction with a liposuction device might yield different results. Further studies may be useful to answer this hypothesis.

Centrifugation was used in all samples as a part of the process to determine viability. Although this might appear contradictory in a decantation study, it is a necessary step in assessing viability. Furthermore, the lipoaspirate was decanted according to the timepoints of the study (i.e., 0,

60 and 120 min) and then processed equally. Thus, if centrifugation might decrease viability at a certain level, this would be equal in all samples, allowing us to measure the difference between each of the timepoints.

Nevertheless, the aim of the study was to determine how the decantation time affects the percentage of viable and apoptotic cells. We have shown that these percentages increased over time and almost doubled after 2 h of decantation.

Conclusions

A long decantation time for adipocytes after liposuction is detrimental to cell viability and apoptosis initiation. We recommend cells should be re-injected within the first hour of aspiration to improve the number of viable cells grafted.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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