

The Effects of Fat Harvesting and Preparation, Air Exposure, Obesity, and Stem Cell Enrichment on Adipocyte Viability Prior to Graft Transplantation

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Abstract

Background: Adipocyte viability is affected by fat preparation and processing methods, but rigorous and objective studies of these relationships are lacking.

Objectives: The authors conducted a comprehensive evaluation of variables affecting adipocyte viability prior to injection of fat at the recipient site.

Methods: Lipoaspirates from 48 patients were processed by high or low vacuum pressure, decantation, electric or manual centrifugation, concentration with cotton gauze, washing, repeated syringe transfer, exposure to lidocaine, and exposure to air. The effects of these variables on adipocyte viability in vitro were ascertained with the MTT assay. The influences of patient obesity (ie, a body mass index [BMI] >30 kg/m²) and enrichment with stem cells on adipocyte viability also were determined.

Results: High vacuum pressure decreased adipocyte viability. Decantation yielded the highest cell viability, followed by washing, concentration with cotton gauze, and centrifugation. Exposure to concentrated lidocaine ambient air exposure, and transfer between syringes significantly decreased viability. Patient obesity was predictive of lower adipocyte viability regardless of processing method, whereas stem cell enrichment significantly improved viability ($P < .0001$).

Conclusions: To maximize adipocyte viability, fat should be obtained with dilute local anesthetics and low vacuum pressure, and the lipoaspirate should be maintained in a closed system. To clear cellular debris and blood, the lipoaspirate should be prepared by washing, and the fat should be enriched with adipose stem cells. Decreased adipocyte viability should be expected when fat is harvested from patients with high BMIs.

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Autologous fat transplantation involves 3 stages: harvesting of the lipoaspirate, preparation of the fat graft, and reinjection. In recent years, many authors have advocated lipotransfer as a means of body contouring and as a vehicle for enrichment of the recipient site with growth factors and adipose stem cells (ASC).¹ Autologous fat may be grafted to reconstruct the breast after cancer, as treatment for radiation dermatitis, and to facilitate tissue healing.¹

Rigorous and objective comparisons of lipotransfer techniques are lacking. Reported outcomes and efficacies of fat preparation methods are based on expert opinions, cases series, and results of the same techniques have been found to vary across practitioners.¹⁻⁶ Objectives of the current study were: (1) to evaluate adipocyte viabilities in vitro after various harvesting and preparation methods; (2) to compare adipocyte viabilities in ASC-enriched and ASC-nonenriched fat samples; and (3) to develop a straightforward protocol to

maximize survival of the fat graft without the need for sophisticated instrumentation.

METHODS

Patients and Study Design

Forty-eight consecutive patients (45 women, 3 men) who underwent liposuction from April 2013 to June 2015 were evaluated in a prospective study of the effects of fat

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preparation techniques on adipocyte viability. The study design was approved by the ethical and clinical study committee of Hospital Universitario Austral (Buenos Aires, Argentina). All procedures were conducted in accordance with guidelines set forth in the Declaration of Helsinki. All patients received information pertaining to this study and provided written informed consent. Patients who had previously undergone abdominal surgery, chemotherapy, or radiotherapy were excluded from the study. Also excluded were patients with a history of chronic corticosteroid therapy, connective tissue disorders, metabolic or hematologic disorders, or diabetes mellitus.

Patients were consecutively allocated to 1 of 7 subgroups according to the date of their initial consultation. For group 1 patients, the effects of decantation vs centrifugation on viability were evaluated under 2 vacuum pressures (220 mm Hg and 720 mm Hg). For patients in groups 2 through 7, lipoaspirates were harvested under low vacuum pressure (ie, 220 mm Hg). For group 2 patients, lipoaspirates were processed by decantation (20 minutes), washed with lactated Ringer's solution, concentrated by passage through cotton gauze, and centrifuged at a low (94 g [700 rpm]) or high gravitational force (1421 g [3000 rpm]). For group 3 patients, lipoaspirates were processed by repeated passage across disparate syringe sizes (60 mL and 10 mL) or similar syringe sizes (20 mL and 5 mL). For group 4 patients, lipoaspirates were exposed to ambient air for 0, 15, or 30 minutes. For group 5 patients, 9-mL aliquots of a subset of the lipoaspirates were transferred to a 10-mL syringe containing 1 mL of 2% lidocaine (Xylocain[®] 2%, Astra Zeneca, Argentina) without a vasoconstrictor. For group 6 patients, adipocyte viability was determined in patients with obesity (body mass index [BMI] > 30 kg/m²) and was compared with viabilities associated with decantation and with high and low centrifugation (group 2). For group 7 patients, a subset of the lipoaspirates was enriched with stem cells, and viability was assessed before and after 7 days of cell culture.

Colorimetric MTT Assay of Viability

We did not rely on cell counting by visual inspection to assess viability because the presence of nonviable intact cells could bias the count, as we have shown before with modified annexin D/propidium iodide staining.⁷ Instead, the MTT assay was applied to all analyses of adipocyte viability. This assay involves reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to formazan by the mitochondrial enzyme succinate dehydrogenase. Metabolically viable cells reduce MTT, which is evident as a color change from yellow to purple. The amount of living cells is proportional to the amount of formazan produced.

All fat samples were prepared from lipoaspirates (120 mL) obtained from the abdomen and flanks of study patients by means of syringe liposuction. A 100- μ L aliquot of fat tissue

(from lipoaspirate) was diluted in 1000 μ L of DMEM culture medium (Dulbecco's Modified Eagle's medium [DMEM], Gibco, ThermoFisher Scientific, Waltham, MA, USA). The resulting solution was seeded into a 96-well plate at 50 μ L per well, 130 μ L of medium was supplemented with 10% fetal bovine serum, and 20 μ L of MTT was added (final volume, 200 μ L/well). The reaction proceeded for 4 hours at 37°C and 5% CO₂, and results were determined with a spectrophotometer at a wavelength of 570 nm.

Lipoaspirate Harvest and Fat Preparation

Fat was aspirated through straight cannulae (15 cm long, 3-holed, 3-mm hole diameter, G.E. Lombardozzi, Argentina) under low vacuum (220 mm Hg) or high vacuum (720 mm Hg) with a 10-mL syringe (Luer lock tip syringe, Terumo, Somerset, NJ, USA) with plunger set at 2 mL or with a 60-mL syringe (Luer-lock tip syringe, Terumo, Somerset, NJ, USA) with plunger set at 60 mL, respectively. Vacuum pressures were determined at these plunger positions by means of a Vacuumeter (High Precision Vacuumeter, class 1, Blondelle, Panimpex, De Panne, Belgium). Lipoaspirates were transferred to 10-mL syringes for transportation to the laboratory.

Group 2 fat samples were obtained by low vacuum pressure and subjected to decanting, washing, gauze concentration,² and manual or electric centrifugation at 94 g (700 rpm) or 1421 g (3000 rpm), respectively, for 3 minutes. The middle portion of centrifuged fat was retained. The bottom layer of cellular debris and the supernatant oil were discarded. Each of the 10 patients contributed one fat sample from which the 5 methods were processed. The decantation subgroup of samples underwent gravity separation by maintaining the syringe in an upright position for 20 minutes. Samples processed by washing were combined with lactated Ringer's solution to remove blood and debris. Specifically, a syringe was filled to 70% capacity with fat, and lactated Ringer's solution was added to 90% capacity of the syringe. The remaining 10% of the syringe volume comprised an air bubble, which allowed for mixing to produce a yellow product. The remaining fat samples in group 2 were concentrated by applying them to cotton gauze for 3 minutes without added instrumentation,⁸ or manual or electric centrifugation.

Group 3 fat samples were aspirated at low pressure and transferred 20 times (ie, back and forth) between a 60-mL syringe and a 10-mL syringe or between a 20-mL syringe and a 5-mL syringe to assess the overall effects of transfer on viability and to determine whether transfer across a larger volume differential had a negative influence on viability. The effects of drying on adipocyte viability (group 4) were evaluated by placing fat samples on Petri dishes (Petri dish, Pyrex, Corning, Inc., Corning, NY, USA) and leaving them uncovered in ambient laboratory air (19°C, 55%

humidity) for 0, 15, or 30 minutes. Contaminating microorganisms were evaluated after 72 hours of sample culture at DMEN with 10% PBS.

Group 5 fat samples were prepared by gravity separation (20 minutes) followed by decantation. The effects of exposure to 2% lidocaine (without vasoconstrictors) on viability then were determined by combining 1 mL of lidocaine with 9 mL of fat in a 10-mL syringe.

For group 6, lipoaspirates were collected from patients with BMIs ranging from 30 to 35 kg/m² and were processed by decantation and manual (94 g) or electric centrifugation (1421 g).

Group 7 samples were prepared by washing and were organized into 4 stem cell-enrichment subgroups. Washing was performed for these samples to ensure removal of traces of blood, debris, and connective tissue.^{9,10} The first subgroup comprised a standard sample of adipose tissue, which naturally includes adipose stem cells. The second entailed adipose tissue from which stem cells had been extracted. Isolated stem cells constituted the third subgroup, and the fourth subgroup consisted of adipose tissue, containing naturally occurring stem cells, that was enriched with additional stem cells derived from subgroup 2. Viability was assessed immediately upon preparation and after 1 week of cell culture. In this group, each of the 8 patients contributed one fat sample from which the subgroups were divided.

Oil Red O Stain

It was used to identify preadipocytes as follows: Prepare fresh tissue sections by placing slides in propylene glycol for 2 minutes and then incubate in Oil Red O Solution (Oil red O Stain, Sigma Aldrich, St. Louis, MO, USA) for 6 minutes. Prepare a mixture of 85% Propylene Glycol in distilled water and differentiate the tissue section in this mixture for 1 minute. Rinse slide in 2 changes of distilled water. Incubate slide in Hematoxylin for 1-2 minutes. Place slide in Propylene Glycol for 2 minutes. Incubate slide in Oil Red O Solution for 6 minutes. Rinse slide thoroughly in tap water. Rinse slide in 2 changes of distilled water. Coverslip using an aqueous mounting medium.

Stromal Vascular Fraction (SVF) Isolation and Identification of ASC

We followed the protocol described by Estes et al.¹¹ Lipoaspirate that had been obtained the same day or the previous day was allowed to settle until the fat and blood had separated, and the crude fat portion (approximately 100 mL) was transferred to a sterile 250-mL plastic centrifuge tube. The fat portion was washed approximately 7 times with a solution of 0.1% (weight [w]/volume [v]) collagenase, 1% (v/v) bovine serum albumin (Fraction V), and 2 mM calcium chloride in Dulbecco's phosphate-buffered saline (D-PBS) that had been warmed to 37°C. Between washes, the fat and D-PBS layers were allowed to separate with

gentle stirring for 3 to 5 minutes. Washing was complete when the D-PBS layer and the fat layer were a similar light pink color. The bright yellow supernatant composed of extracellular oil was removed. The washed fat sample was mixed with warm D-PBS-collagenase (1:1 v/v) and incubated at 37°C with gentle shaking for 1 hour. The sample was centrifuged at 300 g and 21°C for 5 minutes and then was shaken vigorously for 10 seconds to ensure that individual cells were released from strands of fibrous tissue. The sample was centrifuged again at 300 g and 21°C for 5 minutes, and the resultant floating layer, containing mature adipocytes and aqueous D-PBS-collagenase, was aspirated. The sample, comprising the cell pellet and 5 mL of residual D-PBS, was resuspended in 10 mL of stromal medium, and the cell suspensions were pooled into sterile 50-mL centrifuge tubes.

The pooled sample then was centrifuged at 300 g and 21°C for 5 minutes. The resulting supernatant was aspirated, leaving 5 mL of stromal medium on each pellet. The cell pellet was resuspended in 10 mL of stromal medium and pooled contents, and was transferred to a sterile 50-mL centrifuge tube. The sample then was centrifuged at 300 g and 21°C for 5 minutes. The supernatant was aspirated, leaving 5 mL of stromal medium on the cell pellet, and the pellet was resuspended in 105 mL stromal medium per 100 mL of lipoaspirate digest. The resulting cells were plated at an equivalent density of 0.16 mL of liposuction aspirate per square centimeter or 35 mL per 225-cm² flask. Cells then were cultured at DMEN (Dulbecco modified Eagle medium, Gibco, ThermoFisher Scientific, Waltham MA, USA) with 10% fetal bovine serum at 37°C with 5% CO₂. Twenty-four hours after plating, culture medium containing nonadherent cells was replaced with fresh medium. During cell culture, the medium was replaced at 3-day intervals. ASC were identified *in vitro* by their adhesion to the plate edge, their specific duplication, and by identification of surface antibodies by means of flow cytometry.

Enrichment of Prepared Fat with ASC

Isolated ASC were seeded in a 24-well plate at a density of approximately 100,000 cells per well. Fat prepared by washing (100 µL; derived from a 5-mL sample of adipose tissue) was diluted in 1000 mL of Dulbecco's Modified Eagle's medium with 10% fetal bovine serum. A 250-µL aliquot of this solution was added to each well of the 24-well plate, and culture medium was added to 1 mL per well (final dilution, 1:40). Viability was ascertained on days 0 and 7 of cell culture.

Statistical Analysis

Viabilities were expressed as averages and as percentages of decrease relative to the fat preparation method that yielded the highest viability. Decantation was chosen as the standard after group 1 results were obtained. The unpaired

2-tailed Student *t* test and chi-square test were applied for statistical analysis. All analyses were performed using IBM SPSS Statistics 23.0 software (IBM Corporation, Armonk, NY, USA). Statistical significance was defined as $P < .05$.

RESULTS

The current study was not supported by any technical or commercial partnership, and we consider our results to be objective. The results are represented as bar graphs and dot plots to facilitate between-group and intragroup visual comparisons. We evaluated decantation, centrifugation, washing, and cotton gauze concentration because these are the most common methods for fat preparation.²

Forty-eight consecutive patients (45 women, 3 men) were included in this study. The mean age of the patients was 39.1 years (range, 20-55 years). The mean BMI was 25.9 kg/m² (range, 20-29.9 kg/m²) for non-obese patients and 32 kg/m² (range, 30-35 kg/m²) for obese patients. No adverse reactions were observed during tissue harvesting. The patients were grouped as follows: group 1, 5 patients; group 2, 10 patients; group 3, 5 patients; group 4, 5 patients; group 5, 5 patients; group 6, 10 patients; group 7, 8 patients. Decantation was associated with the highest adipocyte viability, so this method was considered the maximum viability standard. Cell viabilities were lower when adipocytes were isolated at a higher gravitational

force or higher vacuum pressure (groups 1 and 2; Figure 1). Washing was associated with the second highest viability ($P < 0.001$), followed by cotton gauze concentration ($P < 0.0001$), and centrifugation at low ($P < 0.001$) and high gravitational forces ($P < 0.0001$), respectively (Figure 2).

Syringe transfer is a typical fat-processing step and is usually accomplished by connecting the ends of 2 syringes and delicately handling the syringes to preserve the vitality of the sample. We evaluated the overall effects of syringe transfer on adipocyte viability as well as the effects of transfer between syringes having a large or small volume differential. We found 30% and 20% reductions in viability when fat was transferred between syringes with a large volume differential (60 mL to 10 mL) compared with decantation ($P < .0001$) or transfer between syringes with less variation in size (20 mL to 5 mL) ($P < .001$), respectively (Figure 3). Transfer between syringes decreased viability. This difference was statistically significant.

Exposure to ambient air is detrimental to adipocyte viability because of drying and potential contamination with prolonged exposure. Contaminating microorganisms were not observed in any sample after 72 hours culture at DMEN with 10% PBS. A significant decrease in viability was noted with a longer duration of air exposure after 15 and 30 minutes (Figure 4).

Application of a dilute anesthetic is routine practice for fat harvesting. We found that exposure to concentrated

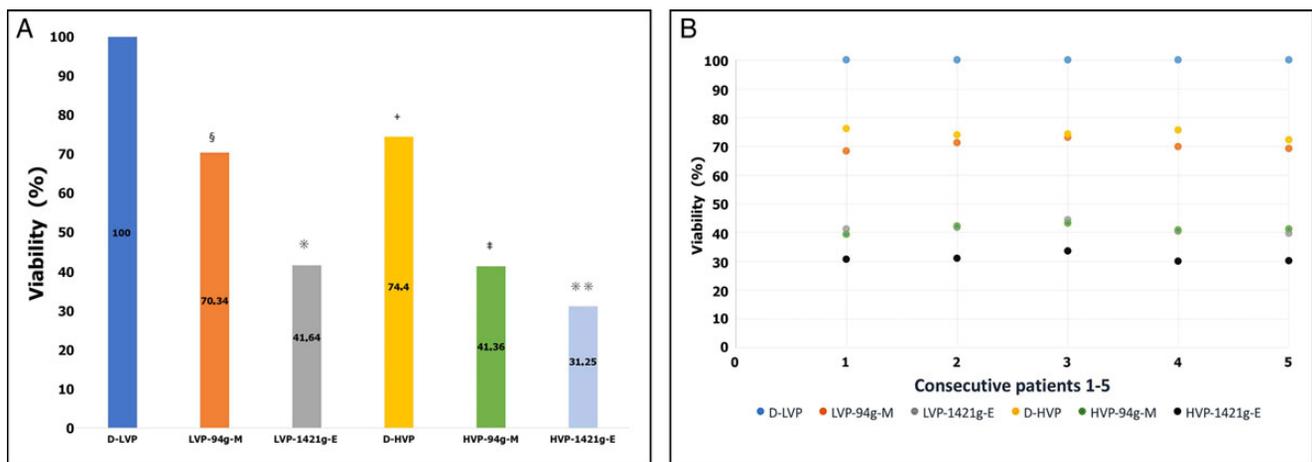


Figure 1. (A) Average viabilities by vacuum pressure for fat samples processed by decantation, manual centrifugation, or electric centrifugation. D-LVP, decantation at low vacuum pressure; D-HVP, decantation at high vacuum pressure; LVP-94g-M, manual centrifugation at low vacuum pressure (94 g [700 rpm]); HVP-94g-M, manual centrifugation at high vacuum pressure (94 g [700 rpm]); LVP-1421g-E, electric centrifugation at low vacuum pressure (1421 g [3000 rpm]); HVP-1421g-E, electric centrifugation at high vacuum pressure (1421 g [3000 rpm]). *P* values represent comparisons of viability with D-LVP as the standard. +, $P < .001$; †, $P < .001$; §, $P < .0001$; *, $P < .0001$; ** $P < .0001$. Viabilities were reduced with electric and manual centrifugation in both vacuum pressure groups. (B) Individual viability distributions by vacuum pressure and gravitational force for fat samples processed by decantation, manual centrifugation, or electric Centrifugation (group 1). D-LVP, decantation at low vacuum pressure; D-HVP, decantation at high vacuum pressure; LVP-94g-M, manual centrifugation at low vacuum pressure (94 g [700 rpm]); HVP-94g-M, manual centrifugation at high vacuum pressure (94 g [700 rpm]); LVP-1421g-E, electric centrifugation at low vacuum pressure (1421 g [3000 rpm]); HVP-1421g-E, electric centrifugation at low vacuum pressure (1421 g [3000 rpm]).

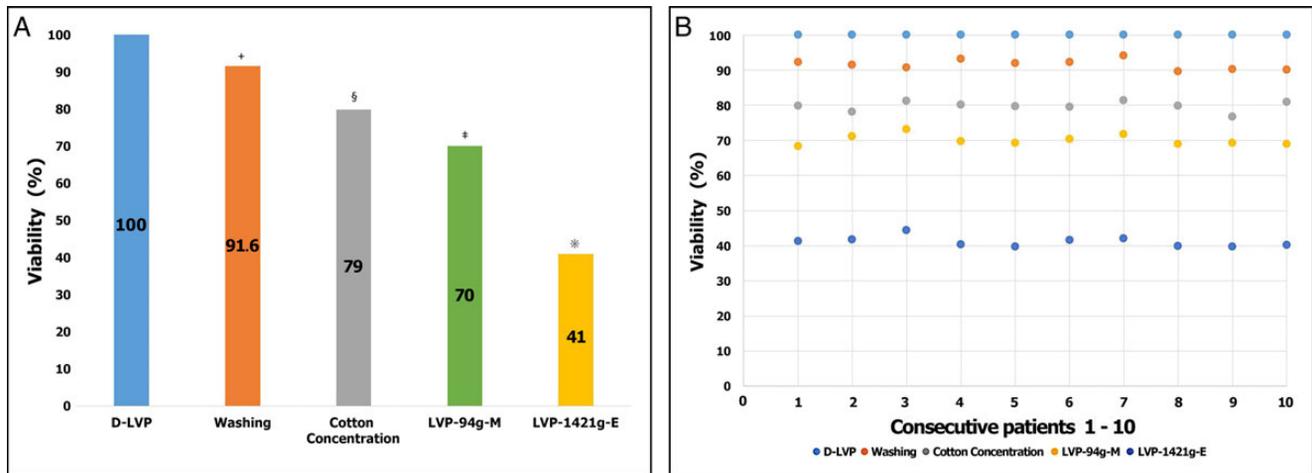


Figure 2. (A) Average viabilities by processing technique (group 2). D-LVP, decantation at low vacuum pressure; LVP-94g-M, manual centrifugation at low vacuum pressure (94 g [700 rpm]); LVP-1421-E, electric centrifugation at low vacuum pressure (1421 g [3000 rpm]). *P* values represent comparisons of viability with D-LVP as the standard. +, $P < .001$; ‡, $P < .001$; §, $P < .0001$; *, $P < .0001$; **, $P < .0001$. (B) Individual viability distributions by processing technique (group 2).

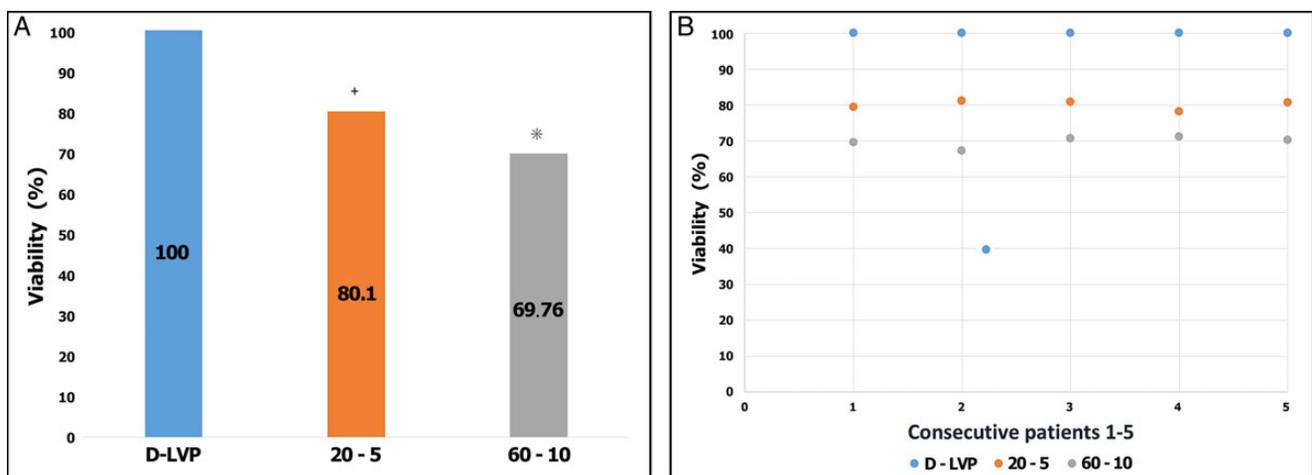


Figure 3. (A) Average viabilities by syringe volume differential (60 mL and 10 mL vs 20 mL and 5 mL), with decantation (group 3) as the control (blue). Decreases in viability of 30% (orange) and 20% (gray) were observed for syringe transfers between large and small volume differentials, respectively. *P* values represent comparisons of viability with D-LVP as the standard. +, $P < .001$; *, $P < .0001$. (B) Individual viability distributions for syringes with large or small volume differentials (group 3).

lidocaine decreased adipocyte viability in vitro by 60%, compared with decantation (Figure 5).

Jo et al¹² found that adipocytes undergo hypertrophic modifications in patients with obesity, and we observed that adipocytes harvested from obese patients were enlarged. The larger size and greater fragility of these cells could result in greater likelihood of rupture during syringe suction and processing. We harvested adipose tissue from patients with BMIs of 30 to 35 kg/m² and processed these samples at various gravitational forces or with decantation (negative control). Overall, high-BMI lipoaspirates had lower viability than lipoaspirates harvested from nonobese patients

BMI range was < 30 for nonobese patients and ≥ 30 for obese patients. 10 samples for each group. (Figure 6).

Washed samples enriched with stem cells exhibited greater viabilities than nonenriched samples on days 0 and 7 of cell culture. This difference was dose-dependent and could be attributed to the higher concentration of preadipocytes that arose in the stem cell-enriched subgroup (Figures 7 and 8).

DISCUSSION

A review of the literature demonstrated that adipocyte viability has been observed after various steps in autologous

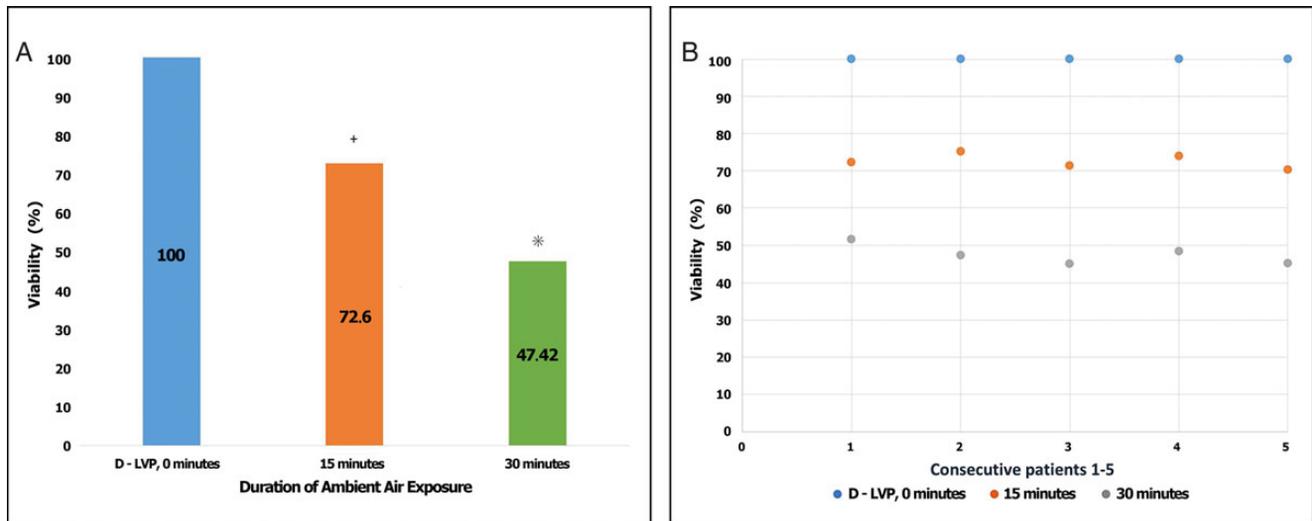


Figure 4. (A) Average viabilities by duration of exposure to ambient air (0, 15, or 30 minutes); (group 4). D-LVP, decantation at low vacuum pressure. Compared with samples exposed for 0 minutes (blue), viability decreased by 28% and 53% after 15 (orange) and 30 minutes (green) of exposure, respectively. *P* values represent comparisons of viability with D-LVP as the standard. +, *P* < .001; *, *P* < .0001. (B) Individual viability distributions for air exposure durations (group 4).

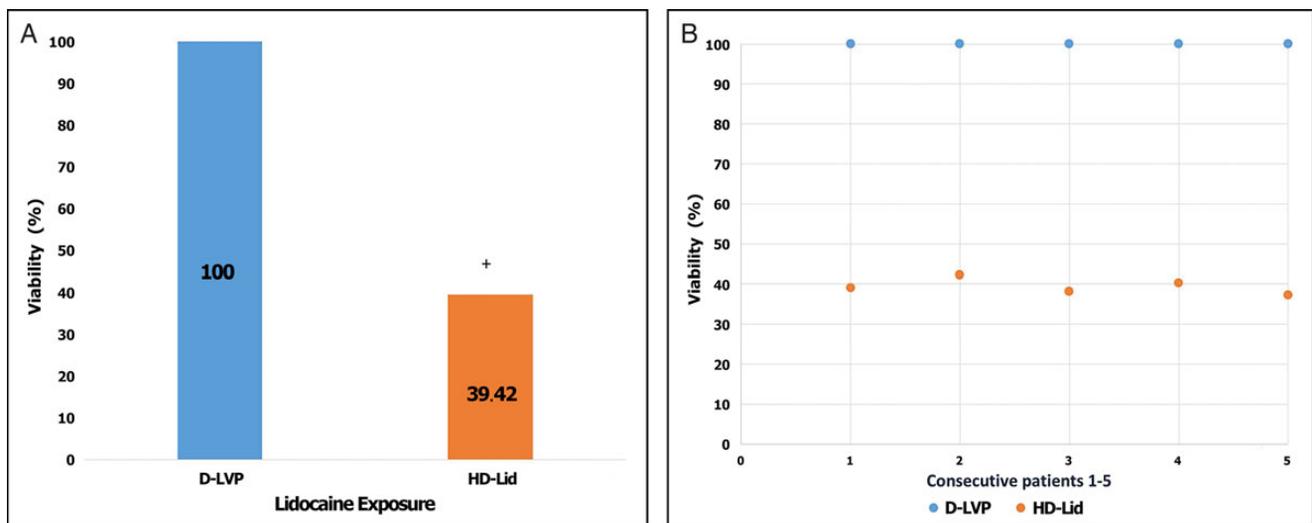


Figure 5. (A) Average viabilities by exposure to lidocaine (group 5). The exposure group entailed 9 mL of fat graft processed by decantation and 1 mL of 2% lidocaine in a 10-mL syringe. The control group consisted of a 10-mL syringe containing 10 mL of fat processed by decantation (blue). A 60% decrease in viability was observed in the lidocaine-exposed group (orange). D-LVP, decantation at low vacuum pressure; HD-Lid, exposure to high-dose lidocaine. *P* < .0001 for viability with D-LVP as the standard. (B) Individual viability distributions for lidocaine exposure (group 5).

fat transplantation.^{1-4,8-10,13} It has been our experience, at numerous scientific meetings, that research teams describe successful results with their techniques for fat transplantation. We maintain that this is not a scientifically rigorous approach to comparing methods, and we advocate objective assessments presented without commercial bias or personal opinion. To our knowledge, this is the first study in which standard fat-harvesting/preparation methods, patient obesity, and stem cell enrichment were evaluated for their effects on

adipocyte viability in vitro. The primary aim of this study was to determine which combinations of processing methods and other variables yielded the highest adipocyte viability. A systematic approach to producing high-viability adipocytes prior to injection can improve graft survival at the recipient site.

In studies of immunosuppressed mice and rabbits,^{14,15} investigators have found fat reabsorption to be unpredictable, and a suitable animal model for lipotransfer has not been established. Other researchers have obtained

unreliable viability results when counting cells under a light microscope.^{4,15} We previously reported that propidium iodide staining is subject to false-positive bias because adipocytes in a state of nuclear death may appear

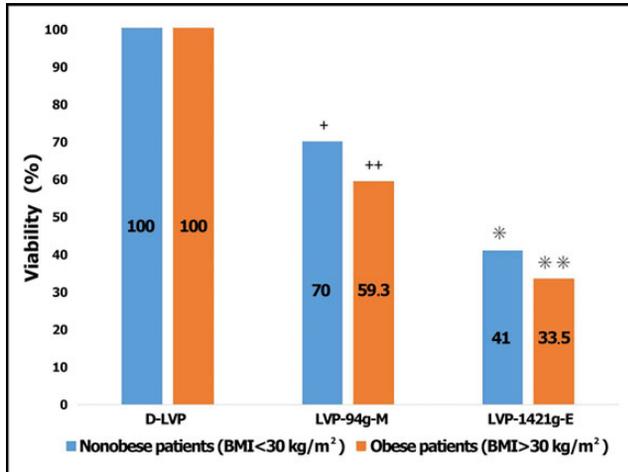


Figure 6. Average viabilities for nonobese (blue) (body mass index [BMI] < 30 kg/m²) and obese patients (orange) (BMI ≥ 30 kg/m²) after manual or electric centrifugation with decantation as the control (group 6). D-LVP, decantation at low vacuum pressure; LVP-94g-M, manual centrifugation at low vacuum pressure (94 g [700 rpm]); LVP-1421g-E, electric centrifugation at low vacuum pressure (1421 g [3000 rpm]). *P* value represents comparisons of viability by obesity status. + *P* < .001; *, *P* < .001; ++, *P* < .001; **, *P* < .0001.

morphologically healthy prior to disintegration of the cell membrane.⁷ We determined adipocyte viability in terms of mitochondrial function with a tetrazolium dye assay (ie, MTT). We analyzed the most common methods of fat processing prior to reinjection, including decanting, washing, cotton gauze concentration, and manual and electric centrifugation.² We did not evaluate whether the donor site affects adipocyte viability because Ullman et al¹⁶ previously demonstrated that fat samples obtained from the thigh, abdomen, and breast of a 48-year-old woman had long-term survival rates and histologic characteristics similar to samples from a nude mouse model. We harvested fat from the abdomen and flanks because these regions typically have sufficient fat, and in turn, many stem cells. We did not compare wet and dry techniques of fat harvesting (ie, with and without tumescent solution, respectively) because Agostini et al¹⁷ had demonstrated that these techniques yielded similar morphometric and viability results for adipocytes. We performed the wet technique because the dry technique poses greater risks of blood loss and hematic contamination and involves more challenging washing procedures.

We determined vacuum pressures for various syringe sizes and plunger positions. A similar analysis was conducted previously with the same results (J. Robles, personal communication, July 1995). We found a direct relationship between vacuum pressure and adipocyte damage. This relationship was corroborated by direct microscopic observation of destroyed structural fat cells.¹⁵ The results of other studies

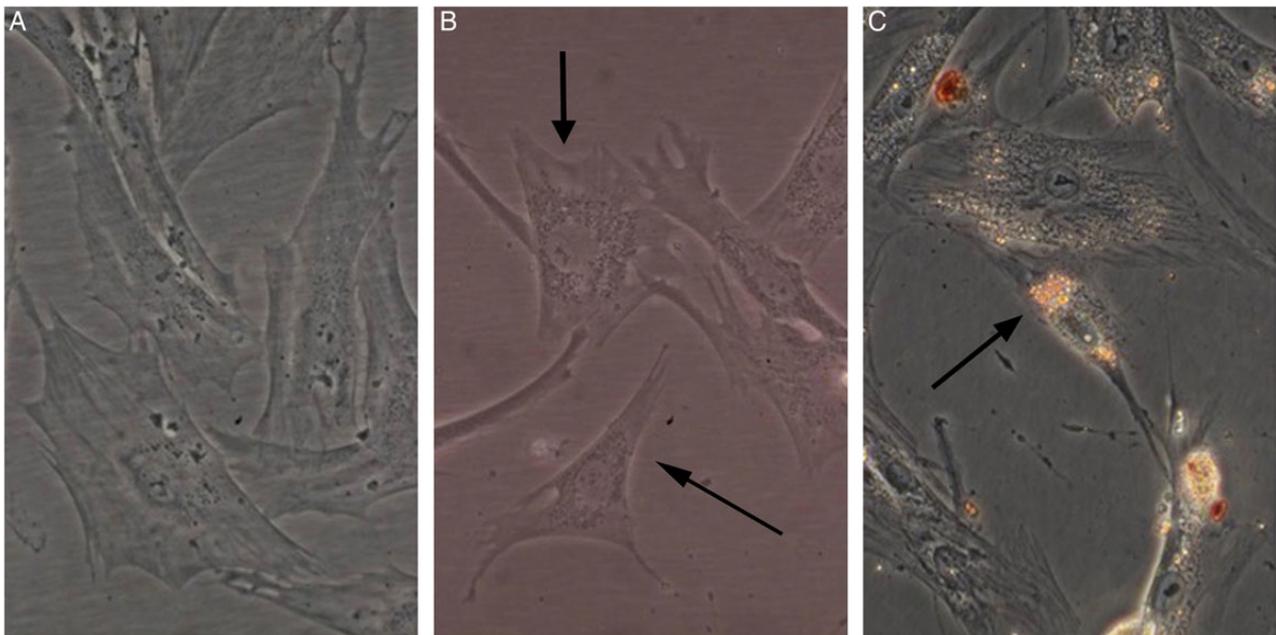


Figure 7. Stem cell culture and differentiation of preadipocytes. (A) Stem cells in culture without adipocytes (control). (B) Stem cells in culture with adipocytes. Arrows indicate potential preadipocytes. (C) Oil Red O staining of stem cells in culture with adipocytes. Arrow indicates lipid droplets that are indicative of spontaneous differentiation to preadipocytes.

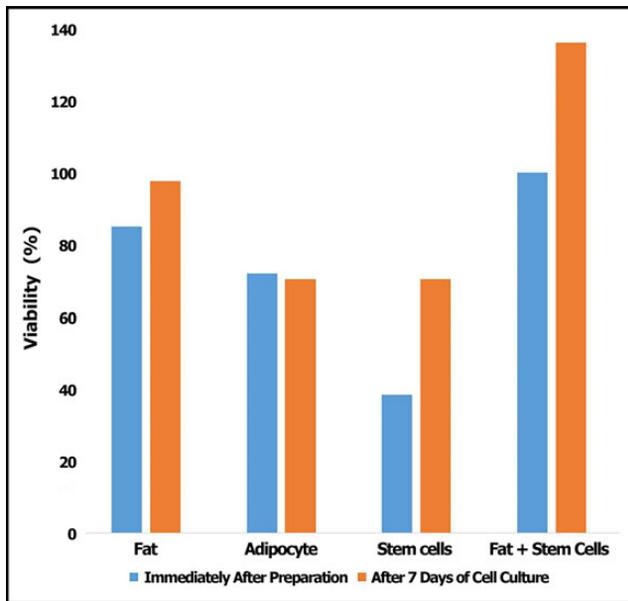


Figure 8. Comparison of adipocyte viabilities with or without stem cell enrichment (group 7). Blue bars indicate viabilities of subgroups immediately after preparation (ie, 0 days of cell culture): fat graft (adipocytes + stem cells), isolated adipocytes, isolated stem cells, fat graft + stem cells (ie, enriched). Orange bars indicate these subgroups after 7 days of cell culture. Improved viabilities were observed in the groups containing stem cells after 7 days of culture. In the enriched subgroup, viability increased by 36%. , + $P < .001$; *, $P < 0,8$; # $P < .001$; **, $P < .0001$.

have indicated that adipocytes are highly resistant to negative vacuum (harvest) and positive pressure (rejection),¹⁸ however, we found that high vacuum pressure reduces viability.

We allotted 20 minutes for gravity separation prior to decantation to simulate routine operating times. We chose to concentrate harvested fat tissue by placement on cotton gauze, rather a metal sieve, because Minn et al⁸ found that the metal sieve decreased adipocyte viability and increased inflammation. Our results at low and high gravitational forces were consistent with those of Condé-Green et al.¹⁰ At high gravitational force, the results of the MTT assay indicated a decrease in adipocyte viability. In addition, adipocyte rupture was increased, as determined with light microscopy and immunofluorescence microscopy, and by visual inspection of the volume of the oil fraction. Despite these findings, the application of a high gravitational force yields a pellet rich in mesenchymal cells, which are considered the most important factor for supporting fat graft survival at the recipient site.⁵

Mesenchymal cells are more resistant to the mechanical stressors of harvesting, processing, and reinjection, and serve as precursors for new adipocytes. In addition, mesenchymal cells help sustain the fat graft by releasing various growth

factors.⁵ We observed a 36% increase in adipocyte viability in the stem cell-enriched group, compared with the non-enriched group, after 1 week of cell culture. Enriched cultures also contained a higher concentration of preadipocytes after 1 week. Because enrichment with high amounts of stem cells has a degenerative effect on the fat graft,⁶ optimization of enrichment protocols is needed.

Our results indicate that simple decantation yields an adipocyte population with the highest viability, followed successively by washing, cotton gauze concentration, and centrifugation. Like decantation, washing involves minimal cell trauma. However, washing also clears cellular debris and blood remnants, which are stimulators of the inflammatory response that can facilitate degradation of the graft.^{13,19} Moreover washing extracts growth factors, but stem cells keep adhered to the stroma.⁹ We observed that washing and cotton gauze concentration yielded high concentrations of stem cells. For these reasons, we chose washing as the fat preparation method for the stem cell-enrichment group. Lactated Ringer's solution was selected because it counteracts acidosis in tissues subjected to hypoperfusion.²⁰

Transfer between syringes decreased viability. As expected, we found that transfer between syringes of disparate volumes (60 mL and 10 mL) decreased viability more than transfer between syringes that are closer in size (20 mL and 5 mL). This was attributed to trauma caused by transferring fat between syringes that differ substantially in size.

Keck et al^{21,22} suggested that lidocaine adversely affects adipocytes and interferes with preadipocytes and their differentiation into adipocytes. Conversely, Moore et al²³ and Shoshani et al²⁴ found that local anesthetics had no detrimental effects on adipocytes. In the current study, exposure to concentrated lidocaine (1 U lidocaine per 9 U fat) substantially reduced adipocyte viability. The application of diluted lidocaine before fat harvesting is likely to have a milder adverse effect, but this effect remains to be quantified in patients.

Fat grafts prepared from obese patients comprise hypertrophic adipocytes (60-150 μm) that are 2 to 3 times larger in diameter than adipocytes from nonobese patients.¹¹ We found that larger cells were more susceptible to mechanical damage than smaller cells; this was especially true for exposure to high gravitational force. All fat samples from obese patients in this study had reduced cell viability. We are planning to evaluate this phenomenon in a larger patient series to achieve greater statistical power and to potentially elucidate the mechanism controlling this difference in viability.

Taken together, our results support the following multi-step procedure for harvesting and preparing a high-viability graft. (1) Harvest fat at a low vacuum pressure (5-mL syringe, plunger pulled to the 5-mL mark; 10-mL syringe, plunger at 2-mL mark; 60-mL syringe, plunger at 10-mL mark); (2) prepare the fat graft by washing in a closed system without exposure to ambient air; (3) if syringe

transfer is necessary, ensure that syringe volumes are similar, and perform transfer in a closed system; (4) enrich the fat preparation with stem cells; (5) ensure soft reinjection at the recipient site; and (6) expect graft viability to be reduced for patients with high BMI.

Study Limitations

At the beginning of this study, fat samples from small groups of patients were organized into comparison groups, and additional samples were included as needed for sufficient statistical power. For this reason, the resultant group sizes varied. We intend to conduct a larger case series to validate the results of the current study. The fat samples in this study were exposed to concentrations of lidocaine that were higher than those applied in vivo; this ensured the detection of any negative influence of lidocaine on viability but was not representative of physiologic conditions. The optimal dose of lidocaine that facilitates fat harvesting without decreasing graft survival remains to be determined.

CONCLUSIONS

We evaluated all conventional methods of fat harvesting and graft preparation for their effects on adipocyte viability in vitro. Our results indicated that decantation yields the highest adipocyte viability, followed by washing, concentration with cotton gauze, and centrifugation at low and high gravitational forces. We found that higher patient BMI was associated with larger adipocytes and decreased viability. We also determined that concentrated lidocaine exposure decreased the viability of fat cells. Finally, we observed in our study a positive correlation between the extent of stem cell enrichment and adipocyte viability. We advocate rigorous and objective assessments of cell viability in lipotransfer as a means to develop a standard procedure for autologous fat transplantation. A concomitant research objective should be to maximize the capacity of the recipient site to support the fat graft.

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REFERENCES

1. Coleman SR, Saboeiro AP. Fat grafting to the breast revisited: Safety and efficacy. *Plast Reconstr Surg.* 2007;119(3):775-785.
2. Condé-Green A, Wu I, Graham I, et al. Comparison of 3 techniques of fat grafting and cell-supplemented lipotransfer in athymic rats: a pilot study. *Aesthet Surg J.* 2013;33(5):713-721.
3. Smith P, Adams W, Lipschitz A, et al. Autologous human fat grafting: effect of harvesting and preparation techniques on adipocyte graft survival. *Plast Reconstr Surg.* 2006;117:1836-1844.
4. Pu L, Coleman S, Cui X, et al. Autologous fat grafts harvested and refined by the Coleman Technique: A comparative Study. *Plast Reconstr Surg.* 2008;122:932-937.
5. Yoshimura K, Sato K, Aoi N, Kurita M, Hirohi T, Harii K. Cell-assisted lipotransfer for cosmetic breast augmentation: supportive use of adipose-derived stem/stromal cells. *Aesthetic Plast Surg.* 2008;32(1):48-55.
6. Paik KJ, Zielins ER, Atashroo DA, et al. Studies in fat grafting: part V. Cell-assisted lipotransfer to enhance fat graft retention is dose dependent. *Plast Reconstr Surg.* 2015;136:67-75.
7. Rieger AL, Nelson KN, Konowalchuk JD, Barreda DR. Modified annexin D/propidium iodide apoptosis assay for accurate assessment of cell death. *J Vis Exp.* 2011;50:e2597.
8. Minn KW, Min KH, Chang H, Kim S, Heo EJ. Effects of fat preparation methods on the viabilities of autologous fat grafts. *Aesthetic Plast Surg.* 2010;34(5):626-631.
9. Condé-Green A, de Amorim NF, Pitanguy I. Influence of decantation, washing and centrifugation on adipocyte and mesenchymal stem cell content of aspirated adipose tissue: a comparative study. *J Plast Reconstr Aesthet Surg.* 2010;63:1375-1381.
10. Condé-Green A, Baptista LS, de Amorim NF, et al. Effects of centrifugation on cell composition and viability of aspirated adipose tissue processed for transplantation. *Aesthet Surg J.* 2010;30:249-255.
11. Estes BT, Diekman BO, Gimble JM, Guilak F. Isolation of adipose-derived stem cells and their induction to a chondrogenic phenotype. *Nat Protoc.* 2010;5(7):1294-1311.
12. Jo J, Gavrilova O, Pack S, et al. Hypertrophy and/or hyperplasia: dynamics of adipose tissue growth. *PLoS Computat Biol.* 2009;5(3):e1000324.
13. Zhu M, Cohen S, Hicok K, et al. Comparison of three different fat graft preparation methods: gravity separation, centrifugation, and simultaneous washing with filtration in a closed system. *Plast Reconstr Surg.* 131(4):873-880.
14. Brucker M, Sati S, Spangerberger A, Weinzwieg J. Long-term fate of transplanted autologous fat in a novel rabbit facial model. *Plast Reconstr Surg.* 2008;122(3):749-754.
15. Crawford J, Hubbard B, Colbert S, et al. Fine tuning lipoaspirate viability for fat grafting. *Plast Reconstr Surg.* 2010;126:1342-1327.
16. Ullmann I, Shoshani O, Fodor A, et al. Searching for the favorable donor site for fat injection: in vivo study using the nude mouse model. *Dermatol Surg.* 2005;10:1304-1307.

17. Agostini T, Lazzeri D, Pini A, et al. Wet and dry techniques for structural fat graft harvesting: histomorphometric and cell viability assessments of lipoaspirated samples. *Plast Reconst Surg.* 2012;130(2):331e-339e.
18. Lee J, Kirkham J, McCormack M, et al. The effect of pressure and shear on autologous fat grafting. *Plast Reconst Surg.* 2013;131:1125-1136.
19. Locke MB, de Chalain TM. Current practice in autologous fat transplantation: suggested clinical guidelines based on a review of recent literature. *Ann Plast Surg.* 2008;60(1):98-102.
20. Griffel MI, Kaufman BS. Pharmacology of colloids and crystalloids. *Crit Care Clin.* 1992;8(2):235-253.
21. Keck M, Janke J, Ueberreiter K. Viability of preadipocytes in vitro: the influence of local anaesthetics and pH. *Dermatol Surg.* 2009;35:1251-1257.
22. Keck M, Zeyda M, Gollinger K, et al. Local anesthetics have a major impact on viability of preadipocytes and their differentiation into adipocytes. *Plast Reconstr Surg.* 2010;126:1500-1505.
23. Moore JH Jr, Kolaczynski JW, Morales LM, et al. Viability of fat obtained by syringe suction lipectomy: effects of local anesthesia with lidocaine. *Aesthetic Plast Surg.* 1995;19:335-339.
24. Shoshani O, Berger J, Fodor L, Ramon Y, Shupak A, Kehat I. The effect of lidocaine and adrenaline on the viability of injected adipose tissue--an experimental study in nude mice. *J Drugs Dermatol.* 2005;4:311-316.

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