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Research

Assessing Adipocyte Viability and Surgeons' Work Efficiency by Comparing Different Liposuction Methods

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Background: Autologous fat grafting is widely used in plastic and reconstructive surgery. Liposuction methods play a key role in surgeons' work efficiency, adipocyte viability, graft survival, and outcomes. We investigated the effect of four liposuction methods on adipocyte viability, debris, and surgeons' work efficiency by measuring the active energy expenditure and changes in heart rate.

Methods: Human lipoaspirate was harvested from patients' removed abdominal flaps using four different liposuction methods, and we counted calories per aspirated volume and surgeons' heart rate. Adipocytes were separated from the lipoaspirate immediately by digestion with 0.1% type I collagenase. After digestion, parts of the cells and debris were measured. Adipocytes were plated in an adipocyte maintenance medium containing Alamar blue reagent. The adipocyte metabolic activity was measured using a spectrophotometer.

Results: After evaluating the active energy expenditure and changes in surgeons' heart rate, the ultrasonic-assisted liposuction (UAL) method was determined to be the most ergonomic liposuction device for surgeons. In addition, adipocyte viability was higher in the UAL group than in the other groups, and debris was the lowest in the power-assisted liposuction 1 group (PAL1).

Conclusions: Adipocyte viability is crucial for improving fat grafting outcomes. This study revealed that the viability of adipocytes is best preserved using the UAL and PAL1 liposuction methods. The UAL and PAL1 methods caused the least damage to the cells. The UAL method yielded the best results for surgeons' work efficiency.
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INTRODUCTION

Autologous fat grafting has become a common procedure in plastic surgery. Several theories tried to explain fat tissue remodeling after transplantation.[1] The most accepted explanation for the varying results is Peer's survival theory, which postulates that the number of viable adipocytes correlates with the fat graft survival volume.[2] It is evident that the more viable cells are transferred, the better chances of retaining the maximum graft volume after injection. The volume of loss and viability after fat grafting can vary and depend on various factors, including liposuction methods, harvesting, decantation time, processing, and transferring.[3–6] The best-analyzed protocol for fat grafting in small-volume transplantation is the Coleman technique[7]; however, the best protocol for large-volume transplantation is still under discussion.

Liposuction techniques have advanced significantly over the last generation of devices.[8] Since its introduction by Illouz et al over 40 years before, suction-assisted liposuction (SAL) has evolved tremendously. It has become one of the most popular aesthetic plastic surgery procedures.[9] Current liposuction technologies include suction-assisted lipectomy and ultrasound-, power-, laser-, water-, and radiofrequency-assisted methods.

Plastic surgeons are at risk of work-related musculoskeletal disorders (MSDs) due to repetitive movements, static or awkward postures, and cervical spine loading with headlamps and loupes.[10] Therefore, the ergonomy of liposuction devices plays an important role. When liposuction is

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performed without unnecessary force by the surgeon, better results and safety are expected. We aimed to determine the most ergonomic liposuction method for fat harvesting regarding surgeon work efficiency and adipocyte viability, which has not been done before, to our knowledge.

METHODS

Ethical Aspects

The study was approved by the Regional Ethics Committee of the Kaunas Biomedical Research, Lithuania (No. BE-2-3). Written informed consent was obtained from each participant.

Patient Population

This prospective randomized trial was conducted between February 2021 and December 2022. Patients who underwent abdominoplasty, mini abdominoplasty or fleur-de-lis abdominoplasty were included in the study.

The exclusion criteria were sex (only women were included), age younger than 18 or older than 60 years, hematologic abnormalities, chronic use of corticosteroids, connective tissue diseases, fat tissue abnormality (lipodystrophies), metabolic diseases, cancer, body mass index less than 18.5 or more than 29.9, secondary abdominoplasty procedure or previous liposuction targeting the abdomen, and smoking 4 weeks before the study.

This study was conducted on 328 specimens [65 in the SAL, 64 in the power-assisted liposuction with Euromi device (PAL1), 64 in the power-assisted liposuction with MicroAire device (PAL2), 66 in the ultrasound-assisted liposuction (UAL), and 69 in the control groups] from patients undergoing plastic surgery procedures. The average age of the patients was 39 years (range 21–57 years), and the average body mass index was 27.9 kg/m^2 (range 22.8–29.9 kg/m^2).

Study Design

Specimens from patients were randomized to undergo traditional SAL, PAL1 or PAL2, and UAL with a vibration amplification of sound energy at resonance (VASER) device (Table 1). The four trial groups were randomized using a sealed envelope system. The same group of surgeons performed all liposuction procedures.

After surgical removal of the tissues, the specimens were immediately transferred for analysis. A C-section and scar areas were excised from each specimen. Only specimens that weighed 500–550 g were used.

The prepared specimen was fixed on a frame to simulate the natural tension and stability of the patient's abdominal tissues (Fig. 1) and infiltrated with 150 mL of a wetting solution (saline). Afterward, a 1-minute cross-tunneling (fat separation and emulsification) was performed with the randomized device without any suction power. Finally, liposuction was performed with a blunt 4-mm cannula, using a regular technique and cannula motion activity. The end point of liposuction was 150 mL of lipoaspirate. The raw lipoaspirate was gently stirred in a collection canister to make different size cells, other particles, and fluid distribute as evenly as possible with no additional manipulation. A 30 cm^3 amount of fat was taken for further analysis. In addition, a 3 × 3 cm sharply cut piece of fatty tissue from the same flap specimen was used as a control for every case (Fig. 1).

Estimation of Surgeon's Work Efficiency

Accelerometry analysis was used to objectively evaluate a surgeon's physical effort using different liposuction methods. Among the accelerometers, researchers use

> **Takeaways**
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> **Question:** What is the effect of four different liposuction methods on adipocyte viability, debris, and surgeons' work efficiency?
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> **Findings:** This study revealed that the viability of adipocytes was best preserved using the UAL and PAL1 liposuction methods. These two methods caused the least damage to the cells. The UAL method yielded the best results for surgeons' work efficiency.
>
> **Meaning:** Investigated parameters depend on liposuction method chosen in semi-in vivo procedure, still the difference is not necessarily of clinical value.

Table 1. Description of Devices Used for the Study Groups

	SAL Group	PAL1 Group	PAL2 Group	UAL Group
Device	—	Lipomatic Eva sp 6 power-assisted machine (Nutational Infrasonic Liposculpture, Euromi, Andrimont, Belgium). This device produces a processional low-frequency cannula movement	PAL-650 from MicroAire (Charlottesville, Va.) handpiece powered by an electric console (5020). This device produces oscillating reciprocal movement of the liposuction cannula tip	Third-generation VASER device (Sound Surgical Technologies, Inc., Louisville, Colo.) was used for ultrasound-assisted fat emulsification. Subsequent fat extraction was performed identically to that of the SAL group.
Cannula	4-mm three-port Mercedes-style cannula	4-mm three-port Mercedes-style cannula	4-mm three-port Mercedes-style cannula	4-mm three-port Mercedes-style cannula
Settings	Cannula was attached to an aspirator (1 atm of negative pressure) via standard suction tubing	Air pressure of 3.9 bar, and 1 atm of negative pressure	The power was set at 80%. 1 atm of negative pressure	Ultrasound-assisted liposuction system was set to 80% amplitude in the pulsed mode. 1 atm of negative pressure

VASER, vibration amplification of sound energy at resonance.

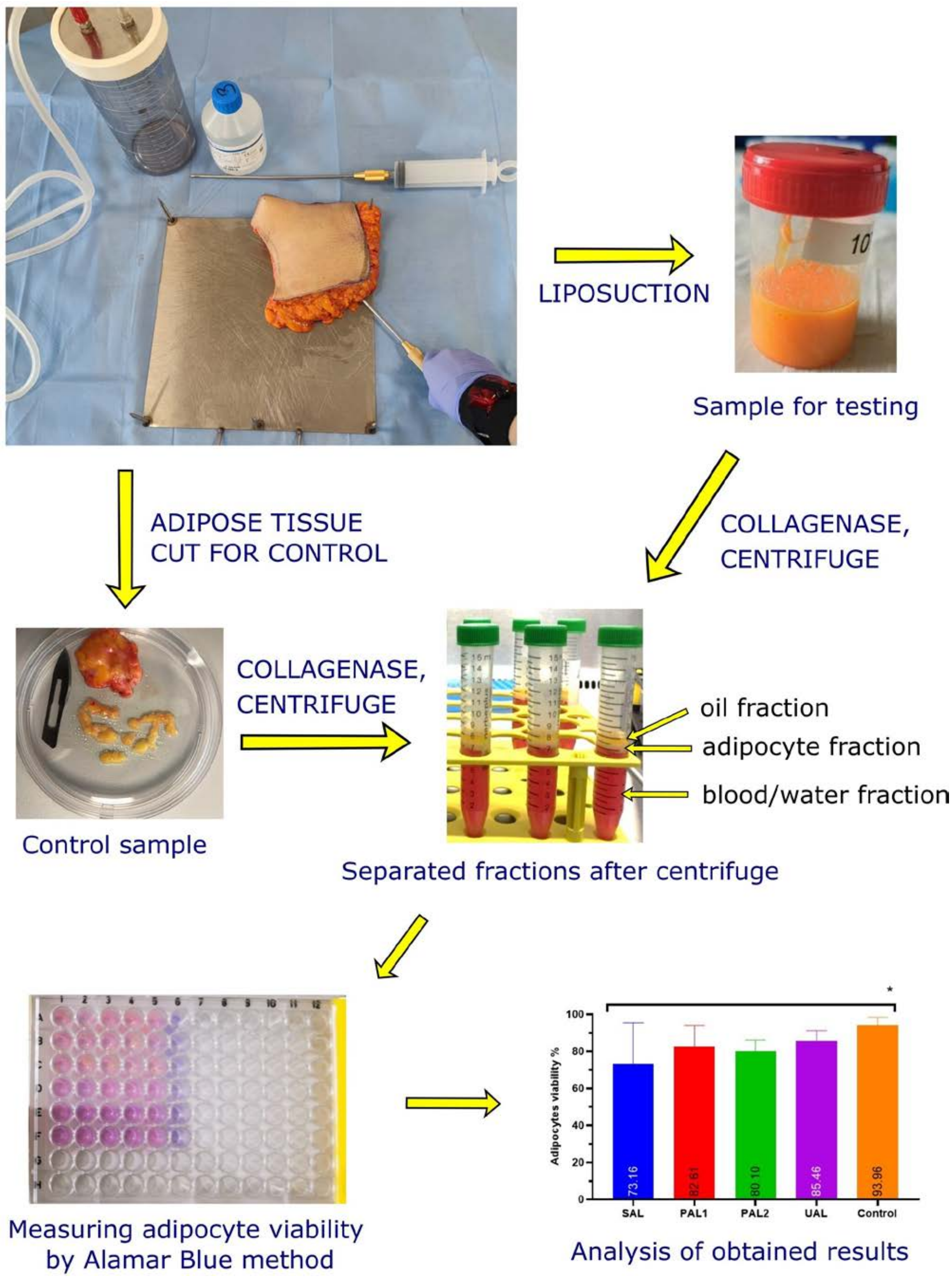

Fig. 1. Schema of the experimental design. The specimen was fixed on a frame. Liposuction was performed with a blunt 4-mm cannula, using a regular technique and cannula motion activity. A 30 cm^3 of fat was taken for further analysis. In addition, a 3 × 3 cm sharply cut piece of fatty tissue from the same flap specimen was used as a control for every case. The cells were cultivated in adipocyte maintenance growing medium and 10% Alamar blue reagent. After 16 hours, absorbance was measured in 96-well plates, only in the transferred medium (without cells). Adipocyte viability was calculated by equating the control sample to 100%.

ActiGraph most frequently, accounting for more than 50% of published studies.[11]

The surgeon was fitted with a GT3XP-BT ActiGraph accelerometer (ActiGraph Corp, Pensacola, Fla.) worn on the wrist.[12] The device is triaxial and detects accelerations in vertical, mediolateral, and anteroposterior axes.

The mean heart rate, the difference between the initial and maximum heart rate, and estimated physical activity energy expenditure (PAEE) were also assessed. The Freedson VM3 (‘11) algorithm was used to calculate PAEE (kcal/min).[13] Only active energy expenditure was calculated; the basal metabolic rate was not taken into

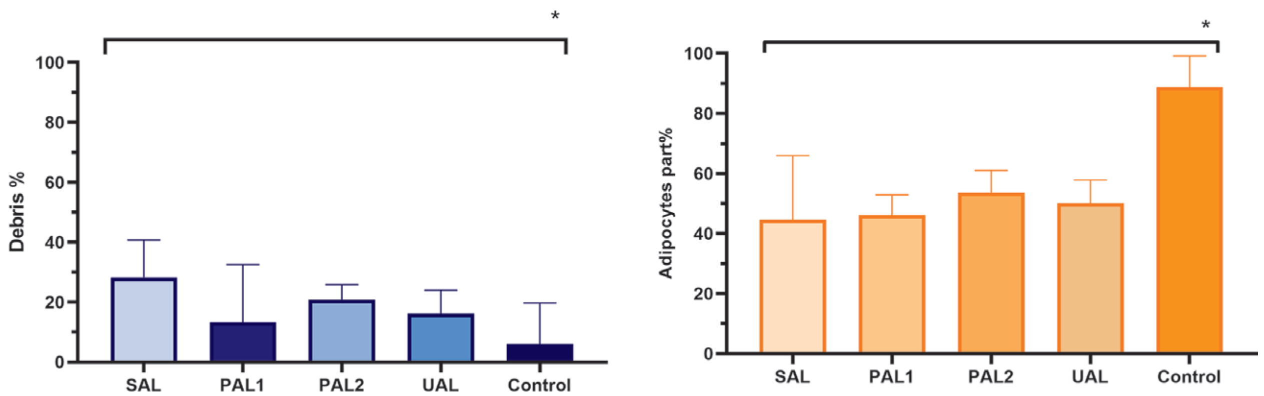

Fig. 2. Lipoaspirate parts. Debris (A) and adipocyte fractions (B) of a different liposuction methods (the control presents a sample without an application of suction). Data are presented as median and interquartile ranges. *P less than 0.05.

account. Data were interpreted using ActiLife 6 software (ActiGraph Corp, Pensacola, Fla.).

Adipocyte Isolation and Measurement Viability

Samples were weighed and mixed with 1:1 volume of digestion medium containing 0.1% collagenase type I (Sigma, C1-22, Germany) and incubated at 37°C while shaking slightly for 60 minutes. Digestion was stopped with a 1:1 volume of Dulbecco modified Eagle medium (Gibco) containing 10% fetal bovine serum (Gibco) and 1% penicillin/streptomycin (Gibco) and centrifuged at 130 × g for 2 min.

Afterward, the specimens were divided into an upper layer of free fat (from broken cells), a middle layer with mature adipocytes, and a bottom aqueous fraction (Fig. 1). The middle layer consisting of adipocytes and the upper layer consisting of debris were weighed separately.

Twenty microliters of the isolated adipocytes was transferred to 1 mL of adipocyte maintenance growing medium and 10% Alamar blue reagent (Invitrogen). The cells were then incubated at 37°C in an incubator with 5% CO_2. Absorbance was measured after 16 hours at 570 and 600 nm in 96-well plates, only in the transferred medium (without cells) (Fig. 1). Adipocyte viability was calculated by equating the control sample to 100%. Afterward, viability was recalculated by evaluating the amount of debris formed in the sample and the viability by percentage using the following formula:

$$\text{Viability } \% = \text{adipocyte viability in total volume } \% \times (\text{mass of adipocyte } \%/100)$$

Statistical Analysis

Statistical analyses were performed using Prism 9.4.1 Program (GraphPad Software Inc., La Jolla, Calif.). After confirming the normal data distribution, comparisons between graft conditions were made using the paired two-tailed t test; if a normal distribution was not confirmed, the Kruskal–Wallis and Friedman tests were used. All data are presented as the mean ± SD or median (interquartile range). We performed the power of analysis while comparing means of control versus separate groups, and alpha was 5%. The power of analysis in all groups was higher than 0.8. For correlation, Spearman test was used. A P value of less than 0.05 was considered statistically significant.

RESULTS

Debris and Adipocyte Fractions

Fractions of debris and adipocytes in the different liposuction groups were unevenly distributed. The median ranged from 6.05% of debris in the control group (the control group presented a sample without the application of suction) to 28.24% in the traditional SAL group (Fig. 2A). The proportion of adipocytes ranged between 44.48% in the SAL group and 88.69% in the control group (Fig. 2B). The group that had the smallest quantity of debris was PAL1 (Fig. 2A), whereas PAL2 had the highest proportion of adipocytes (Fig. 2B) (excluding the control). Results showed no statistically significant difference between the different liposuction methods.

Adipocyte Viability Based on Liposuction Methods

Our data revealed that the UAL group had the highest adipocyte viability median, reaching 85.46% (43.96%–98.49%). The SAL group had the smallest adipocyte viability median 73.16% (26.43%–99.77%). Among the groups, a statistically significant difference was observed between the control group and other groups (Fig. 3). In contrast, there were no statistically significant differences between other groups.

Adipocyte Viability Based on Debris Part and Patients' Age

Regarding the isolated adipocytes' purity, it may be assumed that debris constitutes a large portion of the entire isolate; therefore, debris may strongly influence adipocyte viability. Based on this, we separated the samples into two groups, depending on the quantity of debris: samples with less than 25% debris and samples that contained greater than 25% debris (Fig. 4A). The results revealed that a higher amount of debris was associated with lower viability significantly.

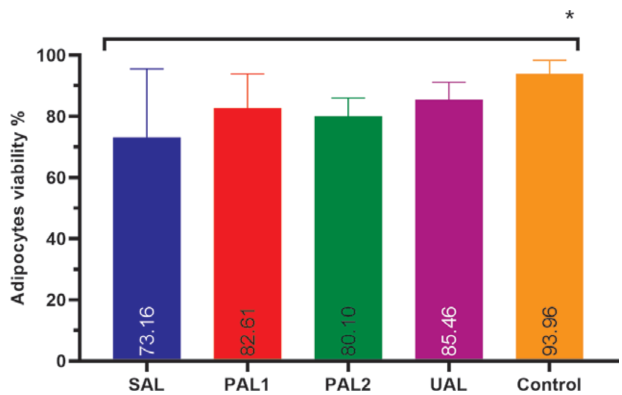

Fig. 3. Adipocyte viability after conversion by debris factor. Data are presented as median with interquartile range. *P less than 0.05.

In all groups, the proportion of debris ranged from 0% to 50.03%. According to the results, the viability of adipocytes varied and did not statistically differ to a greater extent among different liposuction methods in the low debris-containing group than in the liposuction methods in the group with greater than 25% debris (Fig. 4B). In addition, there was a strong negative correlation between adipocyte viability and debris parts (Fig. 4C). Spearman's correlation coefficient was −0.9.

Due to age-related metabolic changes, the patients involved in this study were separated into two groups according to their age: younger than 45 years and 46 years or older. We compared the viability of all adipocytes according to patient age, and observed no differences between these two groups (Fig. 5A). The viability of adipocytes according to the patient's age and liposuction method varied between the different groups but did not differ significantly (Fig. 5B).

Estimation of Surgeon's Work Efficiency by Measuring PAEE and Changes in Heart Rate

The most ergonomic liposuction method based on heart rate and kilocaloric consumption separately was the UAL method, followed by the PAL2 method, whereas the SAL and PAL1 methods had the worst parameters (Figs. 6 and 7). The average relative maximal heart rate reached was 128.6%, 132.9%, 146%, and 141.5% using the UAL, PAL2, PAL1, and SAL methods, respectively (Fig. 6). In comparison, the normalized maximal heart rate differed significantly between the SAL and UAL methods and between the PAL1 and UAL methods. In contrast, no significant difference was recorded between the PAL2 and UAL groups. Kilocaloric consumption of liposuction methods differed significantly between SAL and UAL methods and between PAL1 and UAL methods.

The time spent by the surgeon (time in seconds) to extract 150 mL of lipoaspirate during the application of different liposuction methods revealed that less time was required for the PAL2 (3 minutes 59 seconds) and UAL (5 min) methods. When analyzing the kilocalorie per minute ratio, best ratios 7.1 and 7.2 were reached using the PAL1 and UAL methods, followed by that obtained using the SAL method, with a kilocalorie per minute ratio of 8. Working with PAL2 was the least ergonomic, with a

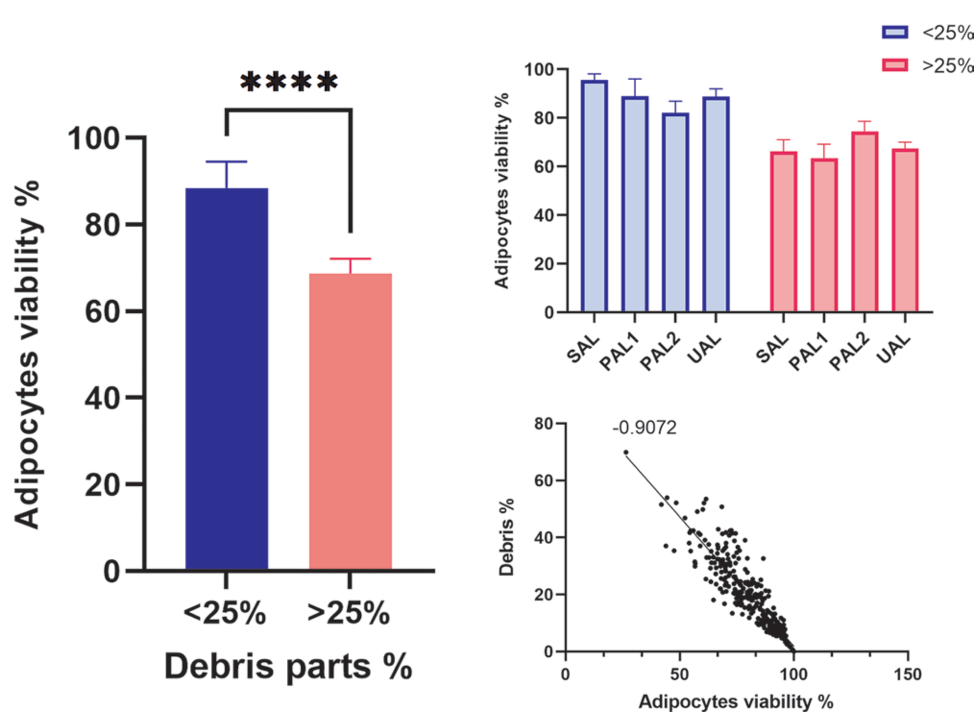

Fig. 4. Adipocyte viability based on the debris part. A, Viability of all adipocytes according to the amount of debris. B, Viability of adipocytes according to the amount of debris and liposuction method. C, Correlation between adipocyte viability and debris. ****$P < 0.00001$.

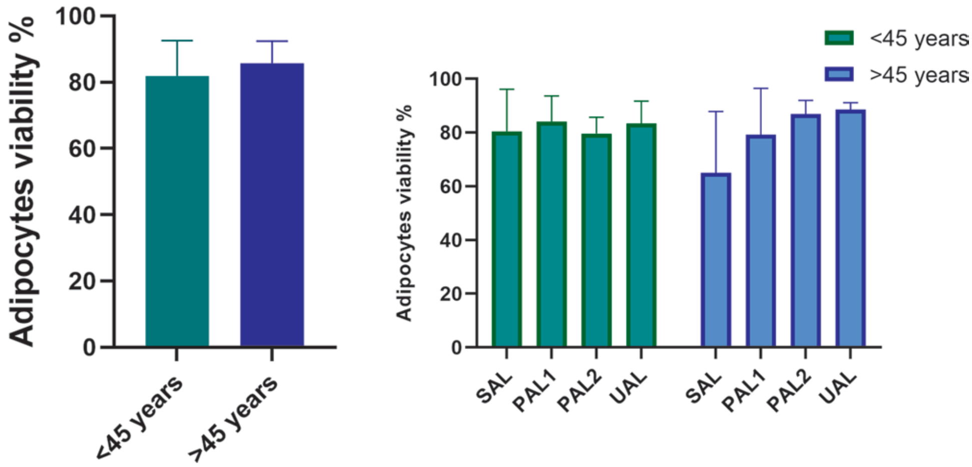

Fig. 5. Adipocyte viability based on patients' age. A, Viability of all adipocytes according to the patient's age. B, Viability of adipocytes according to patients' age and liposuction method.

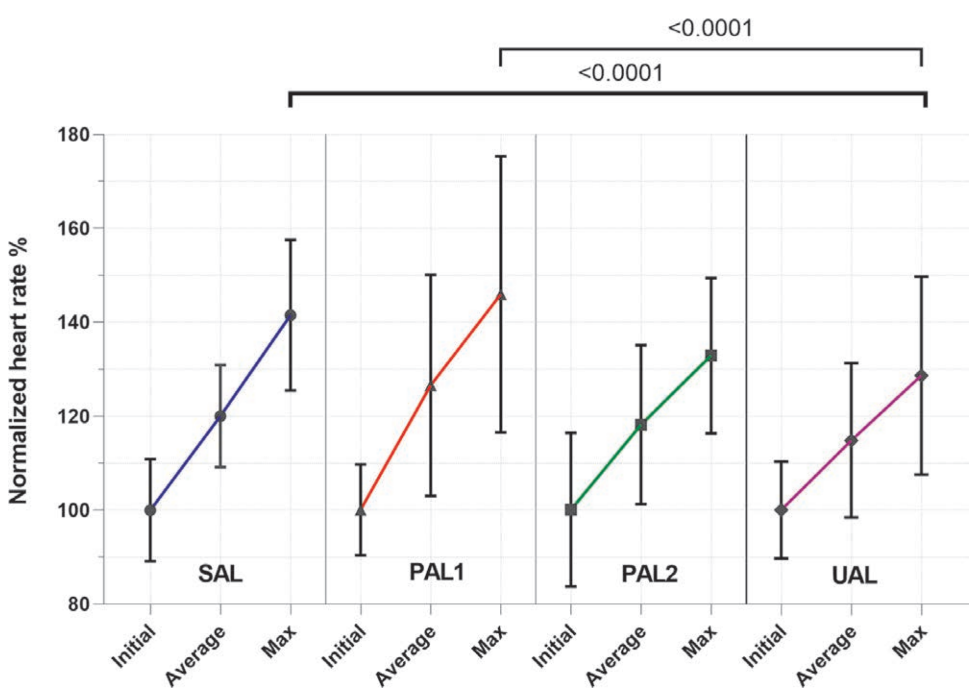

Fig. 6. Normalized initial, average, and maximum heart rates per minute reached by the surgeon while applying different liposuction methods. Data were presented as averages ± SD.

kilocalorie per minute ratio of 9.4 (Fig. 8). Table 2 summarizes the results of this study. We created a "methods scoring system" to evaluate and assist in choosing the most appropriate method for each case.

DISCUSSION

Adipose tissue is a complex tissue composed of adipocytes and the stromal vascular fraction, which includes preadipocytes, blood and endothelial cells, and macrophages. Although other studies mainly analyzed preadipocyte viability, our study model analyzed mature adipocyte viability derived from white adipose tissue. This knowledge may help determine which liposuction method would be the least harmful for the most sensitive adipocyte cells.

Mature adipocytes are fragile cells with short lifespans once harvested.[14] In addition to adipocytes and stem cells, preadipocytes are more resistant to handling and ischemia, and play an important role in long-term outcomes.[15] Moreover, adipose tissue, including mature adipocytes, has been identified as one of the best sources for reconstructive and plastic surgery. Adipose stem cells in the grafted fat play an important role in enhancing angiogenesis,

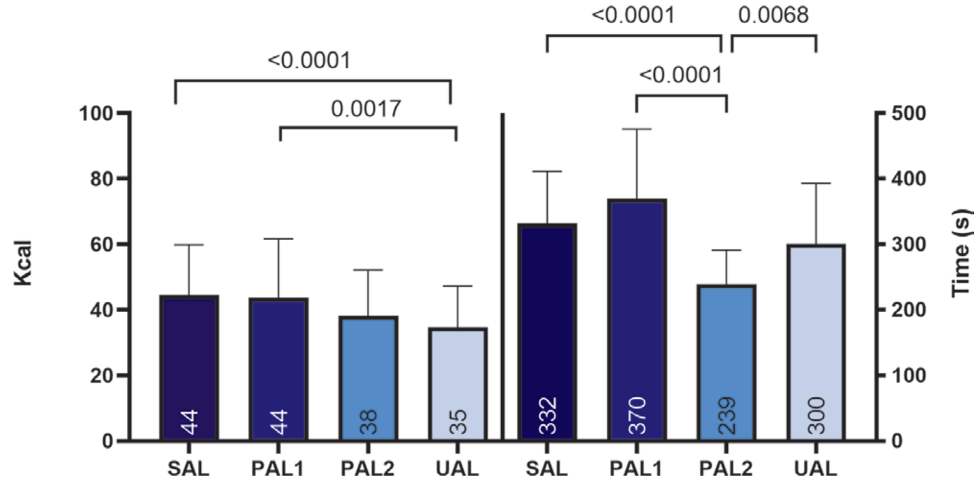

Fig. 7. Kilocalories (kcal) consumed by the surgeon and time (s) required to extract 150 mL of lipoaspirate while applying different liposuction methods. Data were presented as averages ± SD.

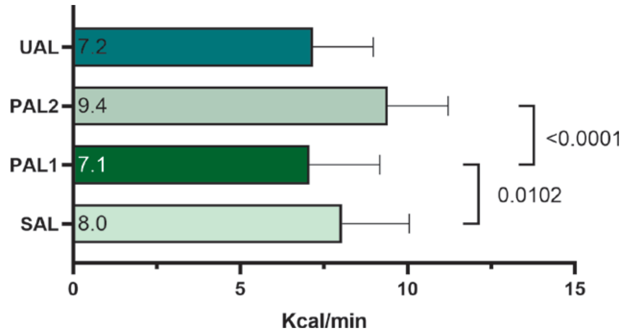

Fig. 8. Kilocalories (kcal) per minute consumed by the surgeon while working with different liposuction methods. Data were presented as averages ± SD.

minimizing the local inflammatory response, etc; however, mature adipocytes make up the bulk of the transplant.[16]

To accurately assess the damage to adipocytes caused by liposuction, the purification protocol and enzymatic digestion must be harmless. The centrifugation technique recommended by Dr. Coleman produces many viable adipocytes and has been the preferred method for lipoaspirate preparation over conventional liposuction.[17] Recent studies have revealed that centrifugation using the standard Coleman technique creates a graded density of fat, with fat in the lower lipoaspirate containing more stromal vascular fraction cells, more viable adipocytes, and higher concentrations of angiogenic/vasculogenic and anti-inflammatory adipokines than those in the upper lipoaspirate.[18] In addition, hand aspiration, low-g-force, and short-duration centrifugation maximize the viable adipocytes.[19]

In addition, the methods used for viability analysis should be appropriate. Accurate analysis of fat cells is challenging because mature adipocytes do not proliferate or adhere well to culture conditions; integrity and viability cannot be tested with standard proliferation tests.[20] Therefore, adipocyte viability is studied under microscope with trypan blue staining.[21] However, this method is limited because advanced skills and experience are required for accurate analysis. Furthermore, dead cells without nuclei or mitochondrial activity may be interpreted as live cells, as the shapes of the cells seem normal under the microscope. This technical bias may be one of the major factors contributing to the wide range of reported results.

Other adipocyte viability analysis methods can be categorized into two groups: membrane integrity assays and functional assays. The integrity assays include trypan blue, Food & Drug Administration/PI staining, and other similar assays.[21–23] The functional assays included MTS, XTT, MTT, BrdU, and Alamar blue. Currently, these techniques are frequently used to analyze adipocyte viability.[14,21,24,25]

In this study, Alamar blue assay was used to assess viability by evaluating mitochondrial function. The reagent is nontoxic; thus, the cells could be incubated up to 24 hours. In our study, adipocytes from the flap removal were placed in the media immediately (within a maximum of 2 hours from flap removal). Once removed from the vascular bed, adipocytes are deprived of blood and oxygen, thereby experiencing reduced viability. We could not find any information on the rate at which adipocytes died if

Table 2. Evaluation of Different Liposuction Methods Used for Different Tasks (Score 1: Worst; 2: Worse; 3: Well; 4: Best)

Method	Adipocytes Viability	Adipocytes Fraction	Debris	Calories	Heart Rate	Time for 150 ml	Kcal/min
SAL	1	1	1	1	1	2	2
PAL1	3	2	4	2	2	1	4
PAL2	2	4	2	3	3	4	1
UAL	4	3	3	4	4	3	3

left untreated before injection. Mecott et al hypothesized that apoptosis occurs in adipocytes and increases over time during decantation. They investigated the percentage of viable and apoptotic cells during the first 2 hours after liposuction, and discovered that apoptosis and mortality of adipocytes increased proportionally with decantation time.[5]

In our study, we also analyzed adipocyte viability while evaluating the amount of oil produced as an indicator of damaged adipocytes while applying different liposuction methods. We discovered that the worst method regarding adipocyte viability and damage was the SAL method; however, the other three methods were not significantly better. We did not find any studies comparing mature adipocyte viability among different liposuction methods. Keck et al revealed that PAL is a feasible technique for harvesting viable adipose-derived stem cells after comparing proliferation rates with those of the cells harvested by SAL. However, they did not analyze mature adipocytes.[26] In addition, they revealed that the fat layer was slightly larger when SAL was used, and it released slightly more oil than PAL. These findings confirm our results.

In contrast, we discovered a strong negative correlation between adipocyte viability and the oil fraction independent of the liposuction method. However, our analysis revealed no correlation between patients' age and adipocyte viability.

High-quality research on health-care professionals and their work-related ergonomics and efficacy is scarce. Plastic surgeons face many risks for work-related MSDs, given long work hours, repetitive movements, static or awkward postures, and cervical spine loading with headlamps and loupes. Epstein et al revealed vulnerability to work-related MSD, sometimes severe enough to end careers.[10,27–29] Frequent surgeon fatigue is a secondary issue that has not been described in detail.[30] If liposuction is performed without unnecessary force and discomfort by the surgeon, better results and safety can be expected.

ActiGraph technology is a tool that can help assess ergonomics; however, in most cases, patients undergoing different surgical procedures are below its scope.[31,32] In addition, no other specific algorithms or prior research related to actigraphy and liposuction were found.

Our research combines the study of surgeons' ergonomics and the viability of removed fat tissue to determine the best liposuction technique for autologous fat transplantation. Surgeons in the UAL group had the lowest average and maximum heart rates per minute, the lowest kilocaloric consumption, and the greatest adipocyte viability. We also discovered PAL2 and UAL superiority compared with SAL to extract 150 mL of lipoaspirate, which suggests a higher level of ergonomics.

A limitation of this study was that only in vitro adipocytes were analyzed; therefore we are not able to state that any difference found in adipocyte viability is clinically relevant, or if this difference would have a meaningful impact on the extent of fat graft take. In addition, other surgical and ergonomic aspects such as safety, swelling, bruising, skin contraction, or final outcome could not be evaluated and require further investigation (in vivo). To normalize different techniques and replicate everyday cases as much as possible, the SAFE[33] algorithm was adapted by performing a pre-cannulation (fat separation) in all groups. Therefore, the results of the UAL seemed relatively good. If the surgeon does not perform fat separation before liposuction, results may vary. In contrast, the intuitive assumption that the PAL makes the surgeon's work much easier is not necessarily correct because the handles of the PAL device are quite heavy, which places a great burden on the surgeon (handle + cannula weight: PAL1 686 g + 24 g; PAL2 500 g + 20 g; UAL and SAL 147 g + 66 g). Moreover, the surgeon's discomfort in using PAL because of the vibration of the instrument was not evaluated. It would be useful to examine ergonomic aspects under real conditions, where a patient is suctioned instead of a belly flap. This would also give more information about surgeons' back or neck pain, as liposuction duration would be longer. Ensuring the best fat grafting survival while keeping the procedure as safe as possible requires constant improvement in every stage of the procedure. Thus, the surgeon's ergonomics, liposeparation, liposuction, and transplant bed[33] must be optimized and constantly re-evaluated.

CONCLUSIONS

Protecting adipocyte viability and ensuring the ergonomics of the surgeon's work are crucial for improving fat grafting outcomes. Our study revealed that adipocyte viability is best preserved using the UAL and PAL1 liposuction methods, although they did not differ significantly from the others. The UAL method had the best results for surgeons' work efficiency; it differed significantly from SAL and PAL1 in terms of kilocaloric consumption and normalized maximal heart rate. In contrast, no significant difference was recorded between the PAL2 and UAL groups.

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DISCLOSURES

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