Comparison of Adipocyte Viability and Fat Graft Survival in an Animal Model Using a New Tissue Liquefaction Liposuction Device vs Standard Coleman Method for Harvesting

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Abstract

Background: The use of autologous fat for augmentation has become common practice among plastic surgeons for both cosmetic and reconstructive procedures. Previously reported data suggest that the method of fat extraction can have profound effects on adipocyte viability and subsequent fat graft survival.

Objective: The authors describe a pilot study comparing a new tissue liquefaction liposuction device (TLL; HydraSolve Lipoplasty System; Andrew Technologies, Irvine, California) with a standard syringe aspiration method with respect to adipocyte viability, fat graft survivability, and fat graft quality.

Methods: Lipoaspirate from 5 patients was harvested using either TLL or the standard method. Samples were centrifuged and assayed for cell viability. All lipoaspirate samples were grafted into nude rats and harvested 42 and 84 days later. Graft survival and quality were assessed.

Results: There was no difference in adipocyte viability between the lipoaspirate conditions. At 42 days, there was no significant difference in fat graft weight and the TLL grafts were more fibrotic than the standard control grafts, but this was improved with the increased centrifuge rate. At 84 days, fat grafts were equivalent with respect to graft weight and histology.

Conclusions: Lipoaspirate harvested with the TLL device and centrifuged at 3000 rpm resulted in fat grafts that were equivalent in weight and histology to those from lipoaspirate harvested with the standard syringe aspiration technique.

Keywords

fat grafting, liposuction, adipocyte, research, survival

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Autologous fat transplantation is widely performed in plastic surgery for soft tissue augmentation in cosmetic and reconstructive surgery. Fat for transplantation has the advantage of being abundant, relatively cheap, easy to harvest, and autogenic.[1] The greatest drawback at the current time is the unpredictable stability and longevity of the graft.[2-6] Current data range from impressive outcomes[1,7] to disappointing long-term results, with up to 80% reabsorption reported.[1,3,8] The wide range in reported rates of successful grafting suggests that the optimum method for harvest, manipulation, and graft placement, at least in the clinical setting, still awaits precise determination.

The cell survival theory suggests that the number of viable cells in the grafted material correlates with the amount of long-term survival of the graft.[9] This theory has been supported by evidence of adipose-derived stem cells in lipoaspirated fat.[10] Further, data have shown that a major factor in fat graft survival is the trauma placed on the adipose tissue during extraction.[11,12] Reports on trauma induced by liposuction vary from no cell damage to 90% adipocyte rupture.[1,13,14] Others have suggested that rates of centrifugation can have a dramatic effect.[15]

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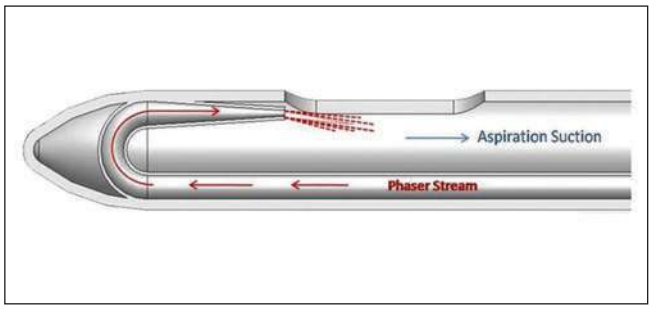

Figure 1. Tissue liquefaction liposuction (TLL) device. Schematic of TLL cannula mechanism during lipoaspiration. Reprinted with permission from Andrew Technologies, Irvine, California, manufacturer of the device tested in this study.

Our study objective was to compare harvesting with a new tissue liquefaction liposuction device (TLL; HydraSolve Lipoplasty System; Andrew Technologies, Irvine, California) with a standard syringe aspiration technique. Lipoaspirate viability and graft survival and quality were assessed.

METHODS

Lipoplasty Adipose Tissue Harvest and Preparation

Human consent was obtained by the surgeon or appropriate member of the study team using a consent form approved by the Institutional Review Board of the University of Texas Southwestern Medical Center (Dallas, Texas). Patients were recruited from those undergoing body contouring procedures by the senior investigator (J.K.). Each patient was infiltrated with a wetting solution consisting of 1 L of Ringer's lactate and 1 ampule of epinephrine (1:1000) so that a 1-mL infiltration-to-aspiration ratio was obtained. Approximately 20 mL of lipoaspirate was collected from each consented patient using the standard Coleman technique (as a control group) or the new TLL device. This new category of liposuction utilizes a stream of warmed, low-pressurized, pulsed saline that is emitted inside the distal end of the cannula and remains inside the cannula until aspirated. The stream causes a targeted phase transition of fat tissue from a solid to a liquid, while non–fat tissue is not liquefied. The fat liquefaction occurs via cell disaggregation: fat tissue is changed from a solid mass into a liquid, multicell suspension composed of clumps of adipose tissue in a saline medium. In TLL, there is no cutting of tissue; the cannula aperture edges are dull, and they are manufactured with a rounded radius of curvature and therefore cannot cut tissue (Figure 1).

The lipoaspirate harvested with the TLL device was divided into 3 samples and centrifuged at 0, 500, or 3000 rpm (TLL-0, TLL-500, and TLL-3000, respectively) for 1 minute. The control group's lipoaspirate was centrifuged at 3000 rpm for 1 minute. The adipose tissue was then transported from the operating room to the appropriate laboratory and distributed into 1.0-mL samples for both analysis and the grafting procedure (Figure 2). Each patient had 4 donor rats with 4 mL in each animal (1 mL/condition: TLL-0, TLL-500, TLL-3000, and control). The remaining tissue was analyzed for further histology and adipocyte viability (see details below; Figure 2).

Lipoaspirate Histology and Clump Size

Small samples (approximately 0.25 mL of the tissue) were fixed in 10% neutral buffered formalin and gently shaken for approximately 48 hours, allowing for the tissue to fix (Figure 2). The samples were then embedded with paraffin, sectioned, and stained with hematoxylin and eosin (H&E). Clump size was determined with pictures of the H&E stains, which were obtained using an Olympus IX51 epifluorescence microscope (Olympus, Center Valley, Pennsylvania) equipped with an Optronics Microfire Color CCD Camera (Optronics, Houston, Texas) and viewed under rhodamine fluorescence. Analysis was performed with National Institutes of Health (Bethesda, Maryland) ImageJ software by analyzing 8 images of clumps from each condition. The average number of adipocytes per clump was determined for each sample of adipose tissue.

Cell Viability

Samples from all 4 testing conditions were collagenase treated (0.5 mg/mL) for 20 minutes at 37°C (Figure 2). Digested samples were then filtered with a 200-µm filter to remove undigested tissue. To test the cell viability of the adipose cells, the Nexcelom Cellometer Auto T4 (Nexcelom, Lawrence, Massachusetts) was used. The sample was mixed 1:1 with 100 µg/mL propidium iodide (pI) intercalating agent and measured. The Cellometer calculates the number of cells with a greater than 30 µm diameter (total adipocytes) and then, of those cells, the number that are pI positive (total dead). Cell viability (%) is calculated by subtracting the dead adipocytes from the total adipocytes (live adipocytes) divided by total adipocytes.

Animals and Graft Implant

Twenty athymic rats (RNU; *Foxn1*^{rnu}) purchased from Charles River Laboratories (Wilmington, Massachusetts) were used for this study. Rats were housed in a temperature-controlled sterile environment at 64 to 79°F using a 12-hour light/12-hour dark cycle. Animals were housed in groups of 2 prior to surgery and singly housed postoperatively. The rats were fed standard chow (#2916; Harlan-Teklad, Houston, Texas), and water was provided ad libitum. At approximately 10 weeks of age, fat grafts were placed on the dorsum of the animal. Animals were anesthetized using a combination of isoflurane gas and oxygen, and fat grafting was performed with a 16-gauge needle through a single stab incision made caudally through the skin to allow passage of the fat graft cannula. The cannula was

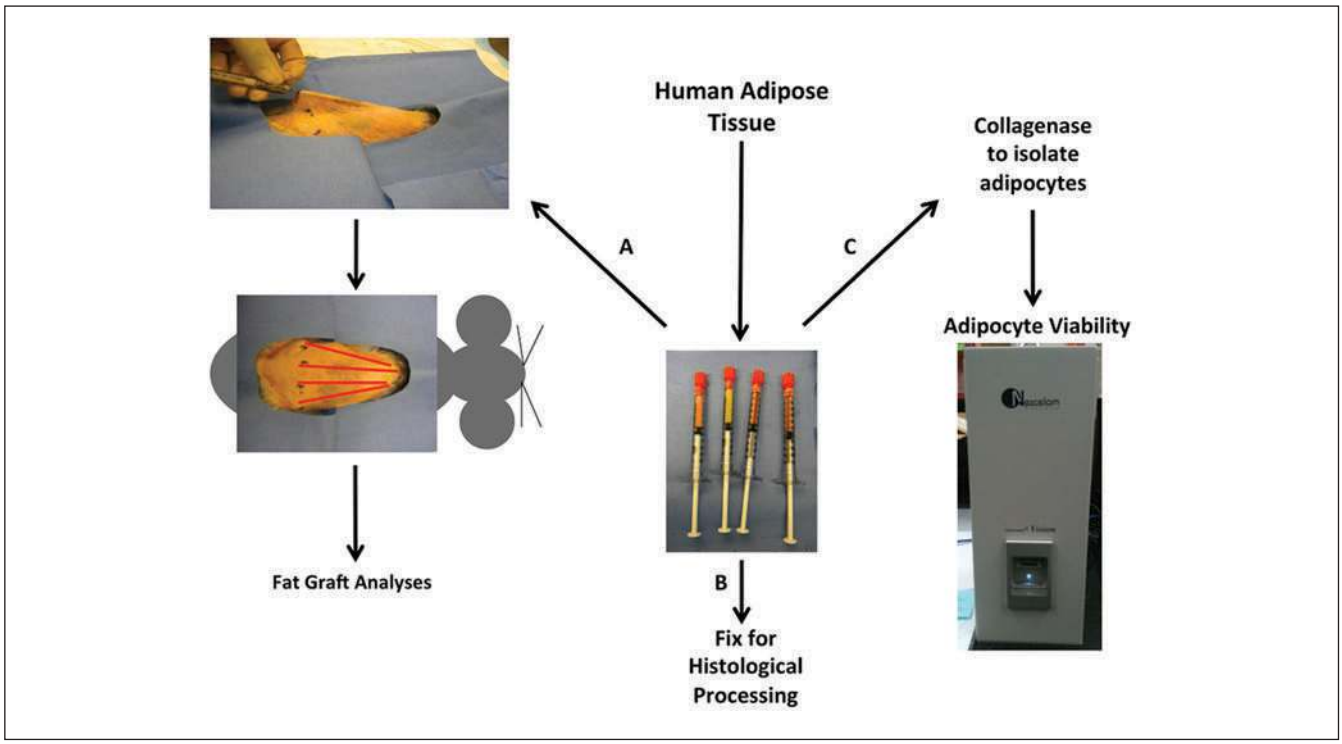

Figure 2. Experimental design schematic. Adipose tissues were harvested using either the control (CNTRL) or tissue liquefaction liposuction (TLL) protocols. Samples were centrifuged at 3000 rpm (CNTRL and TLL), 500 rpm (TLL), and 0 rpm (TLL). Patient lipoaspirate from each of the 4 conditions was grafted into 2 rats; 1 rat from each of 10 patients was sacrificed at day 42 and 1 at day 84, and grafts were harvested. Lipoaspirate from each of 4 conditions was fixed and processed for histological processing. Lipoaspirate from each of 4 conditions was collagenase treated, and adipocytes were assessed for viability using propidium iodide staining and analyzed using the Nexcelom Cellometer (Lawrence, Massachusetts).

inserted through the incision and tunneled under the epidermis/dermis of the skin to deposit the human fat subcutaneously in the formed tunnels. Each fat graft was placed at least 1 cm from other grafts, through individual stab incisions. A total volume of 0.5 mL of lipoaspirate for each experimental condition was deposited per row. No sutures were needed to close the skin due to the single stab incision. Rats were administered 0.01 mg/kg buprenorphine and given 4.4 mg/kg carprofen wafers after the procedure. Buprenorphine doses were also administered every 8 to 12 hours after the procedure for the following 48 hours. Tissues were harvested at either 42 or 84 days after grafting. The tissues were weighed and analyzed for graft survival. Care of all animals and procedures were approved by the University of Texas Southwestern Medical Center.

Graft Explant

Rats were deeply anesthetized, and a 320-mg/kg dose of phenobarbital barbiturate was injected intracardiac for sacrifice. An incision was made on the dorsum along the tail base left to right, and the dorsal skin layer was lifted to visualize the grafts. Gross analyses and general appearance observations were taken at this time and recorded in the study binder. The grafts were then removed individually by the surgeon, left to right of the animal, and the weights of the grafts were recorded.

Graft Histological Analyses

The grafts were cut into 2 sections (cranial and caudal) and each placed in 10% neutral buffered formalin and gently shaken for approximately 48 hours, allowing for the tissue to fix. The samples were then embedded with paraffin, sectioned, and stained with H&E. Scanned photographs from each slide were printed and evaluated by 3 blinded reviewers and rated 1 to 5 for fibrotic and adipose tissue content. The more adipose tissue content, the higher the rating. Figure 3 shows 5 example grafts, with their score based on the aforementioned scoring system and the percent makeup of adipose and fibrotic tissue in each graft.

Statistics

The data were calculated as mean ± SEM. After confirming normal distribution of data, comparisons between graft conditions were made by the paired 2-tailed Student t test. $P < .05$ was considered to be

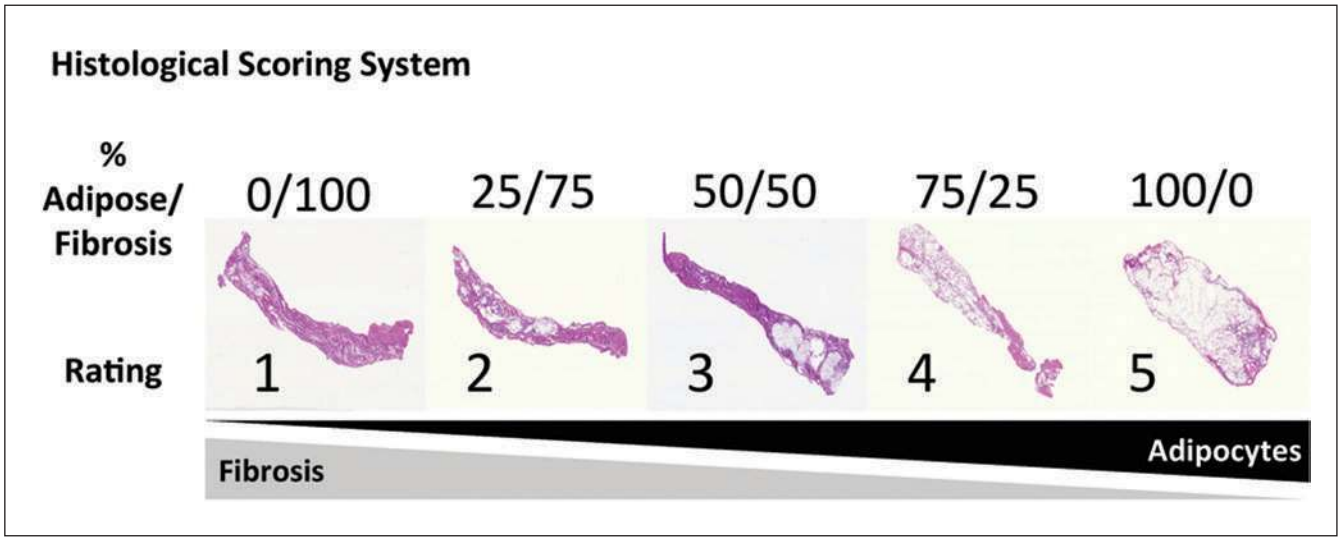

Figure 3. Histological scoring system scale. Fat grafts from each rat were stained for hematoxylin and eosin and assessed by 3 blinded reviewers. Reviewers were provided the scale demonstrating adipose/fibrosis levels and asked to assign each graft a rating.

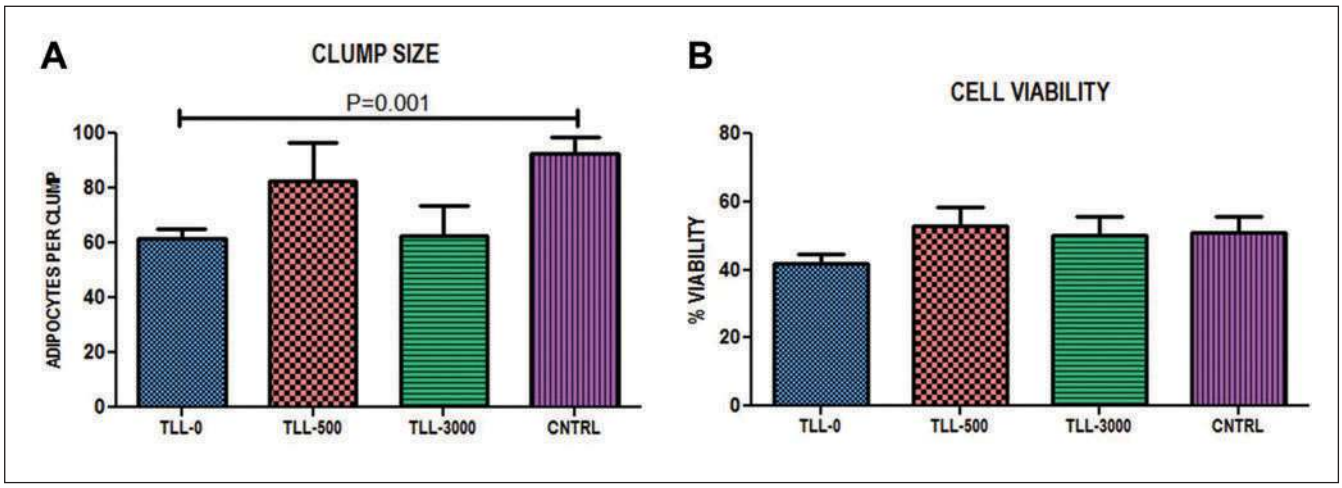

Figure 4. Lipoaspirate clump size and adipocyte viability comparing tissue liquefaction liposuction centrifuged at 0, 500, and 3000 rpm vs the standard control technique. (A) Analysis of the lipoaspirate clump size from each condition. (B) Percent cell viability as assessed using the Nexcelom Cellometer. Data are presented as mean ± SEM; if significant ($P < .05$), P value is noted.

statistically significant. P values were noted where significances occurred.

RESULTS

Effect of Different Liposuction Techniques on Lipoaspirate Viability and Consistency

Lipoaspirate from 5 patients taken using both the TLL and control methods was assessed for clump size using histological methods (Figure 4A). Data demonstrated that with centrifugation (TLL-500, TLL-3000), there was no difference in lipoaspirate clump size between the TLL and control groups. However, with no centrifugation (TLL-0), the clumps found in lipoaspirate harvested with TLL were significantly smaller than those found in the control samples. Samples of lipoaspirate with differential centrifugation speeds were compared with control (Figure 4B). There was no difference in adipocyte viability between any of the tested conditions.

Effect of Different Liposuction Techniques on Fat Graft Survival at 42 and 84 Days After Implantation

Equal weights of lipoaspirate from each of the 4 conditions were implanted on the dorsum of nude rats. At day 42

postimplantation, half of the animals were sacrificed and grafts were dissected and weighed. The grafts from both the uncentrifuged TLL and the TLL centrifuged at 500 rpm were smaller than the control grafts; however, the TLL-3000 and control grafts were equivalent (Figure 5A). Representative images of fat grafts are shown (Figure 5B-5E). To determine the quality of the implanted grafts, histological processing and H&E staining were performed. Three independent, blinded reviewers scored stained sections from each graft. Data demonstrated that the control technique produced grafts with a higher adipose tissue/fibrotic tissue ratio than the TLL technique at day 42 postimplantation (Figure 5F). Representative images of fat grafts are shown in Figure 5G-J.

At day 84 postimplantation, the second half of the animals were sacrificed and their grafts dissected and weighed. At day 84, the TLL-3000 and control samples were equivalent with respect to graft weight, but the TLL-500 and TLL-0 grafts were significantly smaller compared with the control grafts (Figure 6A). Representative images of fat grafts are shown in Figure 6B-E. Similar results were observed when measuring graft quality (Figure 6F). The TLL-0 and TLL-500 conditions produced grafts with significantly more fibrotic tissue than the control grafts. However, the grafts from the TLL-3000 and control lipoaspirate systems were equivalent. Representative images of fat grafts are shown (Figure 6G-J).

DISCUSSION

There are many methods of fat harvest, from traditional tumescent liposuction to ultrasound and water-assisted methods. It has been suggested that conventional liposuction may have a more detrimental effect on fat cells than syringe aspiration for fat graft harvest and that fat grafts harvested with conventional liposuction may have fewer viable fat cells.[13,16,17] More recently, ultrasound-assisted liposuction has been demonstrated to preserve adipose stem cell viability.[18] It has been suggested that water-assisted liposuction (WAL) may spare certain anatomical structures such as blood vessels and nerves and induce less trauma than traditional liposuction. Araco et al[19] performed a study of 60 patients randomly assigned to water-assisted or traditional liposuction. Postoperative pain was assessed by visual analog scale (VAS) score and dose of pain medication required.[1,19] Sasaki[7] demonstrated safety with respect to volume of instilled fluid, lidocaine dosing, and aspiration volumes. In that study, the author also suggested comparable results with fat grafting from WAL lipoaspirate, but further studies are required as this assessment was subjective. Stutz and Krahl[1] published a study using WAL to treat 30 patients with lipoedema. They demonstrated that damage to the lymph vessels can be avoided with WAL. Further, adipocytes remained largely intact with WAL. In our current study, we obtained results from a new TLL device, which provides a localized stream of warmed fluid that remains inside the cannula and may result in less volume being injected into the patient. Our study attempted to determine whether this new technology was a useful adjunct to current fat harvesting techniques.

Fat graft survival is a multifactorial process. Harvest technique and centrifugation rates have been demonstrated to alter fat graft survival.[20] Further, higher numbers of adipocyte-derived stem cells have been shown to enhance graft take.[21] In addition, the presence of cytokines and growth factors (whether endogenous or ectopically produced) also plays a role.[22] In this study, we chose to focus on differences in harvest technique and centrifugation rates. It is possible that different harvesting and centrifugation techniques had effects on other factors responsible for graft survivability. However, the aim of this study was to test techniques as they would be used clinically.

We performed a pilot experiment to test adipocyte viability, fat graft survival, and fat graft quality from lipoaspirate harvested with the TLL device as compared with standard control techniques. Further, we compared the outcomes of differential centrifugation speeds on the TLL lipoaspirate. Our results demonstrate that there is no impact on adipocyte viability based on technique, no matter the centrifugation rate (Figure 4B). There was a difference in clump size, but only between the TLL uncentrifuged lipoaspirate and the control technique (Figure 4A). The adipose tissue clump sizes did trend larger in the control lipoaspirate compared with those from the TLL also centrifuged at 3000 rpm, thus suggesting that there may be innate differences in the physical state of the lipoaspirate. To the best of our knowledge, there are no studies that analyze lipoaspirate clump size and fat graft survival. Analyses of these data suggest that larger clump size is correlated with increased graft take. We could hypothesize that there is a delicate balance between clumping of adipocytes, matrix formation, and vascularity of the graft. Tissue clumps would provide a scaffold on which to rebuild tissue. This rebuilding process would be much less efficient and much more time-consuming if cells had to rebuild the matrix. There is likely a limit to this correlation as adipose tissue clumps with too large a volume will result in necrotic tissue at the center due to poor vascularization.

When fat grafts from the 4 different lipoaspirate conditions were explanted 42 days after grafting, we found that those from the control technique were larger than those from the TLL device with no centrifugation or centrifugation at 500 rpm (Figure 5B-E). There was no statistical difference in graft size between the TLL and CNTRL conditions centrifuged at 3000 rpm, suggesting that with similar centrifugation conditions, results from these 2 techniques are similar. However, measures of graft quality suggest that grafts from lipoaspirate harvested with the control technique have more adipose tissue composition while those from the TLL device have more fibrotic tissue at 42 days (Figure 5F-J), the clinical implications of which are unknown. While slight differences appear at day 42 postimplantation, results suggest equivalency between the 3000-rpm centrifuged TLL and CNTRL techniques by day 84. It is apparent that using the TLL technique for lipoaspiration with no or low rpm centrifugation speeds results in smaller and more fibrotic grafts. As demonstrated

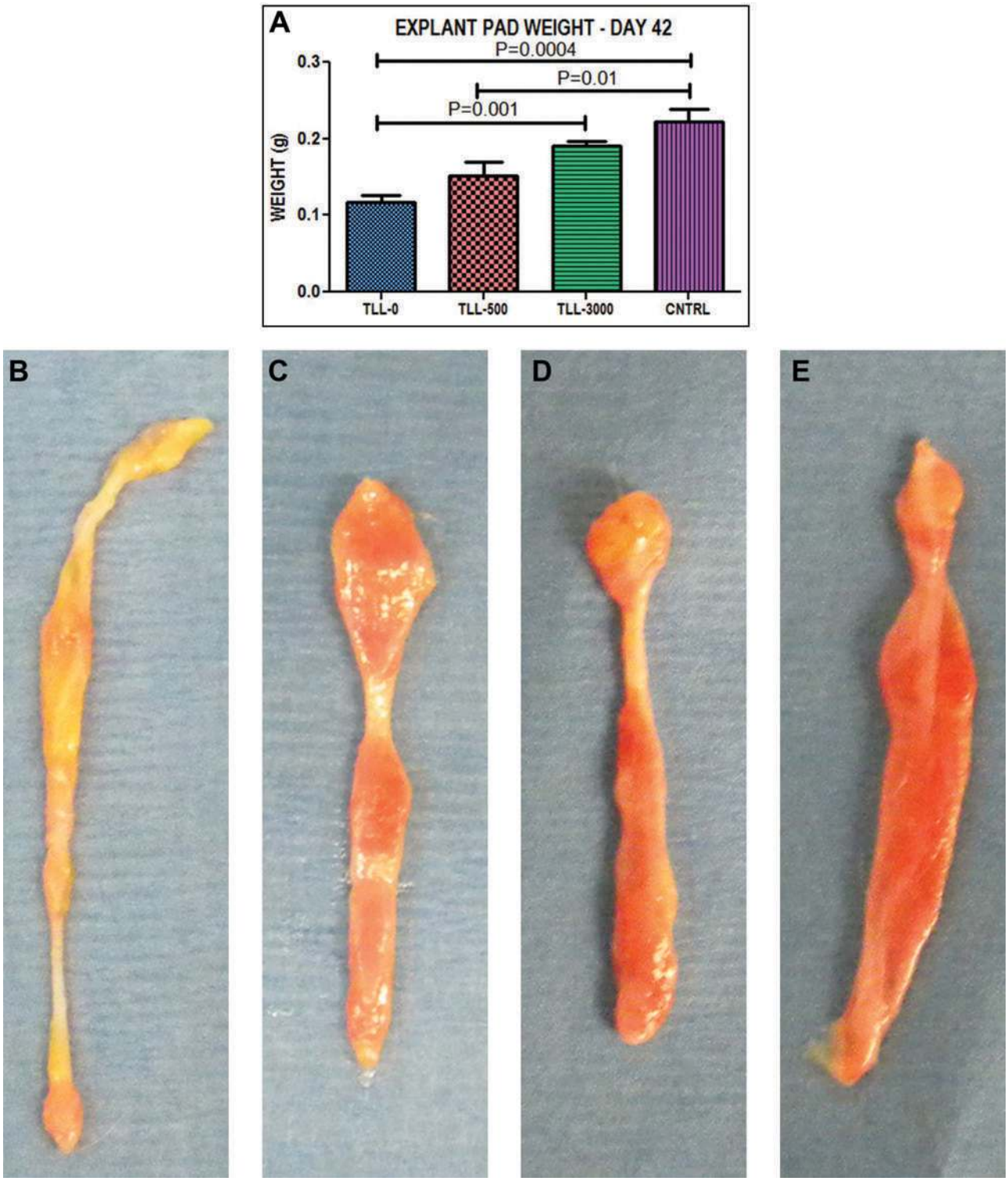

Figure 5. Explant pad weight and histological scoring at day 42 postimplantation from tissue liquefaction liposuction (TLL) and standard control harvested lipoaspirate. (A) Average graft weight (g) at explant and a representative image from each centrifuge condition: TLL-0 (B), TLL-500 (C), TLL-3000 (D), and control (E). (F) Average histological score for each condition and representative histological images of each condition: TLL-0 (G), TLL-500 (H), TLL-3000 (I), and control (J). Data are presented as mean ± SEM; if significant ($P < .05$), P value is noted.

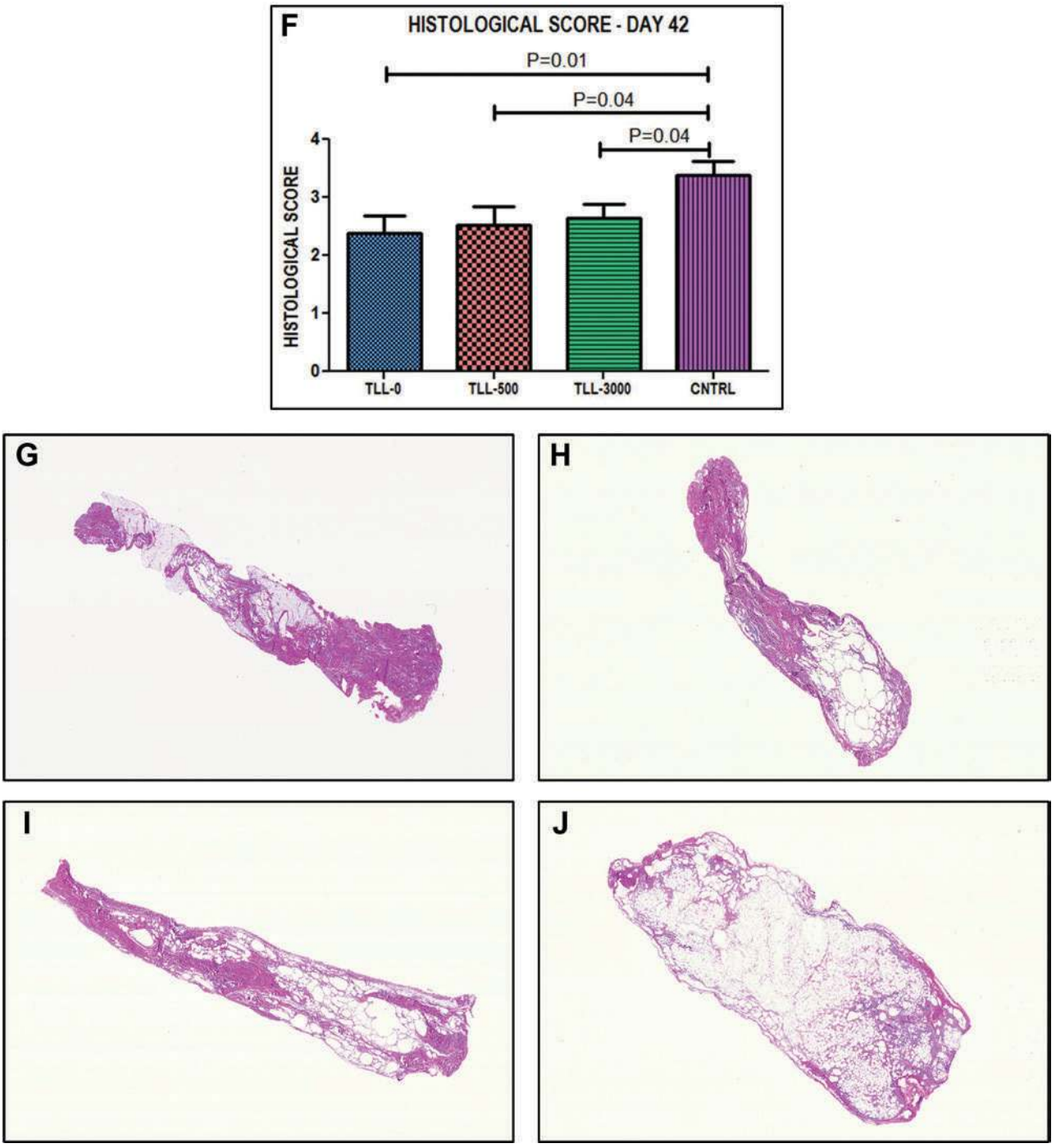

Figure 5. (continued) Explant pad weight and histological scoring at day 42 postimplantation from tissue liquefaction liposuction (TLL) and standard control harvested lipoaspirate. (A) Average graft weight (g) at explant and a representative image from each centrifuge condition: TLL-0 (B), TLL-500 (C), TLL-3000 (D), and control (E). (F) Average histological score for each condition and representative histological images of each condition: TLL-0 (G), TLL-500 (H), TLL-3000 (I), and control (J). Data are presented as mean ± SEM; if significant ($P < .05$), P value is noted.

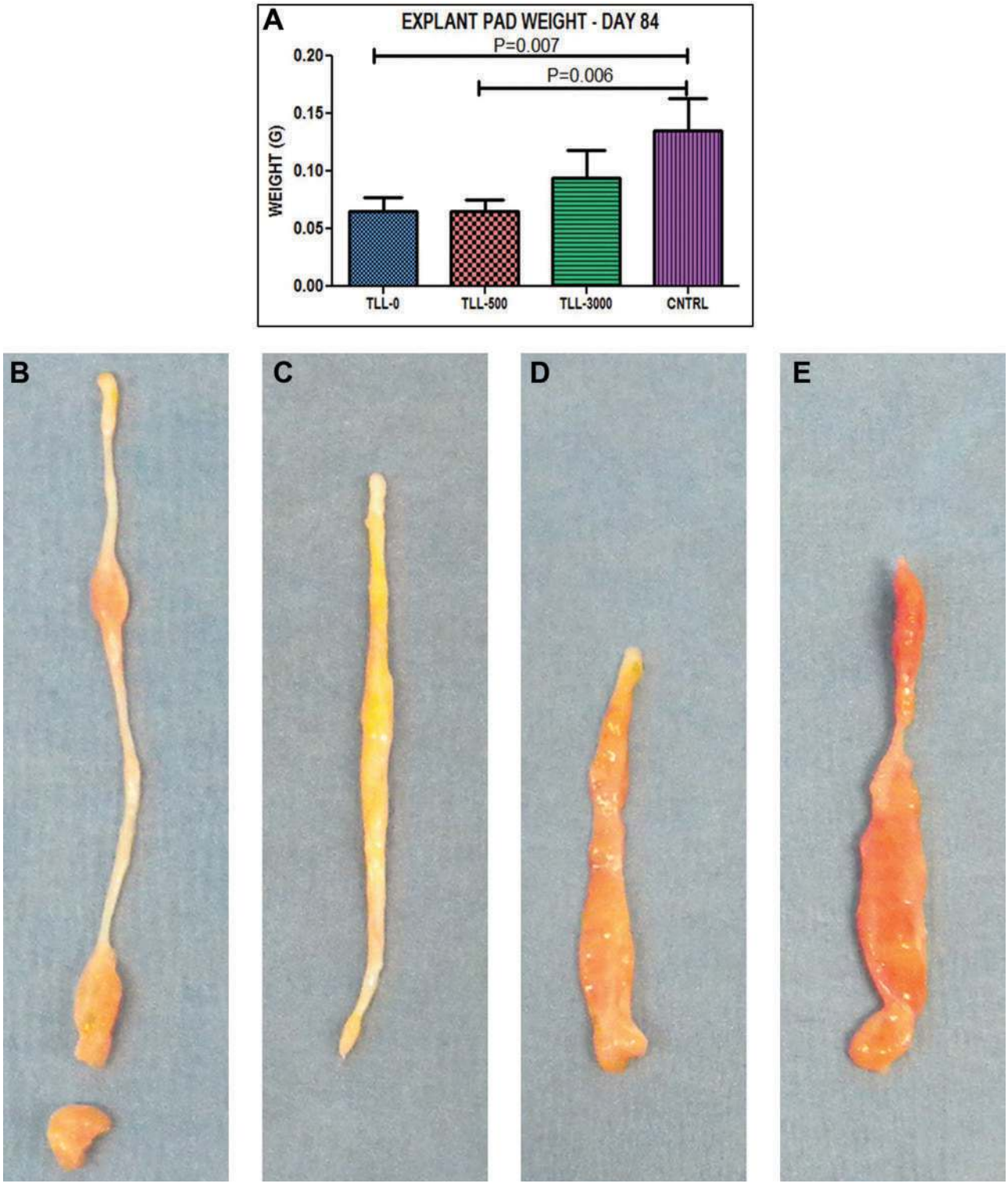

Figure 6. Explant pad weight and histological scoring at day 84 postimplantation of fat grafts from tissue liquefaction liposuction and standard control harvested lipoaspirate. (A) Average graft weight (g) at explant and a representative image from each centrifuge condition: TLL-0 (B), TLL-500 (C), TLL-3000 (D), and control (E). (F) Average histological score for each condition and representative histological images of each condition: TLL-0 (G), TLL-500 (H), TLL-3000 (I), and control (J). Data are presented as mean ± SEM; if significant ($P < .05$), P value is noted.

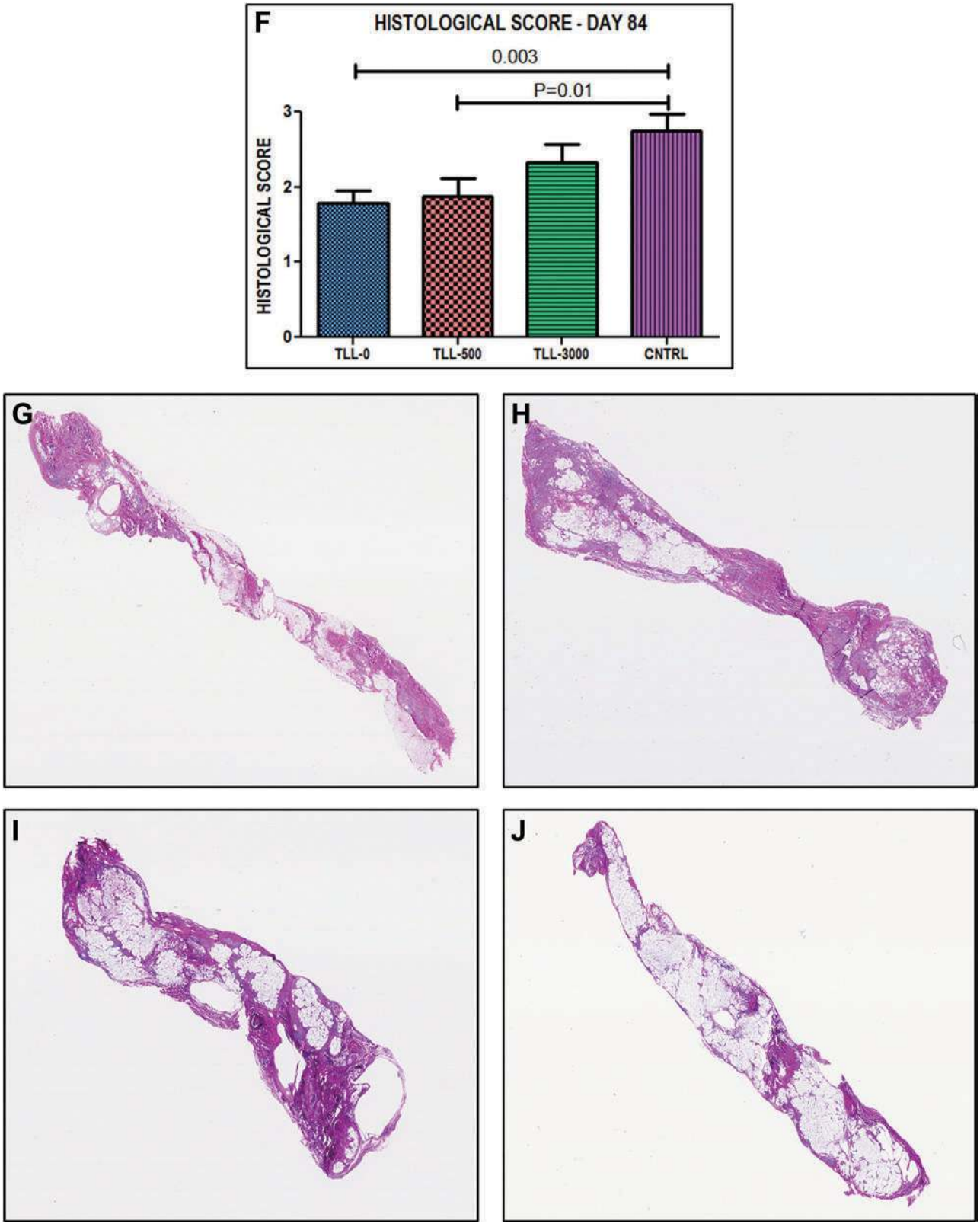

Figure 6. (continued) Explant pad weight and histological scoring at day 84 postimplantation of fat grafts from tissue liquefaction liposuction and standard control harvested lipoaspirate. (A) Average graft weight (g) at explant and a representative image from each centrifuge condition: TLL-0 (B), TLL-500 (C), TLL-3000 (D), and control (E). (F) Average histological score for each condition and representative histological images of each condition: TLL-0 (G), TLL-500 (H), TLL-3000 (I), and control (J). Data are presented as mean ± SEM; if significant ($P < .05$), P value is noted.

previously, the formation of oily cysts and fibrotic connective tissues are evidence of late-stage fat necrosis and result in the need for repeat grafting procedures.[3,8,9] Here we support previous studies that suggest that lipoaspirate centrifugation is critical for graft survival and quality.[20]

There are many techniques for fat harvest and transfer available to the plastic surgeon. Our study examined the ability of a new TLL device to harvest fat that is suitable for fat transfer. Our study confirmed that TLL fat centrifuged in a similar fashion as described by Coleman yields comparable viable volume and similar histology in a rat model. Further work is necessary to determine how this technology compares with other similar technologies, including power-assisted liposuction, water-assisted liposuction, and ultrasound-assisted liposuction, among others.

CONCLUSIONS

The data presented here demonstrate the equivalency between TLL and standard syringe lipoaspiration techniques. There are conflicting data as to the effects of water-assisted lipoaspiration compared with the standard tumescent technique.[1,7,19,23,24] These studies have a broad range of outcomes, including patient outcomes, measures of viable adipose tissue, and numbers of stem cells. We demonstrated here that for grafting purposes, the lipoaspirate from the TLL device and the control technique is equivalent and produces similar outcomes with respect to graft survival and quality in this animal model. These studies were limited with respect to determining why certain harvesting and centrifugation techniques resulted in different fat grafting outcomes. However, the data presented here demonstrate that the established protocols for the techniques tested produce mostly equivalent outcomes. Further studies will need to be performed to determine why the observed differences in graft survival and quality do exist.

Disclosures

Dr Kenkel is a paid investigator for Allergan, Erchonia, and Ultrashape and is on the Advisory Board of Kythera. The other authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

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