

# Autologous Fat Grafts Harvested and Refined by the Coleman Technique: A Comparative Study

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**Background:** The viability of fat grafts obtained by even a well-established technique remains poorly studied and unknown. This study was designed to determine the viability of fat grafts harvested and refined by the Coleman technique.

**Methods:** Sixteen adult white women were enrolled in this study. In group 1 ( $n = 8$ ), fat grafts were harvested and processed with the Coleman technique by a single surgeon from the abdomen of each patient according to his standardized method. In group 2 ( $n = 8$ ), fat grafts were harvested with the conventional liposuction by another surgeon. After centrifugation, the resulting middle layer of tissue was collected. All fat graft samples were analyzed for the following studies: trypan blue vital staining for viable adipocyte counts, glycerol-3-phosphatase dehydrogenase assay, and routine histologic examination.

**Results:** The higher viable adipocyte counts were found in group 1 compared with group 2 ( $4.11 \pm 1.11$  versus  $2.57 \pm 0.56 \times 10^6$  cells/ml;  $p < 0.004$ ). The level of glycerol-3-phosphatase dehydrogenase activity was significantly higher in group 1 compared with group 2 ( $0.66 \pm 0.09$  versus  $0.34 \pm 0.13$  U/ml;  $p < 0.0001$ ). Histologic examination showed normal structure of fragmented fatty tissues in both groups.

**Conclusions:** Although fat grafts obtained by both methods maintain normal histologic structure, the Coleman technique yields a greater number of viable adipocytes and sustains a more optimal level of cellular function within fat grafts and should be considered superior to conventional liposuction as a preferred method of choice for fat graft harvesting. (*Plast. Reconstr. Surg.* 122: 932, 2008.)

Autologous fat grafts have been used successfully for structural fat grafting in facial, lip, and hand rejuvenation and body contour improvement.<sup>1-5</sup> Most investigators believe that fat, as autologous tissue, can be considered the ideal soft-tissue filler because it is abundant, readily available, inexpensive, host compatible, and can be harvested easily and repeatedly. If fat grafts can truly survive after transplantation, structural fat grafting may present a safe, long-lasting, natural-appearing method for soft-tissue augmentation in

patients.<sup>6-10</sup> However, one of the main concerns after fat grafting can be the potential high rate of absorption over time in the grafted site, which may reach up to 70 percent of the filled volume.<sup>6,7</sup> The most acceptable explanation for absorption has been based on the Peer's cell survival theory, which states that the number of viable adipocytes at the time of transplantation may correlate with ultimate fat graft survival volume.<sup>11</sup>

To obtain long-term survival of transplanted autologous fatty tissue, the harvested and processed fat grafts must remain viable before im-

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plantation. In 1994, Coleman first described his technique, which uses a syringe, cannula, and centrifuge, for structural fat grafting.<sup>1</sup> He later refined and popularized his technique for fat graft harvesting and processing with the Coleman instruments and centrifuge and a centrifugation protocol, often referred to as the Coleman technique.<sup>2,3</sup> By using his established technique for fat graft harvesting and processing along with his refined placement technique, many surgeons are able to achieve good long-term results with structural fat grafting.<sup>1-5,12</sup>

Although several studies were performed in the past searching for improvement of fat transfer,<sup>13-15</sup> the viability of fat grafts harvested and processed by even a well-established technique remains poorly studied and unknown. The present study was therefore designed to evaluate the viability of fat grafts immediately after harvesting and refining by an experienced, reputable surgeon with the Coleman technique using a syringe, cannula, and centrifuge.

## MATERIALS AND METHODS

### Fat Graft Harvesting and Refining

Sixteen adult white women, aged 23 to 57 years, who had no major systemic metabolic diseases or lipid disorders were enrolled in this study, and the study was approved by the university's institutional review board. In group 1 ( $n = 8$ ), fat grafts were harvested with the Coleman technique by a single surgeon (S.R.C.) from the abdomen of each patient according to his well-described and standardized method.<sup>1,2,8</sup> Briefly, through a small incision, a mixed solution (0.5% lidocaine with 1:200,000 of epinephrine in lactated Ringer's solution) was infiltrated into the lower abdominal donor site using a blunt Lamis infiltrator (Byron Medical, Inc., Tucson, Ariz.). The solutions were infiltrated at a ratio of 1 cc of solution per cubic centimeter of fat grafts to be harvested. The fat grafts were harvested through the same incisions made previously. The harvesting cannula was 3 mm in diameter and 15 or 23 cm in length, with a blunt tip (Byron Medical). It was connected to a 10-cc Luer-Lok syringe. Gently pulling back on the plunger of a 10-cc syringe provided a light negative pressure while the cannula was advanced and retracted through the harvested site. After filling the syringe with harvested tissue, the cannula was removed from the syringe. A Luer-Lok plug was twisted onto the syringe to seal the Luer-Lok aperture, and the plunger was removed from the barrel of the syringe and the body of the filled syringe was placed into a centrifuge (Byron Medical) and spun at

3000 rpm for 3 minutes. After centrifugation, the oil layer (upper level) was decanted and the aqueous layer (lower level) was also drained out of the syringe. The middle layer, composed of predominantly fat grafts (Fig. 1), was studied subsequently.

In group 2 ( $n = 8$ ), fat grafts were harvested from the abdomen of each patient according to a standard liposuction method with conventional liposuction (suction-assisted lipectomy) by another experienced surgeon (L.L.Q.P.). Through a small incision, a mixed solution (0.5% lidocaine with 1:200,000 of epinephrine in lactated Ringer's solution) was infiltrated into the lower abdomen. A Byron aspiration cannula (3 or 4 mm in diameter) was connected to a liposuction machine (Byron Medical) and used to harvest adipose aspirates by the surgeon as if one were performing liposuction. The negative pressure of the machine during liposuction was set up at a pressure setting of no greater than 20 cmH<sub>2</sub>O. The adipose aspirates (approximately 100 cc) were collected in a bottle at the time of liposuction and transferred immediately to the laboratory. The specimens were then spun at 500 rpm for 10 minutes on a large-capacity centrifuge (Mistral 3000i; Curtin Matheson Scientific, Inc., Houston, Tex.) to separate adipose tissue from oil and soluble liquid. The middle layer of adipose aspirates after centrifugation, which contained more viable adipocytes,<sup>16</sup> was taken as fat grafts for the subsequent comparative studies (Fig. 2). All fat graft samples in this study were analyzed within 30 minutes after they were harvested and processed.



**Fig. 1.** Fat grafts were harvested and processed with the Coleman technique and spun at 3000 rpm for 3 minutes according to his protocol. After centrifugation, both upper and lower levels of components were removed and the remaining fat grafts within syringes were studied subsequently.



**Fig. 2.** Fat grafts were harvested with conventional liposuction and spun at 500 rpm for 10 minutes. After centrifugation, the resulting middle layer of adipose aspirates was then studied for comparison.

## Evaluation of Fat Grafts

### Viable Adipocyte Count

One gram of fat graft specimen from each patient was collected in both groups 1 and 2. Each specimen was washed three times with phosphate-buffered saline. It was then mixed with 1 mg/cc of type I collagenase (Sigma, St. Louis, Mo.) in phosphate-buffered saline containing 5% bovine serum albumin (Sigma) for digestion and incubated at 37°C in a carbon dioxide incubator. After 1 hour of incubation, the digestion was terminated with 10% (volume/volume) fetal calf serum (Sigma), and any remaining tissue fragments were removed by straining the digested fatty tissues through a piece of large-weave gauze. The digested fat grafts were fractionated into mature adipocytes (top layer) and stromal pellet (bottom) after a centrifugation at 200 *g* for 10 minutes. The viable adipocytes were determined after 0.4% trypan blue vital stain (Sigma) from a 100- $\mu$ l sample with 1:1 dilution with trypan blue. The number of viable adipocytes was then counted with a hemocytometer under a microscope with 400 $\times$  magnification.

### Glycerol-3-Phosphate Dehydrogenase Assay

Glycerol-3-phosphatase dehydrogenase assay was chosen in this study to assess cellular function of fat grafts because it is relatively simple but is adipocyte specific. According to the instructions from the manufacturer (Kamiya Biomedical Co., Seattle, Wash.), glycerol-3-phosphatase dehydrogenase activity within fat grafts was evaluated using a spectrophotometric assay. Briefly, 1 g of fat graft specimen was mixed with 4 ml of 0.25M cane sugar solution and homogenized. The mixture was then

spun at 700 *g* at 4°C for 10 minutes and the supernatant was taken to the special centrifuge tube, which was again spun but at 54,000 *g* for 60 minutes. The supernatant obtained after the second centrifugation was diluted approximately 20 to 100 times with an enzyme-extracting reagent. Final procedures of the assay were as follows: the substrate reagent (400  $\mu$ l) was dispensed into an assay well and heated to 25°C; the diluted supernatant was also heated to 25°C and 200  $\mu$ l of it was added to the well and mixed with the substrate reagent; the optical absorption at 340 nm was measured for 3 to 10 minutes and plotted on a graph; the change in optical density per minute from the linear position of the curve was obtained; and glycerol-3-phosphatase dehydrogenase activity was calculated based on the formula glycerol-3-phosphatase dehydrogenase activity (units per milliliter) = change in optical density at 340 nm/minute  $\times$  0.482) and the value expressed as units per milliliter.

### Histology

Each fat graft specimen (approximately 3 g) was fixed immediately in 10% buffered formalin, concentrated by gravity filtration through a porous paper, processed through graded alcohols and xylene, embedded in paraffin, sectioned at 5  $\mu$ m in thickness, and stained with hematoxylin and eosin. All histologic slides were examined by an experienced pathologist in a single-blinded fashion for architectural disruption, adipocyte degeneration, or necrosis.

### Statistical Analysis

All data in this study are expressed as mean  $\pm$  SD. A two-tailed unpaired *t* test was used to assess the difference between the two groups. A value of  $p < 0.05$  was considered statistically significant.

## RESULTS

In this study, the total number of viable adipocytes was  $4.11 \pm 1.11 \times 10^6$  cells/ml in group 1 and  $2.57 \pm 0.56 \times 10^6$  cells/ml in group 2. The higher viable adipocyte count was found in group 1 compared with group 2. The difference of viable adipocyte counts between the two groups was found to be statistically significant ( $p < 0.004$ ).

Glycerol-3-phosphatase dehydrogenase assay was used in this study to assess cellular function of fat grafts in each group. The higher the enzyme activity level, the better the cellular function of adipocytes within fat grafts. The glycerol-3-phosphatase dehydrogenase activity was  $0.66 \pm 0.09$  U/ml in group 1 and  $0.34 \pm 0.13$  U/ml in

group 2. The higher level of the enzyme activity was found in group 1 compared with group 2. The difference of glycerol-3-phosphatase dehydrogenase assay between the two groups was found to be significantly significant ( $p < 0.0001$ ).

There was no evidence of fatty tissue degeneration or necrosis in either group. Normal structure of fragment fatty tissues was found primarily, and the basic structure of fragmental fatty tissues appeared to be maintained in both groups. No distinguishable differences were seen histologically in group 1 compared with group 2 (Fig. 3).

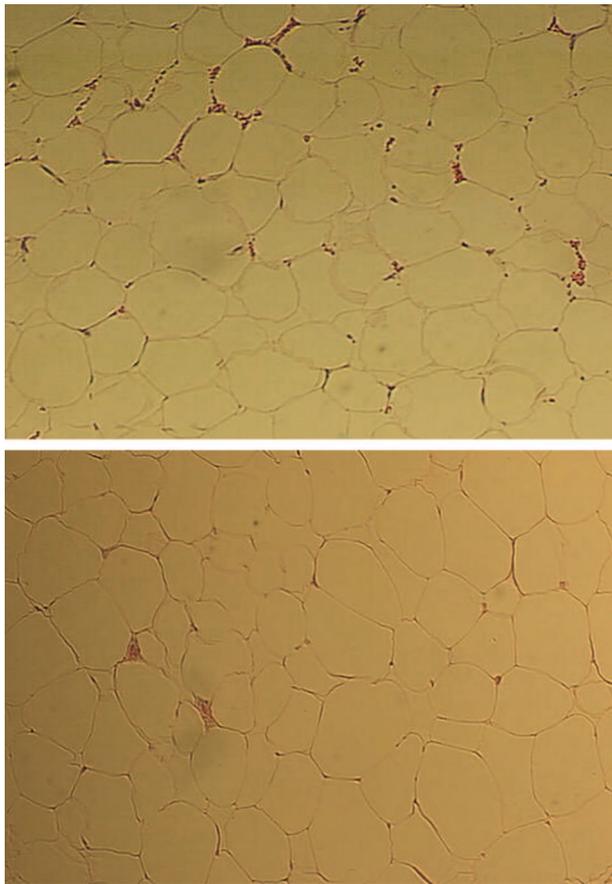
### DISCUSSION

The results from the present study demonstrated for the first time that there were more viable adipocytes and better cellular function of fat grafts harvested and processed by a well-established technique, the Coleman technique, than those of fat grafts harvested by conventional liposuction per-

formed by an experienced surgeon and then processed with centrifugation, a technique used by some surgeons with various modifications.<sup>4,12,16-18</sup> However, fat grafts harvested and processed with both techniques were able to maintain normal histologic structure as fragmental fatty tissues, with no distinguishable differences. The above findings indicate that the Coleman technique may be superior to conventional liposuction for harvesting and processing fat grafts because the Coleman fat grafts have a greater number of viable adipocytes and sustain more optimal cellular function than fat grafts harvested with conventional liposuction performed by an experienced surgeon. One of the explanations for our findings can be attributed to the less traumatic method of the Coleman technique when it is used to harvest fat grafts because the syringe aspiration can only generate very low but consistent negative pressure. Such a low pressure may minimize the force exerted on the adipocytes; therefore, the integrity of these cells within the fat grafts can be preserved.<sup>19</sup> In addition, higher negative pressure (20 cmH<sub>2</sub>O) generated by conventional liposuction may lead to suboptimal cellular function of adipocytes within fat grafts, even if their structures remain intact.<sup>20</sup>

Despite a growing interest in autologous fat grafting, there is no consensus and agreement on what is the best technique with which to obtain fat grafts. Based on a recent survey of the members of the American Society for Aesthetic Plastic Surgery, the Coleman technique was the most common method of autologous fat transfer (54 percent), followed by the standard liposuction method (25 percent), other syringe techniques (16 percent), and direct excision (5 percent).<sup>12</sup> However, no previous scientific studies have been conducted to compare the viability of fat grafts obtained by the Coleman technique and conventional liposuction, two of the most common techniques used by plastic surgeons to harvest fat grafts in the United States. The findings from the present study provide scientific evidence that the Coleman technique is a better technique with which to obtain fat grafts and should be the preferred method in autologous fat grafting.

Other methods have been reported in the literature and used clinically to obtain fat grafts. Har-Shai et al. advocated an integrated approach for increasing the survival of autologous fat grafts. In their study, fat grafts were harvested with conventional liposuction and processed by slow centrifugation (200 g). The fat grafts after centrifugation were suspended with cell culture medium. In 15 patients who were enrolled in the study, the amount of graft taken



**Fig. 3.** Histologic examination (hematoxylin and eosin stain; original magnification,  $\times 100$ ) showed primarily normal structure of fragmented fatty tissues, with no obvious differences between the Coleman fat grafts (*above*) and fat grafts harvested with conventional liposuction (*below*).

in the recipient sites ranged between 50 and 90 percent clinically at 6 to 24 months' follow-up.<sup>19</sup> Ramon et al. reported their preferred technique for obtaining fat grafts. In their study, fat grafts were harvested with conventional liposuction. The fat grafts were then placed on a sterile cotton towel, called the "towel" technique. Significantly less fibrosis was noted with this technique compared with the centrifugation technique.<sup>14</sup> Hu et al. reported their modified technique, including syringe technique for fat graft harvest, separation of adipose tissues with gravity, and additional purification with cotton sticks. They observed good volume maintained after fat injection in a total of 17 patients who were followed for 13 to 37 months.<sup>21</sup> However, no study was conducted comparing their preferred method and the Coleman technique.

The findings from the present study support a previously accepted hypothesis 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.<sup>22,23</sup> However, contrary to our findings, Smith et al., in their recent study, concluded that no significant difference in adipocyte viability was observed with regard to harvesting techniques using syringe aspiration and standard liposuction.<sup>15</sup> Their finding was supported by a previous study that found that conventional liposuction fat harvest might not result in increased fat cell damage compared with fat harvested by direct excision.<sup>24</sup> A recent study from our laboratory found that although fat grafts harvested by conventional liposuction were able to maintain normal structure with near the same number of viable fat cells compared with fresh fatty tissues harvested by direct excision, they had a less-than-optimal level of cellular function.<sup>20</sup> Therefore, controversy remains regarding whether higher suction pressure used in conventional liposuction can truly cause any significant degree of cellular damage within fat grafts. Further studies may be warranted to determine the effect of higher pressure used during harvest on the viability of fat cells within fat grafts.

Because the Coleman technique can be time-consuming and operator dependent for fat graft harvest if it is performed by less experienced surgeons, the method may be limited to obtain only a small amount of fat graft. The technique may not be the preferred technique by many surgeons for obtaining a large amount of fat graft for fat grafting to the breasts or buttocks because at least several hundreds cubic centimeters of fat graft is needed for these procedures.<sup>5,27</sup>

Some surgeons still routinely harvest fat grafts with liposuction for fat grafting.<sup>4,17,18</sup> However, those fat grafts may need to be optimized by further treatment with culture medium, insulin, or growth factors to improve their viability before or during implantation.<sup>26–29</sup> Nevertheless, the findings from the present study clearly support the use of the Coleman technique for obtaining fat grafts because the Coleman fat grafts have better viability of adipocytes within harvested fat grafts.

## CONCLUSIONS

Our results for the first time demonstrate that although fat grafts obtained by both methods maintain normal histologic structure, the Coleman technique yields a greater number of viable adipocytes and sustains a more optimal level of cellular function within harvested fat grafts than conventional liposuction performed by an experienced surgeon. Therefore, the Coleman technique should be considered as a standard and preferred method of choice for fat graft harvesting and processing. Future studies may be warranted to possibly develop a more speedy technique for obtaining fat grafts or to see whether fat grafts harvested by conventional liposuction can be optimized in vitro before being used as fat grafts for in vivo injection.

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