Fatty Acid Analysis of Transplanted Adipose Tissue

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Objective: To determine whether autologously transferred human adipose tissue maintains viability in vivo for prolonged periods.

Design: Six healthy female patients (mean age, 61.5 years; mean body mass index, 23.4 kg/m²) received autologous fat transplants from the gluteus to the nasolabial folds. Subcutaneous fat was sampled from facial and gluteal sites 4 times in 1 year.

Setting: Private practice, basic science research center.

Intervention: After local anesthesia, 10 g of subcutaneous adipose tissue was harvested from the right buttock of each patient. Ten milligrams of adipose tissue was aspirated from the right nasolabial fold. Five grams of gluteal fat was then injected into each nasolabial fold using a uniform monolayer threading technique with no overcorrection. As controls, 10 mg of adipose tissue was obtained from the opposite left buttock and left cheek. Adipose tissue from the transplanted and control facial and gluteal sites was sampled at 4, 6, and 12 months after transplantation.

Main Outcome Measurements: Gluteal fat has more monounsaturated fatty acids and less saturated fatty acids than facial fat. This unique site-specific fatty acid pattern was used to assess the course of the survival of transplanted adipose tissue in the nasolabial region. In all fat samples, the percent area (weight percentage) was obtained for each fatty acid (C₁₂:₀ to C₂₂:₆ w-₃) using capillary gas chromatography. Clinical results were also analyzed by macrophotographs.

Results: As expected, gluteal fat had significantly more monounsaturated fatty acids and less saturated fatty acids than facial fat. In 5 of 6 patients, at 4, 6, and 12 months after transplantation, the fatty acid pattern at the transplanted recipient site was similar to the pattern of the control facial site. However, at 4 months, 1 patient had a fatty acid pattern in the transplant recipient site that was similar to the pattern of her gluteal fat. This pattern persisted for 1 year. Fat retention at the transplant site was corroborated by photographic assessment.

Conclusions: Long-term adipocyte survival is an achievable goal following fat transfer. The importance of harvesting and injection techniques as well as adipose tissue characteristics require further study.

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T RANSPLANTED, autologous adipose tissue is an increasingly popular material for soft tissue augmentation in aesthetic and reconstructive surgery. Advances in the techniques of liposuction surgery, the relative ease of adipose tissue harvesting, and the avoidance of the antigenic and inflammatory responses from allogenic or alloplastic materials are factors that have increased interest in adipose tissue as a successful filler. Although most commonly used for correcting facial rhytides, fat transplants have also been used for augmentation of congenital defects; scarring diseases such as linear morphea, lupus erythematosus, atrophoderma of Pasini and Pierini, and acne; postradiation scars; and augmentation of aging hands and breasts. Despite the expanding use of transplanted adipose tissue, the literature is devoid of objective studies that document the persistence of the grafted material and the time course of resorption. A review of the literature in human fat transfer with multiple techniques for both harvesting and transplantation suggests a wide range of graft survival rates of 0% to 80%. In animal models, prolonged survival of fat transplants has been demonstrated with and without the addition of nutrients such as vitamin E and fetal bovine serum. Past studies of assessing adipocyte viability have been based on histologic studies or evaluation by photography and other imaging techniques.

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The fatty acid composition of adipose tissue primarily depends on the type of dietary fat consumed during the previous 2 to 3 years. However, there are small site-specific differences in fatty acid composition, which are useful as a “fingerprint” to mark the persistence of transplanted fat. Subcutaneous fat from the face,
PATIENTS AND METHODS

Six female patients with a mean age of 61.5 years (range, 51-67 years) were entered into the study after giving informed consent. All patients were in excellent health and were taking no medications. The mean body mass index (a measure of weight in kilograms divided by the square of height in meters) was 23.4 (range, 17.5-30.0), with no recent weight loss or gain (Table 1). The patients had not been treated previously with filler agents. No patient had previous facial surgery or inflammatory disease of the face such as acne. The results of screening blood tests, including complete blood cell count, platelet count, prothrombin time, partial thromboplastin time, and glucose, cholesterol, and triglyceride levels, were normal. This study was performed under institutional review board peer review (Rockefeller University, New York, NY).

TRANSPLANTATION TECHNIQUE

The details of the procedure using a closed syringe tumescent technique are as follows. The face and the gluteal regions were cleansed with povidone-iodine (Betadine), then draped in sterile fashion. After local injection of 5 mL of 1% lidocaine to anesthetize the right gluteal area, a 14-gauge Klein liposuction cannula (HK Medical, San Juan Capistrano, Calif) attached to a 30-mL syringe was used to aspirate 10 g of subcutaneous adipose tissue from the right buttck (midline from the iliac crest, midinfragluteal fold). The tissue was allowed to separate and a portion was saved for fatty acid analysis. The supernatant was discarded. The fat was immediately transferred into 3-mL syringes at room temperature.

A total of 3 mL of 1% lidocaine was used to anesthetize the facial donor areas (left and right nasolabial folds). A 10-mg aliquot of adipose tissue from the numbed right nasolabial fold was aspirated through an 18-gauge needle attached to a 10-mL syringe. The midline of the infranasal crease and angle of the mouth were used as reference for future biopsies. Local pressure was applied to stop the small amount of bleeding.

A 16-gauge needle and syringe (Becton Dickinson, Franklin Lakes, NJ) was used to subcutaneously inject 5 g of gluteal fat into each nasolabial fold using a uniform monolayered threading technique with no overcorrection. A 10-mg aliquot of adipose tissue was also obtained as controls from the opposite (left) buttck and left cheek (3 cm from the transplant site) after the local injection of 3 mL of 1% lidocaine.

After rinsing with saline, the fat samples were stored at −20°C until analyzed.

FATTY ACID ANALYSIS OF ADIPOSE TISSUE

Serial fat aspirates were obtained from facial (treated and control) and gluteal (donor and control) sites at approximately 4, 6, and 12 months after transplantation. The donor transplant site was the right buttck and the control site was the left buttck. The transplant recipient site was the right nasolabial fold and the recipient control site was the left cheek. The adipose tissue fragments were thawed and extracted with Folch method (chloroform-methanol, 2:1), and the lipid (99% triglyceride) was transmethylated with 9% methanolic hydrochloride. A gas chromatograph (model 5890; Hewlett-Packard Co, Palo Alto, Calif) equipped with a flame ionization detector and a 100-mm×0.25-mm (0.2-µm coating) SP2360 fused silica column (Supelco Inc, Bellefonte, Pa) was temperature programmed as described previously, with slight modifications. Forty-three peaks were identified from C12:0 to C22:6. The percent area (weight percentage) was obtained for each fatty acid. The intrasample coefficient of variation was less than 1% for fatty acids contributing more than 5% of the total area. Quantitative fatty acid methyl standards (Nu Chek Prep, Inc, Minneapolis, Minn) were run monthly and indicated that no area response correction factors were required.

PHOTOGRAPHIC EVALUATION

Clinical results were evaluated by 2 blinded observers using macrophotography. Photographic documentation was performed with the Yashica Medical Eye II (Tokyo, Japan) equipped with a macrolens, a shooting area of 24 × 360 mm, and shooting distance of 15.3 cm, using identical lighting and patient positioning with respect to anatomic landmarks.

STATISTICAL METHODS

Results are expressed as mean weight percentage ± 1 SD for each fatty acid. The differences between cheek and gluteal sites at the time of transplantation were compared with a 1-tailed paired t test (Excel 97 software; Microsoft Corp, Redmond, Wash). P <.05 was considered statistically significant.

RESULTS

The fatty acid compositions of 16 fat samples obtained from 4 sites at 0, 4 to 5, 7 to 10, and 10 to 17 months were analyzed from each of the 6 patients. As expected, gluteal fat had more monounsaturated and less saturated fatty acids than facial fat (Table 2). In 5 of 6 patients at the first 4-month biopsy, the transplant recipient and control facial sites had similar fatty acid compositions; this was true at subsequent biopsies. How-
ever, in 1 patient (patient 2) at 4 months, the transplant recipient pattern was similar to the pattern in gluteal fat rather than facial fat (Table 3, Figure 1, and Figure 2). This persisted through the last biopsy at 1 year. There was no unique clinical characteristic of this patient, except that she had the lowest body weight and body mass index.

In all patients, the fatty acid compositions of the control sites (left gluteal and left cheek) remained stable over time. There were no significant illnesses or change in body weight during the study. One patient (patient 3) had a facial bruise treated with oral antibiotics 1 week after the 4-month biopsy. The patient with biochemical evidence of fat retention had the best suggestion of retention by photographic assessment at all time points (Figure 3).

The literature is laden with subjective reports of long-term augmentation following fat implantation. However, few reports document the scientific basis of this assumption. This is the first report, to our knowledge, to use the site-specific fatty acid pattern of adipose tissue to document the persistence of transplanted fat. Although evident in only 1 of 6 patients, this study supports the long-term potential of this natural autologous filler.

There are 2 theories to explain clinical success after fat transplantation. The host replacement theory claims that the fat graft tissue survives after the host reaction subsides. The surgically transplanted fat becomes ischemic following transfer into the recipient site. Some cells die, some survive as adipocytes, and others differentiate from preadipocyte cells. After recovery from the transfer process, the preadipocyte cells become functional adipocytes and accumulate fat once the fat graft regains its blood supply from the periphery.

Published histologic studies from humans have demonstrated both healthy and fibrous fatty tissue after fat transplantation. The relative importance of fibrosis vs fat engraftment for the long-term clinical filler effect of autologous transplanted fat remains unknown. An in vitro study evaluating the long-term viability of human adipocytes and preadipocytes harvested by a closed syringe tumescent liposuction technique such as that used in this study showed that a significant percentage of cells can survive the harvest and thrive in cell culture. A more recent report documented that human adipocytes maintained their viability in culture for up to 2 months after closed syringe liposuction harvest.

Other studies have attempted to document the persistence of transplanted fat with imaging techniques. Horl et al examined 10 patients for 1 year with magnetic resonance imaging. They noticed a 55% loss at 6 months, with regular negligible decreases in volume between 9 and 12 months as documented by the quantitative imaging technique. The transplantation techniques and donor and recipient sites were similar to those in our study.

Goldman et al used ultrasound to document the retention up to 1 year of transplanted fat in patients with facial depression syndromes (ie, Romberg syndrome and lupus). Long-term correction of lipodystrophy and coup

Comment

Table 1. Fat Transfer Study

<table>
<thead>
<tr>
<th>Patient No./Age, y</th>
<th>Date of Fat Transfer</th>
<th>4-mo Follow-up</th>
<th>6-mo Follow-up</th>
<th>1-y Follow-up</th>
<th>Complications</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>BMI, kg/m²</th>
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<td>1/57 3/69/96</td>
<td>7/27/96</td>
<td>10/24/96</td>
<td>3/8/97</td>
<td>None</td>
<td>165.24</td>
<td>81</td>
<td>30.0</td>
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<tr>
<td>3/51 4/24/96</td>
<td>8/29/96</td>
<td>10/10/96</td>
<td>4/17/97</td>
<td>Bruising</td>
<td>155.08</td>
<td>60.75</td>
<td>24.6</td>
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<tr>
<td>4/62 11/20/96</td>
<td>2/10/97</td>
<td>5/14/97</td>
<td>11/30/97</td>
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<td>166.51</td>
<td>50.85</td>
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<tr>
<td>5/66 1/8/97</td>
<td>4/30/97</td>
<td>7/12/97</td>
<td>1/14/98</td>
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<td>157.62</td>
<td>66.60</td>
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<tr>
<td>6/66 6/23/97</td>
<td>11/24/97</td>
<td>4/13/98</td>
<td>2/0/99</td>
<td>None</td>
<td>162.70</td>
<td>63.90</td>
<td>23.7</td>
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</table>

* All patients were in excellent health and taking no medications.

Table 2. Fatty Acid Compositions of Cheek and Gluteal Fat

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>Donor Transplant Site</th>
<th>Transplant Recipient Site</th>
<th>Recipient Control Site</th>
<th>Transplant Recipient Site</th>
<th>Recipient Control Site</th>
<th>Transplant Recipient Site</th>
<th>Recipient Control Site</th>
<th>Transplant Recipient Site</th>
<th>Recipient Control Site</th>
<th>Transplant Recipient Site</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:0†</td>
<td>1.9 (0.3)</td>
<td>2.2 (0.4)</td>
<td>2.3</td>
<td>1.6</td>
<td>2.1</td>
<td>1.3</td>
<td>1.4</td>
<td>2.0</td>
<td>1.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18:0†</td>
<td>16.4 (3.4)</td>
<td>19.8 (2.0)</td>
<td>20.7</td>
<td>20.2</td>
<td>20.8</td>
<td>19.3</td>
<td>19.2</td>
<td>19.2</td>
<td>19.2</td>
<td></td>
<td></td>
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<tr>
<td>C18:1ω-9†</td>
<td>5.5 (1.5)</td>
<td>4.1 (2.0)</td>
<td>3.6</td>
<td>3.6</td>
<td>3.6</td>
<td>4.3</td>
<td>3.3</td>
<td>3.5</td>
<td>3.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18:2ω-6†</td>
<td>3.2 (0.9)</td>
<td>4.6 (1.5)</td>
<td>5.5</td>
<td>5.7</td>
<td>5.9</td>
<td>7.5</td>
<td>6.4</td>
<td>4.9</td>
<td>5.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18:3ω-6</td>
<td>40.9 (3.1)</td>
<td>39.5 (2.7)</td>
<td>39.8</td>
<td>41.1</td>
<td>40.1</td>
<td>40.5</td>
<td>40.3</td>
<td>37.9</td>
<td>38.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14:0</td>
<td>17.5 (1.5)</td>
<td>16.9 (3.0)</td>
<td>15.2</td>
<td>16.9</td>
<td>18.0</td>
<td>18.2</td>
<td>19.0</td>
<td>18.6</td>
<td>18.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total saturated</td>
<td>21.6 (3.1)</td>
<td>26.6 (2.7)</td>
<td>28.5</td>
<td>27.6</td>
<td>28.9</td>
<td>28.2</td>
<td>27.1</td>
<td>26.2</td>
<td>26.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total monounsaturated</td>
<td>46.4 (4.4)</td>
<td>43.6 (4.7)</td>
<td>43.4</td>
<td>44.7</td>
<td>43.7</td>
<td>44.9</td>
<td>43.7</td>
<td>41.4</td>
<td>41.6</td>
<td></td>
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</tr>
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</table>

* Values are given as mean (SD) weight percentage for each fatty acid for patients 1 and 3 through 6 (n = 5).
†P < .05 for donor transplant vs transplant recipient control. Cheek and gluteal fatty acid compositions did not change over time.
de sabre deformities have also been reported by Glogau18 by an ultrasound imaging technique.

Harvesting and injection methods may influence the longevity of correction associated with fat transfer. Important variables may include syringe and cannula size, anesthesia, degree of overcorrection, frequency of treatments, donor sites, depth and technique of placement, sample washing, and sterility. McCurdy42 analyzed fat cell survival clinically and concluded that the following technical characteristics prolonged graft survival: (1) low vascularity of the donor site, (2) high vascularity of the recipient site, (3) low-pressure technique of aspiration of fat, (4) filtered and washed harvested adipocytes, (5) use of 2-mm or larger cannulae for injection to minimize adipocyte injury, (6) multilayered deposition of fat, and (7) overcorrection of recipient site. However, using cell culture techniques, Schiffman43 found that the size of the cannulae used to remove fat did not significantly affect fat cell integrity. Brandon and Newman44 also found that the type of anesthesia, needle shape, and suctioning technique did not influence fat survival. Takasu and Takasu45,46 found that freezing of fat for variable periods did not improve survival.

Brandon and Newman44 state that body fatness and physical fitness may influence the survival rate of transplanted fat. They theorized that smaller fat cells with higher quantities of collagen surrounding the globules decrease the trauma during fat transfer and thus improve the quality of the graft once it reaches the recipient site.

Clearly, more studies are required to explain the lack of retention of fat in the other patients in this study. In these patients, it should be noted that it is possible that the transplanted fat was retained in the face but converted to the fatty acid pattern of the surrounding cheek fat. If true, the underlying reason for site-specific differences in adipose tissue fatty acid composition may be due to effects from locally secreting substances rather than innate cell differences.

CONCLUSIONS

One of 6 patients presented in this study had persistence of fat after autologous fat transfer for 1 year. This was documented by the persistence of the unique gluteal fatty acid pattern in the facial region. Other than a thin constitution, this patient had no distinguishing factors from other patients exhibiting lower fat survival. The reason(s) for the long-term success of fat transplantation in this patient cannot be determined from this study but warrants future investigation. Further studies elucidating the role of harvesting and implantation variables and adipose tissue.

![Figure 1](http://www.archdermatol.com/) Total saturated fatty acid (C14:0, C16:0, C18:0) in transplanted recipient site, control recipient site, and transplant donor site at start and 4, 7, and 12 months in patient 2.

![Figure 2](http://www.archdermatol.com/) Total monounsaturated fatty acids (C16:1 and C18:1) in transplanted recipient site, control recipient site, and transplant donor site at start and 4, 7, and 12 months in patient 2.

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>Start</th>
<th>4 mo</th>
<th>7 mo</th>
<th>12 mo</th>
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</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>2.4</td>
<td>1.6</td>
<td>2.5</td>
<td>2.0</td>
</tr>
<tr>
<td>C16:0</td>
<td>15.9</td>
<td>18.4</td>
<td>19.2</td>
<td>15.8</td>
</tr>
<tr>
<td>C16:1(9)</td>
<td>4.7</td>
<td>3.2</td>
<td>2.7</td>
<td>4.9</td>
</tr>
<tr>
<td>C18:0</td>
<td>4.9</td>
<td>5.9</td>
<td>6.2</td>
<td>4.4</td>
</tr>
<tr>
<td>C18:1(9)</td>
<td>41.3</td>
<td>41.0</td>
<td>39.3</td>
<td>42.5</td>
</tr>
<tr>
<td>C18:2(9,12)</td>
<td>19.6</td>
<td>18.0</td>
<td>19.1</td>
<td>19.2</td>
</tr>
<tr>
<td>Total saturated</td>
<td>23.2</td>
<td>26.0</td>
<td>28.0</td>
<td>22.4</td>
</tr>
<tr>
<td>Total monounsaturated</td>
<td>46.1</td>
<td>44.3</td>
<td>42.1</td>
<td>47.4</td>
</tr>
</tbody>
</table>

*Values are given as weight percentage for each fatty acid.

Table 3. Fatty Acid Compositions of Cheek and Gluteal Fat for Patient 2
characteristics are in progress. These preliminary results based on objective data suggest that long-term adipocyte survival is an achievable goal following fat transfer.

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REFERENCES