A dipose tissue transfer is a powerful yet unpredictable tool for the plastic surgeon. Some authors have found that fat grafts after breast cancer reconstruction retain 45 to 59 percent of their original volume at 49 days and 27 to 54 percent at 140 days. Fat graft resorption is common, and patients frequently undergo at least two or three rounds of grafting to achieve adequate volume correction. Two phenomena limit the viability of transferred lipoaspirate. The first is the trauma associated with tissue procurement and purification. The cannula insertion and tissue disruption injure the donor cell population. Second occurs when the remaining cells are then subjected to a hypoxic insult.

Both insults contribute to the variable results that ensue after fat grafting. Remote ischemic preconditioning is an inexpensive, noninvasive technique that has been used in animal models and...
multicenter clinical trials to protect organ systems (i.e., heart, lung, nerve, intestine, muscle, and kidney). Preconditioning has been shown to limit the cellular injury before acute ischemia associated with tissue transfer. In brief, remote ischemic preconditioning involves subjecting an area away from the site of graft harvest or placement to a prescribed period of ischemia, and reperfusing that area to disseminate currently unknown protective molecular mediators. To date, most protocols have involved intermittent extremity tourniquet application at various intervals and durations. We recently demonstrated through a novel murine model that when donor adipose tissue is subjected to remote ischemic preconditioning before transfer, there is increased viability of fat grafts at 28 days. No work has been performed to date to analyze the effect of recipient remote ischemic preconditioning on the recipient wound bed before fat transfer. Furthermore, there are no data describing whether remote ischemic preconditioning treatment of fat graft donor or recipient sites plays a more substantial role in cell viability.

**MATERIALS AND METHODS**

**Animals**

Native FVB mice and those harboring the CAG-luc-eGFP L2G85 transgene were obtained from The Jackson Laboratory (Bar Harbor, Me.) at 6 to 7 weeks of age and acclimated to the animal facility over a 2-week period. The mice received a high-fat diet (25 percent carbohydrate, 60 percent fat, and 15 percent protein, volume/volume) to foster subcutaneous adipose formation for 6 to 8 weeks before adipose transfer and during the 4-week period after tissue transfer.

**Fat Grafting**

We anesthetized each animal with oxygen supplemented with 2% isoflurane gas. Animal abdomens and hindlimbs underwent hair removal, germicidal scrub, sterile preparation, and draping. Next, a wetting solution composed of 1 cc of epinephrine (1:1000) and 30 cc of 1% lidocaine in 1 liter of lactated Ringer solution was prepared. From this mixture, 1- to 2-cc aliquots were instilled into the inguinal abdominal fat pad designated for procurement through 30-gauge needles. Stab incisions using a sterile 18-gauge needle were made in the skin through the dermis for subcutaneous access. The donor animals' subcutaneous fat was tunneled using a 16-gauge, Coleman no. 2 cannula immediately before harvest (Fig. 1). The abdominal and inguinal subcutaneous fat was then excised directly by means of a midline abdominal incision. The tissue was then morselized, serially homogenized with Coleman cannulas (16, 18, and 19 gauge), and allowed to settle in open centrifuge tubes for 5 minutes. The samples were then centrifuged at 500 g for 2 minutes.

*Fig. 1. Demonstration of technique. (Left) Subcutaneous placement of wetting solution and fat tunneled using a Coleman cannula immediately before harvest. (Center) The abdominal and inguinal subcutaneous fat was then excised directly, morselized, serially homogenized with Coleman cannulas (16, 18, and 19 gauge), and allowed to settle in open centrifuge tubes for 5 minutes. The samples were then centrifuged at 500 g for 2 minutes after removal of supernatant/oil, the fat is placed in the subcutaneous dorsal skin folds of the recipient animals using Coleman microcannulas.*
at 25°C. We removed the remaining supernatant/oil and transferred the samples to 1-cc syringes with 19-gauge Coleman no. 1 microcannulas for subcutaneous placement in the recipient animals.

**Remote Ischemic Preconditioning**

Treatment occurred by means of three cycles of a 5-minute ischemia as we have described previously. In brief, we induced ischemia with a ¼-inch Penrose tourniquet applied to the animals’ proximal hindlimbs below the area of adipose harvest or tissue placement. Ischemia was verified visually by lack of paw capillary refill. Lack of capillary refill was noted immediately after tourniquet application, and thus ischemia was documented from the moment tourniquet use started and only the time of use was standardized between animals. After 5 minutes of ischemia time, the tourniquet was removed and distal capillary refill was assessed. All animals had return of perfusion. After 5 minutes of reperfusion, the tourniquet was applied. After the final 5 minutes of ischemia, fat procurement or placement occurred.

**Treatment Groups**

Remote ischemic preconditioning was carried out in a stepwise fashion. The adipose in the control-control group experienced no remote ischemic preconditioning before fat transfer in either the donor or recipient animals. The adipose in the remote ischemic preconditioning–control group experienced remote ischemic preconditioning before fat procurement in the transgenic donor mouse but the wild-type recipient did not. The control–remote ischemic preconditioning group did not experience remote ischemic preconditioning in the adipose in the transgenic donor mouse before tissue harvest, but the wild-type recipient experienced remote ischemic preconditioning immediately before fat transfer. Finally, both the transgenic donor mouse and the wild-type recipient underwent remote ischemic preconditioning before tissue procurement and transfer in the remote ischemic preconditioning–remote ischemic preconditioning group.

**In Vivo Viability Evaluation**

Whole-body real-time scans of anesthetized mice were acquired in supine position on postimplantation days 1, 7, 14, and 28. All dorsal hair was removed by means of a chemical depilatory agent as needed over the course of the experiment. The recipient mice were placed in an IVIS Lumina small animal scanner (Perkin Elmer, Waltham, Mass.). Bioluminescence from each area of subcutaneous transplanted adipose tissue was measured 40 minutes after intraperitoneal injection of 3 mg of luciferin and recorded to document the volume and viability of transferred tissue. The bioluminescence measurements were captured with Live Image v4.0 software (Perkin Elmer). Identical illumination parameters (exposure time, 9 seconds; f/stop, 1.2; field of view, 25; and small binning) were used for each image acquisition. Intensity of bioluminescence from each area of subcutaneous transplanted adipose tissue was quantified and recorded in triplicate.

**Histologic Analysis**

Fat grafts were explanted with the dorsal skin and subcutaneous tissue en bloc on day 28. The tissue was fixed for 15 minutes in Bouin solution and then placed into 10% neutral buffered formalin. The tissues were paraffin embedded and sectioned at 3 µm and stained with hematoxylin and eosin. Immunohistochemical staining was performed using the DAKO Autostainer with Envision Flex Detection System and anti–green fluorescent protein (Santa Cruz Technologies, Santa Cruz, Calif.). Histologic slides were imaged with a Nikon DS-Fi2 digital camera (Nikon Corp., Tokyo, Japan).

**Statistical Analysis**

Multiple comparisons between remote ischemic preconditioning and control groups were performed by a two-way $t$ test analysis. A probability value of $p < 0.05$ was considered statistically significant. Each experiment was run at least three times for internal validation.

**RESULTS**

**Bioluminescence**

Bioluminescence was detectable on days 0, 1, 7, 14, and 28 in all groups (i.e., control-control, remote ischemic preconditioning–control, control–remote ischemic preconditioning, and remote ischemic preconditioning–remote ischemic preconditioning) (Fig. 2). We tabulated the total flux from each region of interest and then plotted both their average measurements and the standard error of the mean for each time point. This curve demonstrates that there was a clear statistically significant difference in bioluminescence at every time point between the various treatment groups (Fig. 3). On day 0, the animals that experienced remote
ischemic preconditioning to the recipient bed had approximately 50 percent of the bioluminescence of their control recipient counterparts. Of those animals, the remote ischemic preconditioning–treated donor tissue (remote ischemic preconditioning–control) again demonstrated greater bioluminescence immediately after insertion over control (control-control). Bioluminescence increased greatly for all groups on day 1. Remote ischemic preconditioning donor groups (remote ischemic preconditioning–control and remote ischemic preconditioning–remote

Fig. 2. Bioluminescence from each area of subcutaneous transplanted adipose tissue specimens measured 40 minutes after intraperitoneal injection of 3 mg of luciferin (control, red, on the left; remote ischemic preconditioning, green, on the right, for each animal).

Fig. 3. Average bioluminescence of control and remote ischemic preconditioning–treated donor adipose tissue from FVB-Tg(CAG-luc,-GFPI2G85Chco/J mice into the dorsal skin fold of control and remote ischemic preconditioning–treated FVB/NJ wild-type mice over 28 days.
ischemic preconditioning) demonstrated the highest increases. However, remote ischemic preconditioning donor groups (remote ischemic preconditioning–control and remote ischemic preconditioning–remote ischemic preconditioning) continued to demonstrate higher levels of bioluminescence throughout the course of the experiment. These effects were even pronounced with the addition of remote ischemic preconditioning on the recipient wound bed (remote ischemic preconditioning–remote ischemic preconditioning). Both remote ischemic preconditioning recipient groups (control–remote ischemic preconditioning and remote ischemic preconditioning–remote ischemic preconditioning) demonstrated increased bioluminescence throughout the course of the experiment. At the final time point, day 28, the remote ischemic preconditioning–remote ischemic preconditioning group showed a statistically significant elevated amount of bioluminescence.

We then directly compared those groups that represent a potential true clinical comparison. Patients are only able to undergo fat transfer with or without remote ischemic preconditioning to both donor and recipient wound beds. Adipose transfer from allogenic sources is not currently a clinical reality. Thus, we specifically compared the complete control group (control-control) and the complete treatment group (remote ischemic preconditioning–remote ischemic preconditioning) (Fig. 4). The amount of bioluminescence present on day 0 was higher in the control group. However, the remote ischemic preconditioning treatment group had greater bioluminescence for the remainder of the experiment. This difference was statistically significant on days 14 and 28. It was at that point that the control group demonstrated attrition of bioluminescence to below insertion values, whereas the treatment group demonstrated values well above insertion values.

To gain further perspective, we plotted the bioluminescence values over the course of the experiment in relation to their insertion values (Fig. 5). On day 1 after insertion, both control-control and remote ischemic preconditioning–remote ischemic preconditioning groups demonstrated increased values to 150 percent and 400 percent, respectively. However, by day 28, the control group retained approximately 30 percent of its bioluminescence, whereas the remote ischemic preconditioning group was nearly 300 percent. Considered together, both the absolute and relative quantities of viable, bioluminescent adipose were higher in the remote ischemic preconditioning–remote ischemic preconditioning group compared with the control-control group.
Tissue Analysis

We have previously demonstrated that at days 0 and 1 there is similar appearance of histologic adipose architecture among the remote ischemic preconditioning and control adipose tissue groups. We performed histologic analysis on day 28 on all four treatment groups (i.e., control-control, remote ischemic preconditioning–control, control–remote ischemic preconditioning, and remote ischemic preconditioning–remote ischemic preconditioning). On day 28, once again, there was significant liposclerosis in the control-control group and almost none in the remote ischemic preconditioning–remote ischemic preconditioning group (Fig. 6). The mixed treatment groups (remote ischemic preconditioning–control and control–remote ischemic preconditioning) demonstrated intermediate levels of liposclerosis. The explanted tissue was stained for green fluorescent protein (Fig. 7). Only tissue that was subjected to some form of remote ischemic preconditioning (remote ischemic preconditioning–control, control–remote ischemic preconditioning, and remote ischemic preconditioning–remote ischemic preconditioning) continued to retain green fluorescent protein. The dense, non–green fluorescent protein–labeled interstitial cells in the clinical control graft supports the likely local ingrowth from the wild-type recipient animal. The control–remote ischemic preconditioning group had modestly more inflammatory cells than either of the other two remote ischemic preconditioning groups (remote ischemic preconditioning–control or remote ischemic preconditioning–remote ischemic preconditioning).

DISCUSSION

Fat grafting is a powerful tool available to the plastic surgeon. It can address contour deformities and improve skin quality. However, inconsistencies in transfer, trauma associated with procurement, and local ischemia before the development of recipient circulation all contribute to highly variable long-term results. Several methods to improve fat transfer viability have been suggested. These include various methods of harvest, size of liposuction cannula, centrifugation, and enzymatic processing before delivery. Negative-pressure therapy, growth factor administration, and stem cell supplementation have also been suggested. These techniques are meant to foster lipoaspirate tissue proliferation and neovascular ingrowth. However, no approach to date has targeted improvements to local tissue ischemia by increasing the adipose tissue’s ability to tolerate the insult. In addition, those modalities that foster tissue growth through either direct or indirect growth factor modulation may present challenges when fat grafting is used in anatomical regions previously occupied by cancer.
Remote ischemic preconditioning has been studied in several species and has been shown to have protective effects against ischemia for several organ systems. Although the exact mechanism is not known on how remote ischemic preconditioning influences cell survival during an ischemic insult, hypotheses from several studies suggest that it has a complex multimodal mechanism. We previously demonstrated in a murine model that when adipose undergoes remote ischemic preconditioning before transfer, it displays several hallmarks consistent with ischemic tolerance (i.e., increased viability and less liposclerosis). This work suggests that remote ischemic preconditioning may play a meaningful role in increasing retention and viability of adipose grafts by affecting the remote recipient wound bed. Kraemer et al. demonstrated that remote ischemic preconditioning improves distant capillary bed tissue oxygen saturation and perfusion. It is perhaps this action that in essence prepares the recipient site for tissue engraftment and helps limit ischemic injury to the transferred tissue.

We previously demonstrated that donor remote ischemic preconditioning–treated liposaprate retains twice the bioluminescence of control samples on posttransplantation days 0 and 28. However, that information in isolation is not clinically relevant. In clinical practice, patients who undergo autologous fat transfer are both the donor and the recipient. Therefore, if treated by this method, they would undergo both donor and recipient remote ischemic preconditioning. To examine this issue directly, we compared the role remote ischemic preconditioning plays on the clinically relevant control group (i.e., control-control) and the clinically relevant remote ischemic preconditioning group (i.e., remote ischemic preconditioning–remote ischemic preconditioning).

Remote ischemic preconditioning played a role in augmenting bioluminescence of the remote ischemic preconditioning–remote ischemic preconditioning transferred fat over the 28-day experiment. By day 28, there was a nearly 9-fold difference in values between the control-control and remote ischemic preconditioning–remote ischemic preconditioning groups. Although the control-control group bioluminescence decreased below insertion levels, implying cellular attrition, the remote ischemic preconditioning–remote ischemic preconditioning group demonstrated increased...
bioluminescence over insertion values, suggesting possible cellular growth and/or proliferation.

Subgroup analysis of the non–clinically relevant groups (i.e., control–remote ischemic preconditioning and remote ischemic preconditioning–control) is more difficult to analyze and interpret. The remote ischemic preconditioning–control group displayed increased bioluminescence throughout the course of the experiment over control (i.e., control-control). It demonstrated bioluminescence attrition down to nearly insertion values by day 28. This group also displayed both less liposclerosis and increased green fluorescent protein retention than control. The control–remote ischemic preconditioning group displayed increased bioluminescence throughout the course of the experiment over control (i.e., control-control). However, the control–remote ischemic preconditioning group did not demonstrate bioluminescence attrition by day 28. This group also displayed both less liposclerosis and increased green fluorescent protein retention compared with control. By day 28, both the control–remote ischemic preconditioning and remote ischemic preconditioning–control groups demonstrated less bioluminescence than the remote ischemic preconditioning–remote ischemic preconditioning group. It is not clear why this occurred. Without knowing the specific molecular mediators involved in the pathway, it is difficult to isolate and control the variations of these non–clinically relevant subgroups. However, we do learn that remote ischemic preconditioning does have some benefit to both the donor and recipient aspects of the fat transfer process.

The goal of our study was to determine the impact of remote ischemic preconditioning on adipose tissue transfer. We have demonstrated that remote ischemic preconditioning increased adipose survival and retention rates. Further study is needed to elucidate the longevity (i.e., viability beyond 28 days) of this methodology and whether these murine results can be replicated clinically. If these benefits carry over into the clinical area, then RIPC may be an inexpensive, widely available means to augment the predictability of adipose transfer. After screening patients that can tolerate intermittent tourniquet application (risk of venous thrombosis and release of
ischemic byproducts), clinicians may one day be able to increase the predictability of fat grafting by intermittent and cyclic extremity tourniquet application completely away from the operative field.

CONCLUSIONS

In this work, we used a murine model to quantitatively evaluate adipose transfer in clinically relevant patterns of remote ischemic preconditioning. This model demonstrated that remote ischemic preconditioning increases the viability of fat transfer and decreases interstitial fibrosis.

Andrew A. Gassman, M.D.
Department of Plastic Surgery
University of Texas Southwestern Medical Center
1801 Inwood Road
Dallas, Texas 75390-9132
andrew.gassman@utsouthwestern.edu

ACKNOWLEDGMENTS

This work was supported by the Plastic Surgery Foundation (Pilot Research Grant number 274376) (to A.A.G.) and the Jean Perkins Foundation (to J.C.L.).

REFERENCES