necessary. Using this approach, we have been able to obtain very satisfactory results.
DOI: 10.1097/PRS.0b013e3182827a84

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DISCLOSURE

The author is a paid consultant of and receives royalties from Mentor Worldwide LLC, and is a consultant for Novus Scientific and Greer Medical.

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Observations on the Survival and Neovascularization of Fat Grafts Interchanged between C57BL/6-gfp and C57BL/6 Mice

Sir:

We read with interest the article by Zhao et al.,[1] and we congratulate the authors for their elegant study design and choice of experimental model. In particular, emphasis should be placed on the adoption of immunocompetent inbred strains with syngeneic background by means of which, despite adopting homologous transplantation, outcomes could resemble those of autologous transplantation as it normally occurs in clinical practice. Importantly, most preclinical research on adipose tissue grafting is based on homologous transplantation in immunosuppressed animal models, thus showing results significantly biased by the lack of any inflammatory/immunologic reaction. Nonetheless, despite a substantial lack of focused experimental evidence, fat graft survival by means of revascularization of cells has been widely confirmed by clinical experience and is not a new finding.[2] The authors describe the origin of the novel blood supply (host-driven), but they do not investigate, clarify, or discuss the possible mechanisms of neoangiogenesis. Is the grafted fat revascularized by means of a hypoxia-mediated pathway or are the transplanted adipocytes/preadipocytes responsible for secreting proangiogenic growth factors such as vascular endothelial growth factor? Is fat survival dependent on diffusion of nutrient substances until angiogenetic processes begin? Adipose tissue is known to act as a "secretome," being a source of numerous cytokines. Inflammatory infiltrate may also play a relevant role, and further data regarding histologic assessment of inflammatory cells could be helpful in evaluating this issue.[3] These processes are likely to correlate with the total volume of grafted adipose tissue, and this key point may represent a relevant bias that should be addressed. The authors could transplant different amounts of fat, analyzing the likely different evolution in terms of absorption and revascularization. Moreover, adipose tissue harvested from different body areas has been proven to possess different qualities in terms of density of mature or multipotent cells: in this study, the authors report the use of both inguinal and axillary fat pads. In a small experimental animal, fat pads are likely to show similar properties; the authors could analyze the composition of harvested tissue and comment on the eventual differences in behavior of different subpopulations of adipose tissue. The study design does not consider other variables that notably differ in the clinical setting, such as transplantation of adipose tissue in a donor pathologic site less prone to provide an adequate vascular supply (e.g., postsurgical scar tissue, burn tissue, sclerodermic tissue) or manipulation of the fat (adoption of vasoconstrictors such as adrenaline during harvesting), including centrifugation.[4,5] The latter, in particular, should be more accurately investigated to assess the respective roles in fat survival and trigger to revascularization of adipose cells (adipocytes and preadipocytes) and cells contained in the stromal vascular fraction. These variables could all be easily integrated in the described experimental model. Indeed, in our opinion, the authors have provided interesting preliminary results that should be more extensively analyzed to lead to more exhaustive correlations with actual clinical experience, thus providing a more fruitful discussion on therapeutic prospects.
DOI: 10.1097/PRS.0b013e3182827a97

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DISCLOSURE

The authors have no financial interest to declare in relation to the content of this communication.

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Adipocyte Damage in Relation to Different Pressures Generated during Manual Lipoaspiration with a Syringe

Sir:

We found the recently published work of Rodriguez and Condé-Green entitled "Quantification of Negative Pressures Generated by Syringes of Different Calibers Used for Liposuction" extremely interesting.[1] Fat grafting has recently been experiencing an exponential growth of its surgical indications.[2] However, it is commonly thought that, in contrast to liposuction with a cannula by a continuous vacuum system, harvesting through small syringes (e.g., 10-cc syringes) allows injury to be avoided and the viability of adipocytes to be preserved. These uninjured adipocytes can then be injected, after processing, into the recipient site, maximizing long-term surgical results.

Rodriguez and Condé-Green demonstrated that negative pressure generated by a syringe is determined by the volume or number of cubic centimeters introduced in the syringe by pulling back the plunger. Another surprising observation is that syringe caliber should be considered a secondary factor. The pressure increase inside the syringe as the plunger is pulled back is not linear but parabolic, with rapid increases in negative pressure generated initially at low volumes and leveling off only after approximately 13 cc of plunger pull-back. Thus, the greatest increase in pressure is registered during one of the most commonly used harvesting methods, namely, harvesting with a 10-cc syringe.

Rodriguez and Condé-Green use a graphic to help surgeons apply the desired negative pressure regardless of the syringe used, without quantifying the viability decrease in relation to the different pressures. Aiming to fill this gap, we compared the level of damage between samples of fat harvested with 5 (sample A) or 10 cc (sample B) of plunger pull-back in a 10-cc syringe and with 15 (sample C) and 30 cc (sample D) in a 30-cc syringe.

The samples were collected manually, through a two-hole Coleman blunt microcannula attached to Luer-Lok syringes (Becton Dickinson, Franklin Lakes, N.J.), from the same donor site (thigh) performing a wet technique of harvesting. Adipose tissue samples were fixed in 4% formaldehyde in isotonic phosphate-buffered saline for 24 hours. Tissue sections of 5 mm were dehydrated in graded ethanol, cleared in xylene, pelleted by centrifugation at 100 *g* for 5 minutes, and embedded in paraffin.

On a microphotograph (20×), with a corresponding field of 125,000 μm^2, counting of adipocytes with cy-

Table 1. Results

Sample*	Nonviable Adipocytes (%)
A (5 cc)	0–25
B (10 cc)	25–50
C (15 cc)	50–75
D (30 cc)	>75

*Adipose tissue represented 97 percent of each sample, fibrovascular tissue 3 percent.

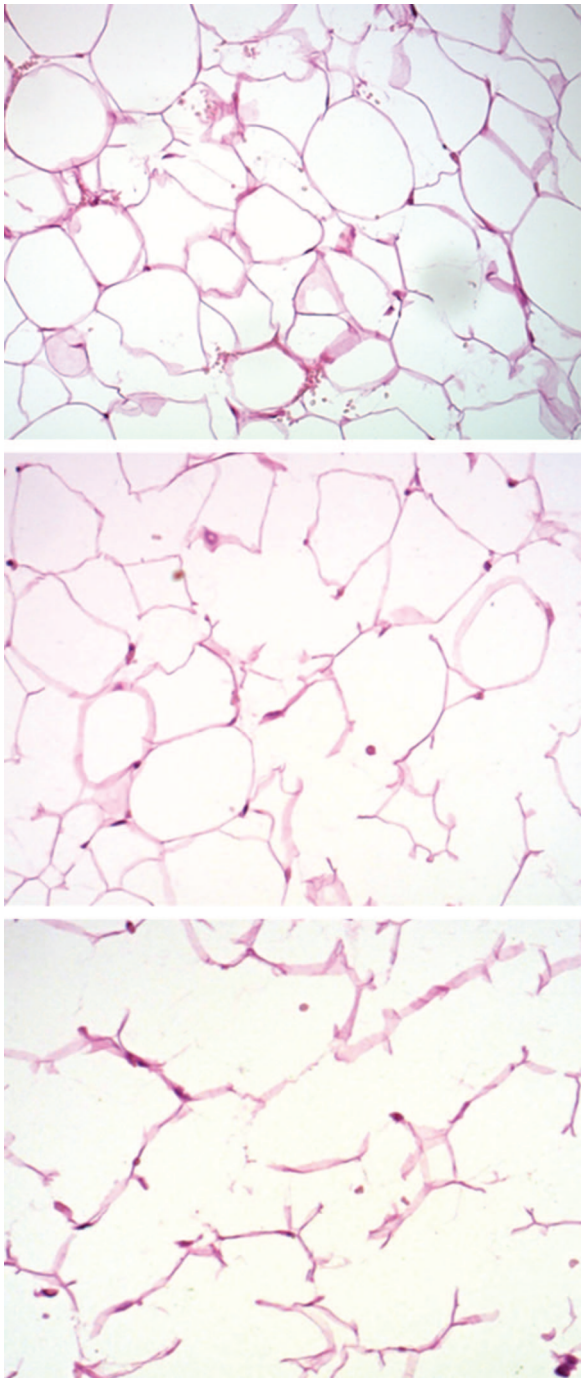

Fig. 1. Microphotographs (EE stain; original magnification, × 20) of 5 cc of plunger pull-back (sample A) (*above*), 10 cc of plunger pull-back (sample B) (*center*), and 30 cc of plunger pull-back (sample D) (*below*).