Fat grafting: Early hypoxia, oxidative stress, and inflammation developing prior to injection

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Autologous fat grafting is widely used during plastic surgery but unpredictable fat retention remains a major concern. Although many studies have explored whether fat harvesting, processing, and injection affect adipocyte viability, the oxygenation and inflammatory status of grafted fat remain unknown. Unlike vascularized tissue transfer, fat grafting is a non-vascular procedure. Fat initially lacks vascular support and receives oxygen and nutrients only via diffusion until neovascularization develops. However, even when fat is re-injected in small aliquots, the oxygen diffusion level may not adequately fulfil the metabolic needs of grafted fat, especially in the first days after injection. Very little is known about tissue damage developing within the first hour after surgery, which is nearly the average time between harvesting and injection in operation theater. For example, no study has yet measured oxygen saturation immediately after fat harvesting; this would aid assessment of fat status prior to injection.

In this experimentation, human adipose tissue was collected via liposuction from the abdomen and flanks of seven patients undergoing routine elective procedures. Tumescence was induced by infiltration of saline and epinephrine 10 min prior to skin incision, followed by power-assisted liposuction (PAL system) using a 4-mm-diameter cannula. Fat was rinsed using the Revolve system with lactate Ringer's solution at 37°C. Oxygen concentration was measured using a single-channel oximeter placed within the Revolve system, together with a temperature sensor over 1 h. This time limit corresponds to the average waiting time before injection in the theater. Fat tissue samples were collected after harvest and 1 h later, and O_2 levels were measured. The samples were then snap-frozen in liquid nitrogen and stored at -80°C prior to analysis. Gene expression assessed via quantitative real-time polymerase chain reaction and total RNA was extracted from 100 mg fat samples using an RNeasy Lipid Tissue Mini Kit (Qiagen). We used an 8-isoprostane ELISA kit (Cayman Chemical) to measure oxidative stress measurement. All statistical analyses were performed with the aid of

Statistica software. The normality of data distribution was assessed. Gene expression levels were compared using one-way ANOVA with appropriate post-hoc testing. 8-isoprostane concentrations were compared using the Wilcoxon test. Results are expressed as means ± standard deviations. A p-value <0.05 was considered to reflect statistical significance.

All samples evidenced rapid oxygen partial pressure falls over 1 h. The mean PO_2 after 1 h was 8.1±4.3 mmHg. We compared the expression levels in fat snap-frozen just after harvest and 1 h later. The 1-h samples exhibited significantly higher levels of the pro-inflammatory cytokines IL-6 (p<0.05), IL-1β (p<0.05), and MCP-1 (p<0.05). The TNF-α and IL-10 levels also increased, but not significantly (p=0.08 and p=0.07 respectively). The 1-h 8-isoprostane levels were significantly higher than those at harvest (0,82±0,09 vs. 0,67± 0,19 pg/mg) (p<0.05).

Fat oxygenation status was measured during the first hour after collection. Previous studies [1,2] have shown that the PO_2 of subcutaneous adipose tissue ranged from 23 to 84 mmHg. In this study, oxygen partial pressure of fat tissue was 8.1 mmHg 1 h after collection. Thus, at the time of injection, the tissue was severely hypoxic, and therefore heavily stressed. Of all adipose tissue cells, adipocytes are the most susceptible to severe ischemia. [3] Adipose-derived stem cells survive for up to 3 days and differentiate into adipocytes in graft zones wherein vascularization and oxygenation are optimal [3]. In contrast, both adipocytes and adipose-derived stem cells die in non-vascularized zones under ischemic conditions, being replaced by cystic oils and fibrosis, seriously compromising fat volume retention.

Gene expression levels were measured to evaluate the effects of fat processing and hypoxia. Within 1 h of harvesting, a significantly higher-level expression of the proinflammatory cytokines IL-6, IL-1-β and MCP-1, and somewhat greater expression of TNF-α and IL-10 was observed (Fig. 1A, 1B). Such increases have not been reported

previously. Tissue damage can be caused by hypoxia and/or liposuction trauma. IL-6, TNF-α, and MCP-1 are known to initiate, maintain, and enhance inflammation, in turn promoting neutrophil-mediated recruitment of both monocytes and M1 macrophages by grafted tissue. Long-term activation of these cells triggers both necrosis and fibrosis. However, some recent studies have shown that M2 macrophage activation facilitates both angiogenesis and graft survival[4]. Although the 'optimal' level of inflammation remains unclear, enhanced inflammation may promote inflammatory cell recruitment and impair fat graft retention.

The oxidative status of adipose tissue was evaluated immediately after harvest and 1 h later. F2-isoprostanes are produced via free-radical peroxidation of arachidonic acid; their levels are directly proportional to the extent of oxidative stress. 8-isoprostanes are the best characterized F2-isoprostanes, and are optimal biomarkers of oxidative injury. The 8-isoprostane level was significantly higher 1 h after fat collection compared to the level immediately after collection. Increased inflammation and enhanced reactive oxygen species production may compromise adipose tissue structure. Some authors consider that injected fat recruits neutrophils within 24 h. These cells then produce reactive oxygen species and trigger inflammation[5]. We found that reactive oxygen species production and inflammatory cytokine upregulation begin very soon after fat collection.

Thus, is fat re-oxygenation appropriate? Harvested fat rapidly becomes severely hypoxic and exhibits early signs of tissue damage; re-oxygenation may be appropriate prior to injection. Oxygenation would obviate reactive oxygen species formation by limiting hypoxia-reperfusion injury. However, as a high oxygen partial pressure is also deleterious, further experiments are needed to optimize any re-oxygenation strategy.

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