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Early enteral 5% glucose infusion maintains the epidermal growth factor levels in the jejunal flap used for pharyngo-oesophageal reconstruction

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Summary *Background:* Free jejunal flap reconstruction is the main treatment for patients after pharyngo-oesophagectomy. Flaps are unavoidably subjected to ischaemia and reperfusion (I/R) during preparation. Enteral nutrition has been shown to improve the recovery of injured intestine, although the precise underlying mechanism remains unclear. This study was aimed to determine whether early enteral 5% glucose infusion is beneficial for the recovery of flap. Further, the possibility that enteral glucose infusion induces altered mucosal responses was evaluated.

Patients and methods: Patients, who underwent free jejunal flap reconstructions after pharyngo-oesophagectomy, were enrolled. An externalised monitor loop was made to observe the viability of flap and to collect intestinal fluid. Control patients ($n = 11$) received peripheral parenteral nutrition for seven post-operation days. For early enteral-fed patients ($n = 12$), in addition to fluid infusion, administration of 5% glucose (25 ml h^{-1}) via a jejunostomy tube was initiated 6 h after surgery. Blood, flap fluid and mucosal specimens were harvested. Plasma and flap luminal levels of interleukin (IL)-6, IL-10, epidermal growth factor (EGF) and secretory immunoglobulin A (sIgA) were measured. Further, mucosal morphology was examined.

Results: There were no significant differences in either plasma or luminal concentrations of IL-6, IL-10 and sIgA at different time points between groups. The luminal EGF level in the control group reduced markedly from the 3rd postoperative day, contrasting with a well-maintained level in the early enteral-fed group. No significant difference in mucosal histology between groups was observed.

Conclusion: Early enteral glucose infusion does not significantly benefit the ischaemia—reperfusion-injured flap; however, it does preserve EGF levels in the flap lumen.

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Jejunal flaps have been commonly used in the reconstruction of pharyngo-oesophageal defects.[1,2] During the preparation of flaps, segments of the jejunum are excised and subjected to a period of warm ischaemia until revascularisation. Both ischaemia and the subsequent reperfusion lead to tissue injury via a complex, multifactorial pathophysiological process, which involves the actions of nitric oxide, oxygen free radicals, various cytokines and other mediators.[3,4] This structural injury might then compromise functional performance of the flap at the transposed site. In spite of several proposed strategies based on animal studies,[5–7] to date, no effective method is clinically available for resolution of this inherent problem.

Despite some contradictory results, early enteral feeding, initiated mostly within 24 h of surgery, has recently gained more popularity than parenteral feeding, which is the traditional postoperative management of patients undergoing jejunal flap reconstructions.[8–11] The advantages of early enteral feeding, as demonstrated in numerous studies, include reduction in infectious complications,[8] prevention of mucosal atrophy,[12] maintenance of mucosal immunity[13] and improvement in wound healing.[14] While the role of enteral nutrients in maintaining the structural and functional integrity of the small intestine under both physiological and pathophysiological conditions is well appreciated, the underlying mechanisms are not fully understood. There are several plausible mechanisms and a leading one is through the stimulation of humoural factors.[15–17] According to this theory, enteral administration of nutrient(s) might stimulate the intestinal mucosa to release cytokines, growth factors or other mediators, which would exert actions locally and/or subsequently be transmitted via blood circulation to distant places such as the transplanted flap in this study. Consequently, part of the humoural factor-induced mucosal response might be reflected in the flap secretions. To prove this, collecting the fluid secreted from the intestinal flap is essential but difficult in an *in vivo* status; however, an externalised monitor flap in our reconstruction model makes this task feasible (Figure 1). The monitor flap, derived from the same jejunal segment and sharing a common vascular system with the free jejunal flap, acts as a highly reliable monitor for the viability of the buried flap. Secretory function, other than peristalsis, is the most visible inherent characteristic that the denervated flap preserves; nevertheless, components of secreted fluid might not be exactly the same as those of fluid produced in the originated site of the flap, as a consequence of lacking food stimulation and extrinsic innervations. Fortuitously, these limitations made the monitor flap an ideal model for examining, via altered secretions, part of the mucosal response to humoural factors released following enteral food stimulation without the influence of exogenous nerves.

Taken together, the main goal of this study was to investigate whether early enteral nutrient provision is beneficial for the recovery of the ischaemia–reperfusion (I/R)-injured jejunal flap at the transplanted site. Further, the possible effect of enteral feeding-induced humoural factors on the mucosal response was tentatively evaluated. The early enteral nutrient used was 5% glucose, which is safe and well tolerated for stressed gastrointestinal tract and has been demonstrated to be beneficial for the maintenance of mucosal integrity in response to intestinal ischaemia.[18] I/R injury-related parameters including the plasma and flap fluid concentrations of a pleiotropic cytokine (interleukin (IL)-6), an anti-inflammatory cytokine (IL-10) and epidermal growth factor (EGF) were measured. Secretory immunoglobulin A (sIgA) levels in the plasma and flap secretions were determined as an index of mucosal immunity. In addition, alterations in the flap mucosal morphology were monitored.

Materials and methods

Patients and treatments

A total of 23 patients (male: 13; female: 10), who underwent free jejunal flap reconstructions after pharyngo-oesophagectomy, were enrolled in this study. A monitor loop, about 10 cm in length, which did not interfere with the reconstruction process and the final functional performance, was constructed to observe the viability of jejunal flap and to collect intestinal flap fluid (Figure 1). Further, each patient had a feeding jejunostomy insertion after the reconstruction procedure. The study protocol was approved by Institutional Review Board of our hospital and informed written consent was obtained from each patient before surgery. Patients were randomised into two groups. For patients of the early enteral-fed (EEF) group ($n = 11$; mean age: 44 ± 7 years), in addition to regular peripheral parenteral nutrition, administration of 5% glucose ($25 \, ml \, h^{-1}$) via a jejunostomy tube was initiated 6 h after surgery[19,20] and continued for 7 days. Patients of the control group ($n = 12$; mean age: 50 ± 5 years) only received peripheral parenteral nutrition for the same period of time. Fluid secreted from the monitor loop was collected daily for seven consecutive days. Blood and tissue samples were also harvested at different time points. Starting on the 8th postoperative day, both groups of patients were treated identically with regular enteral feeding combined with intravenous fluid infusion.
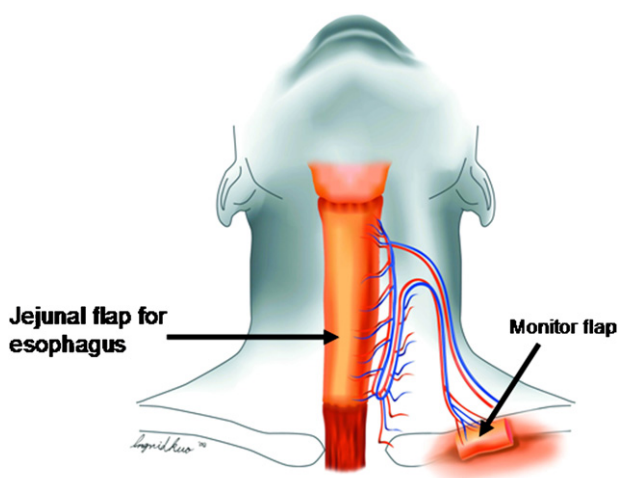

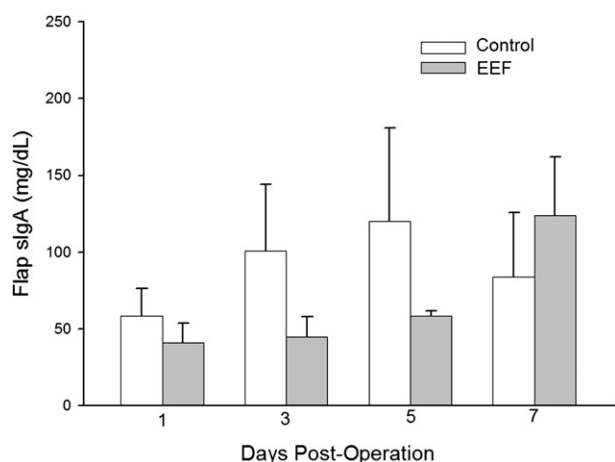**Figure 1** Free jejunal flap for pharyngoesophageal reconstruction.

Figure 2 Alterations in flap luminal levels of sIgA at different days after reconstructive surgery.

Measurements of IL-6, IL-10, EGF and sIgA

Plasma and flap fluid levels of IL-6, IL-10, and EGF were measured using a Quantikine Enzyme-linked Immunoassay System (R&D Systems, Minneapolis, MN, USA). SIgA concentrations in the flap fluid were determined by immunoturbidimetry.

Histological examination

Tissue samples were processed as described previously.[21] Briefly, mucosal tissues were fixed in 6% phosphate-buffered formaldehyde, embedded in paraffin, cut to 5-μm thickness and stained with haematoxylin−eosin. The mucosal damage score, as reported by Ming and Goldman,[22] was used; it consisted of a 0 (absent) to 3 (severe) score based upon the following criteria: (1) oedema, (2) inflammation, (3) mucosal necrosis, (4) shortening of villi and (5) decrease of goblet cell.

Statistical analysis

All data are expressed as means ± standard error of the mean (SEM). Statistical evaluations were made by Student's
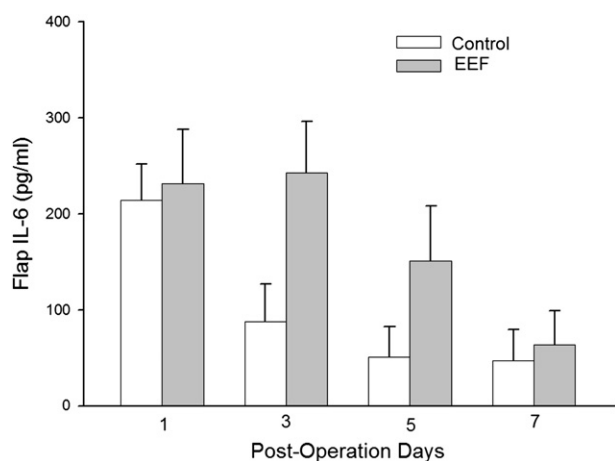

Figure 3 Alterations in flap luminal levels of IL-6 at different days after reconstructive surgery.
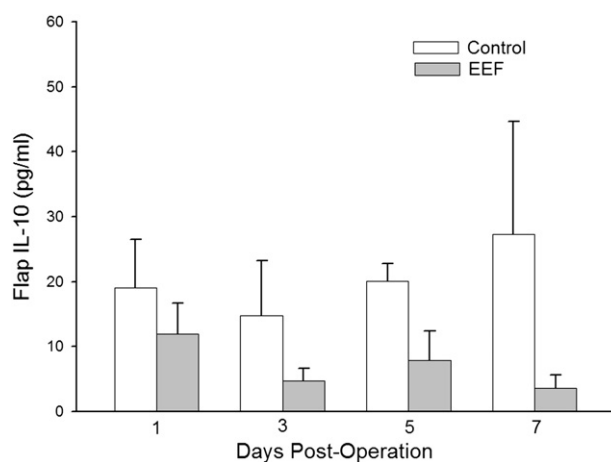

Figure 4 Alterations in flap luminal levels of IL-10 at different days after reconstructive surgery.

t-test or analysis of variance with Tukey's test *post hoc* by SigmaStat Software (Jandel Scientific, San Rafael, CA, USA). A $P < 0.05$ was considered significant.

Results

Before revascularisation, the mean ischaemia time of flaps was 70.8 ± 6.9 min and 61.9 ± 3.9 min $(P > 0.05)$ in the control and the EEF groups, respectively. All the 23 jejunal flaps survived without any vascular compromise. Further, no apparent gastrointestinal symptoms such as nausea, vomiting and diarrhoea were observed in all the patients.

The sIgA levels in the flap secretions were similar in both control and EEF groups at each indicated time point (Figure 2). In both groups, high IL-6 concentrations in the flap fluids were measured on postoperative day 1 with subsequently decreasing levels, and there was no significant difference between groups at any of the time points studied (Figure 3). Similar observations were also made for the IL-6 levels in the plasma (e.g., on postoperative day 1: EEF group 189.8 ± 44.63 vs. control group 221.3 ± 42.8 pg ml^{-1}; day 7: EEF group 40.9 ± 12.0 vs. control group 58.3 ± 26.5 pg ml^{-1};
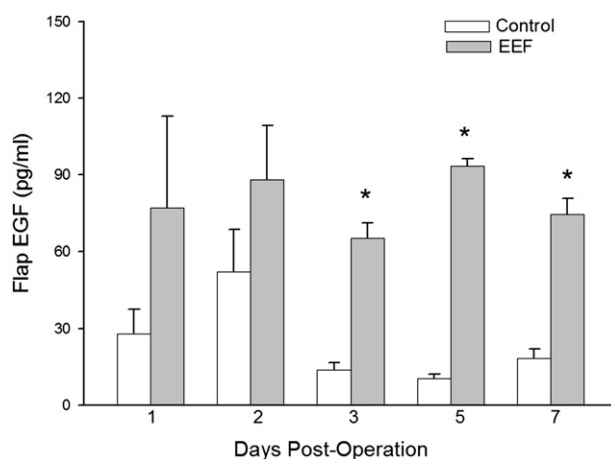

Figure 5 Alterations in flap luminal levels of EGF at different days after reconstructive surgery. $* P < 0.05$ versus control group.

$P > 0.05$). Moreover, the concentration of IL-6 in the plasma was not different from that in the flap fluid at each time point.

With regard to IL-10, the concentrations measured in the plasma at each sampling time were similar in both groups (e.g., on postoperative day 1: EEF group 16.7 ± 5.9 vs. control group 18.1 ± 7.0 pg ml^{-1}; day 7: EEF group 7.6 ± 2.4 vs. control group 14.7 ± 8.5 pg ml^{-1}; $P > 0.05$) and were statistically equivalent to those in the flap fluids. Further, no discernible differences in the flap IL-10 levels between groups were observed (Figure 4).

The plasma concentrations of EGF in both groups were similar at each time point (e.g., on postoperative day 1: EEF group 8.3 ± 3.3 vs. control group 12.8 ± 10.7 pg ml^{-1}; day 7: EEF group 11.2 ± 6.9 vs. control group 13.3 ± 9.0 pg ml^{-1}; $P > 0.05$). On postoperative days 1 and 2, the differences in the flap fluid levels of EGF between groups did not reach statistical significance. However, the EGF levels in the EEF group at subsequent time points were maintained at relatively constant levels and were comparatively higher than those in the control group (Figure 5).

The effect of early enteral glucose provision on the recovery of I/R-injured mucosal tissue was assessed by alterations of mucosal damage scores on different post-operative days. As shown in Figure 6, I/R induced a marked mucosal injury in both groups, and there was no significant difference in the damage score between groups at each sampling time point.

Discussion

It has long been well acknowledged that postoperative enteral feeding is superior to parenteral feeding; however, information regarding the mechanisms whereby enteral feeding may protect gastrointestinal epithelium against injury is still far from complete. The present study, using a unique reconstructive surgery model, demonstrated, for the first time, that early enteral infusion of 5% glucose after pharyngo-oesophageal reconstruction preserves EGF levels in the jejunal flap, which is no longer at its anatomic site.

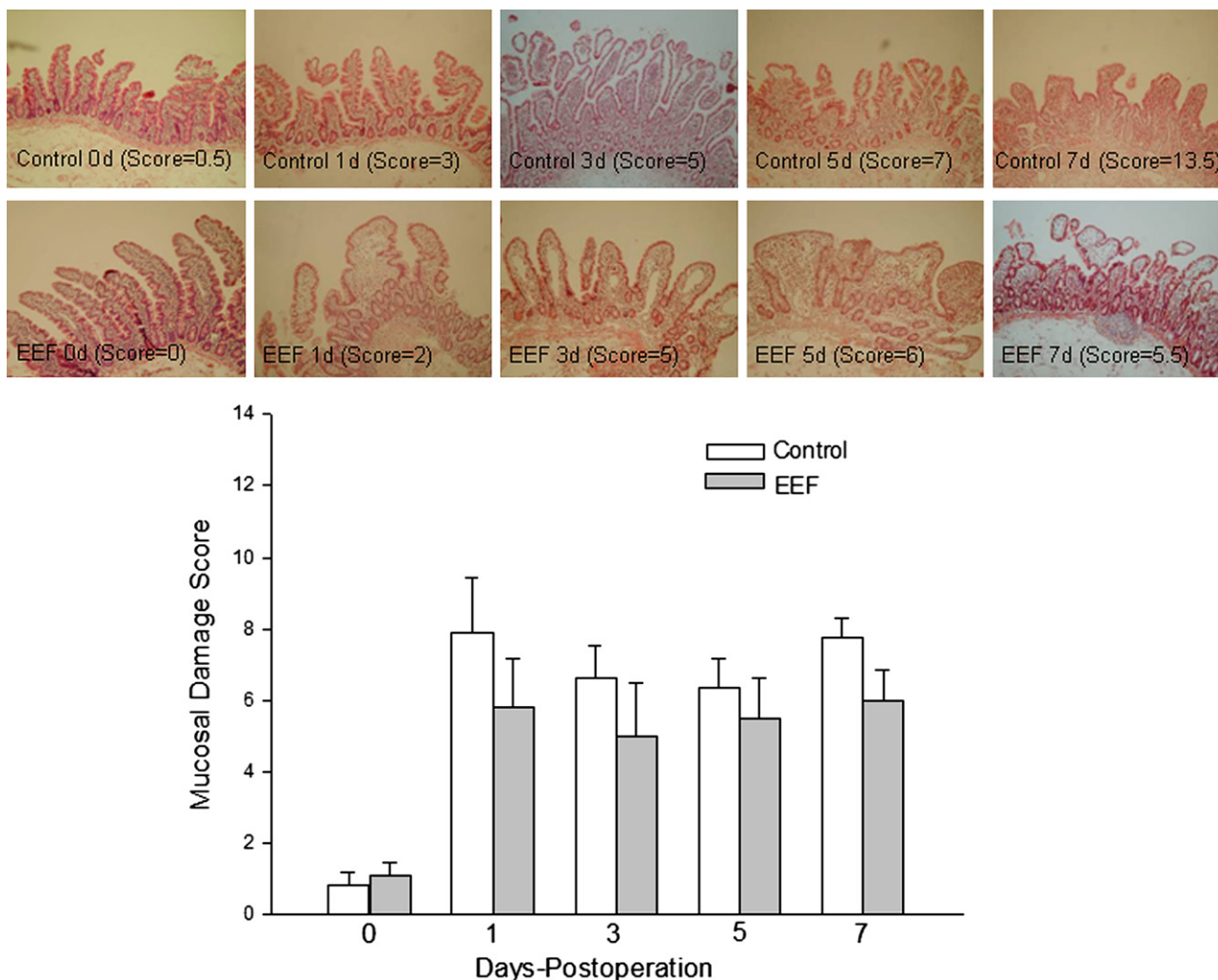Intestinal I/R induces a complex inflammatory cascade. Several pro-inflammatory cytokines (IL-1β, IL-6, tumour

Figure 6 (A). Representative photomicrographs showing histological changes of jejunal flap mucosa at different time points in control and EEF groups. (B). Alterations in mucosal damage scores at 0 (prior to ischemia), 1, 3, 5, and 7 days after reconstructive surgery.

necrosis factor (TNF)-α and IL-8) are sequentially released and IL-6 is the main mediator of the host acute phase responses.[23,24] On the other hand, the anti-inflammatory functions of IL-10 have been demonstrated to play a protective role in various inflammatory models.[25,26] The insignificant difference in the release profiles of IL-6 and IL-10 between the EEF group and the control groups suggests that early enteral glucose infusion does not exert an important influence on the resolution of inflammatory response in our reconstruction model. The observation that the levels of both IL-6 and IL-10 in the plasma are similar to those in the flap fluids indicates that the gut is not a major production site of these inflammation-associated cytokines.

SIgA, the major immunologic product of gut-associated lymphoid tissue (GALT), is a critical component in mucosal immunity and barrier integrity in response to I/R insult.[27] The similar levels of sIgA in flap fluid in both control and early enteral feeding groups suggest that early enteral infusion of 5% glucose, in this present study, does not improve the mucosal immune function and integrity following reconstructive surgery.

EGF, a peptide comprised of 53 amino acids, is mainly synthesised in the salivary glands and Brunner's glands of the duodenum. In the gastrointestinal tract, EGF promotes the growth and differentiation of epithelial cells[28,29] and modulates intestinal ion and glucose transport.[30] In addition, it has been shown to attenuate intestinal mucosal injury by oleic acid[31] and I/R,[32,33] suggesting that it is also cytoprotective. In this present study, the plasma levels of EGF in both groups were relatively constant throughout the study period, and no significant difference between groups was observed. However, patients receiving enteral provision with 5% glucose displayed a flap EGF secretion profile, which was dramatically different from that by control patients. The pathophysiological significance is not clear. We proposed a potential mechanism for this observation; that is, enteral glucose administration might, via either the nonspecific effect of the presence of food in the lumen or the specific effect of glucose, stimulate the gut mucosa to release certain mediators. These mediators are then transported by the blood to the flap and directly act upon the flap mucosa to produce EGF and other factors. Alternatively, an interaction between these mediators and the intrinsic neurons residing in the flap tissue might be required for EGF secretion to be induced.

The considerable difference in the flap luminal EGF levels between groups was not reflected in the alterations of mucosal morphology as evidenced by the similar mucosal damage scores at various time points in both groups. One plausible explanation for this is that we observed the morphological changes for only seven postoperative days: that might be not long enough for the effect of EGF on the mucosal morphology to be manifested. The other is that if the mucosal injury is more serious than that observed in this study due to long ischaemia time during jejunal flap transfer, the maintained high level of EGF might thus be able to demonstrate its protective effect. On the other hand, EGF might exert more subtle effects such as an increase in the activity of ornithine decarboxylase or the synthesis of polyamines,[34,35] which could not be detected using the methodology in this study.

In summary, although early enteral glucose infusion did not demonstrate pronounced benefit on the recovery of I/R-injured flap mucosa, it did maintain the EGF levels in the flap lumen. The pathophysiological significance of the preserved EGF levels and the underlying mechanisms are currently under investigation.

The study protocol was approved by the Institutional Review Board of our hospital and informed written consent was obtained from each patient before surgery.

Conflict of interest

None.

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