Effect of perioperative feeding with fibre & arginine on morphometrics of ileum after surgical stress

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Background & objectives: Major surgeries as well as other types of injury have been shown to affect the gut function. Enteral diets influence intestinal mucosal morphometry to different extents depending on their composition. Little is known about the effects of these defined-formula diets in patients with surgical stress but no malnutrition. This experimental study was undertaken to compare the effects of different enteral diets on the mucosal morphometrics of small bowel in surgically stressed rats without malnutrition.

Methods: Male Wistar-Albino rats (n=84) weighing between 160-220 g were randomised into three groups. Group A received standard rat chow. Group B received a complete balanced nutrition supplemented with fibre, and the rats in Group C were given an isocaloric specialized elemental nutrition enriched with specific combination of nutrients and arginine. The feeding was started two days before the operation and continued until re-operation. Laparotomy, ileal transection, and end-to-end anastomosis was performed as the surgical procedure. The rats were sacrificed on days 0, 2 and 7 post-operatively. One cm of ileal segment containing the anastomosis was examined histologically. Parameters for intestinal mucosal morphometry (number of villi, villous height, mucosal thickness) and number of mucous containing cells were determined.

Results: Number of mucous cells per villus was significantly (P<0.05) higher in group A compared to groups B and C on days 0 and 2 post-operation. On day 7 villous height and mucosal thickness were also significantly higher in group A compared to the other two groups.

Interpretation & conclusion: Laparotomy and a minor surgical intervention such as small bowel transection was not a major surgical stress for intestinal mucosal atrophy in rats without malnutrition. The effect of fibre and arginine enriched defined-formula diets did not seem to improve intestinal mucosal changes in such a surgical stress model compared with normal rat chow.

Key words Arginine - fiber - intestinal mucosa - perioperative enteral nutrition
The key role of enteral feeding on the healing of gastrointestinal tract is well recognized. Intraluminal nutrients were shown to increase mucosal blood flow, preserve gut barrier function, and decrease mucosal atrophy. Several experimental studies demonstrated the beneficial effects of defined-formula diets especially in models of obstructive jaundice and radiation enteritis.

Pre- and post-operative nutritional support during major surgical interventions improves the outcome in patients with moderate to severe malnutrition. The obvious benefits of early enteral nutritional support in abdominal surgery have been shown about two decades ago. Supplementary nutrition may be of great benefit to those whose absorptive surface has been severely reduced since in such cases adaptive mechanism for compensation of absorptive and metabolic loss is triggered.

A variety of intraluminal nutrients may influence small bowel mucosal growth since these are metabolized most rapidly by mucosal cells of the small intestine and are important oxidative fuel for rapidly dividing cells such as enterocytes, colonocytes and lymphocytes. Specialized enteral and parenteral feedings supplemented with such nutrients as arginine, nucleotides, fish oil, and/or structured lipids have been associated with improvements in immune function in critically ill and post-operative patients. These diets contain specific combinations of nutrients that enhance immunocompetence, maintain intestinal integrity, and modify the response to stress. The complex composition of these diets makes it difficult to study the effects of a specific nutrient per se, and their combined effects may not necessarily be simply additive in terms of the number of calories or the amount of protein.

These diets have been introduced for use in patients with increased metabolic needs but little is known about their effects in surgically stressed individuals without malnutrition, particularly because of their higher cost compared with standard diets. The aim of this experimental study was to compare the effects of perioperative feeding with defined-formula diets containing specific combination of nutrients and normal rat chow on the histology of intestinal mucosa in surgically stressed rats without malnutrition.

**Material & Methods**

The protocol of the study was approved by the local ethical committee, and Animal Experiments Ethics Review Committee of the Adnan Menderes University.

**Study groups:** A total of 84 male Wistar-Albino rats weighing between 160-220 g were used. The rats were randomly divided into three groups: Group A (n=28) received standard rat chow, whereas the rats in Group B (n=28) received a complete balanced nutrition supplemented with fibre (Biosorb fiber, Nutricia, Holland), and in Group C (n=28); an isocaloric specialized elemental nutrition enriched with arginine, nucleotides, and essential fatty acids (Impact, Novartis, Switzerland) was given. The animals were housed separately in wire-bottom cages at 24°C room temperature with a 12 h light-dark cycle. In all groups, 8 rats were sacrificed on the day of operation (day 0) and on day 2 post-operation (p.o.). The remaining animals in all groups were sacrificed on the day 7 p.o.

**Enteral diet:** The feeding was started two days before the operation and continued until their relaparotomy. The rats had free access to water during the study. Both of the defined enteral diet formulae had a concentration of 1 kcal/ml.

**Surgical operation:** The rats were anaesthetized by an intramuscular (im) injection of ketamine, 50 mg/kg (Ketalar; Parke Davis, Eczacibasi, Istanbul, Turkey) and xylazine 10 mg/kg (Rompun; Bayer AG, Leverkusen, Germany). All the animals were allowed to breathe spontaneously during the study. Coeliotomies were performed through a midline incision and sterile wet gauze pads were placed over the intestines in order to minimize evaporation. The ileum was transected 8 cm above the ileocecal valve and without removing any ileal segment an end-to-end anastomosis was performed as one layer with interrupted 6/0 stitches (Prolene, Ethicon, UK). During relaparotomy, an ileal segment of 1 cm including the anastomotic site was resected for histological examination.

**Histological examination:** All samples were evaluated by the same pathologist in a blinded fashion. Before the examination, luminal contents of the intestinal segments were washed out with saline solution.
Sections were fixed with formaldehyde, and embedded in paraffin. Afterwards the samples were stained with haematoxylin-eosin to evaluate the number of villi, villous height and mucosal thickness and with PAS to identify mucous containing cells. The number of villi per cm of ileum was counted and recorded separately for each group. The villous height was defined as the measurement in millimeters from the base of the villous to the tip. This was determined at five separate points close to the anastomosis sutures and recorded as the mean villous height. Mucosal thickness, the thickness of mucosa and submucosa, was measured on the individual intestinal segments with the use of an ocular micrometer (Eyepiece micrometer, Olympus, Japan). The measurement of mucosal thickness was done at five representative sites where villi were easily discernible from the base to the tip of each crypt. The number of mucous cells per villous was counted and recorded separately.

**Statistical analysis:** Multiple comparisons between the groups and intra-group evaluation of data were performed with one-way ANOVA and post-hoc tests. Differences between groups were considered significant with $P < 0.05$. Data were analyzed with a personal computer by a statistical software package (SPSS for Windows 9.0; SPSS Inc, Chicago, Ill).

### Results

Eight animals died during the study period (3 in group B and 5 in group C). All of them died between days 3-5 p.o. and autopsy revealed anastomotic breakdown or leakage.

**Changes in intestinal morphometry:** The histomorphometric analysis of ileum at the anastomotic site on days 0 and 2 post operation showed a statistically significant difference in the number of mucous cells per villous which was higher in group A ($P<0.05$) compared to other groups (Tables I, II).

On day 7 p.o. not only the number of mucous cells per villous in group A was significantly higher ($P<0.05$) than the other defined-formula groups but there was statistically significant difference in villous height and mucosal thickness between the same groups ($P<0.05$). However, the increase in the number of villi per cm of ileum was significantly higher in group C than A and B ($P<0.05$) (Table III).

Intra group analysis was also done (Fig. 1). Villous height did not differ in group B, however in groups A and C the increase in villous height was especially significant on 7th post-operative day ($P<0.05$). The
synthesis after injury. Zulfikaroglu et al. reported that enteral immunonutrition using glutamine or arginine, ω-3 fatty acids, and RNA supplemented enteral diet during both pre- and post-operative period decreased atrophy of intestinal mucosal villi and reduced bacterial translocation in rats with obstructive jaundice. Gurbuz et al. found improved mucosal morphometrics with supplemental dietary arginine. L-arginine increased villus height and crypt cell mitosis in the small intestinal mucosa and improved weight gain after massive small bowel resection in rats. However, Welters et al. demonstrated that parenteral arginine supplementation in rats with short bowel syndrome led to a slowing of the adaptive response. Immunonutrient enteral diets have also been shown to improve survival in animal models of gut-derived sepsis and have reduced infectious complications and/or hospital stay in several prospective, randomized clinical studies.

Fermentation of fibre - a key nutrient for intraluminal free fatty acid formation has been shown to be one of the leading factors in the mucosal response to injury. Perioperative fibre or arginine supplemented diet did not significantly improve mucosal reconditioning in the present study.

Surprisingly, the results of our study indicated that the animals nourished with standard rat chow had better results than group supplemented with fibre and arginine. On the other hand, the increase in the number of villi was significantly higher in arginine supplemented group C compared to other groups. One might speculate that defined formula diets at least maintained the number of villi per cm of ileum.

Table III. Mucosal morphometrics of various groups on day 7 post operation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>A* (n=12)</td>
</tr>
<tr>
<td>No. of mucous cells (per villus)</td>
<td>29 ± 7 (22-45)</td>
</tr>
<tr>
<td>No. of villi (per cm of ileum)</td>
<td>5 ± 1 (4-7)</td>
</tr>
<tr>
<td>Villous height (mm)</td>
<td>0.35 ± 0.04 (0.31-0.43)</td>
</tr>
<tr>
<td>Mucosal thickness (mm)</td>
<td>0.48 ± 0.03 (0.44-0.53)</td>
</tr>
</tbody>
</table>

*P<0.05 compared to groups B, C  
**P< 0.05 compared to groups A, B

difference in the number of villi per cm of ileum was not significant in groups B and C on various operation days but the decrease in group A was significant. Mucosal thickness varied significantly on different post-operative days in all the groups.

Discussion

The present study was designed to evaluate the effects of perioperative enteral nutrition with normal rat chow, fibre enriched, and arginine enriched immunoenhancing formulae on the regeneration capacity of the intestine in a minor surgical stress model in rats. Both fibre diets and immunonutrition had been shown to have beneficial effects when given preoperatively in patients with upper gastrointestinal malignancies.

Major surgeries as well as other types of injury have been shown to affect the gut function and nutritional support may modulate these changes in the intestine. Clinical implications of perioperative enteral nutrition with or without defined-formula diets before major surgical stress have been studied earlier. Major complication rates and parameters for postoperative surgical outcome were not significantly different. Despite the similar results of related studies, perioperative enteral nutrition support has been shown to improve outcome of surgical patients in a variety of conditions. However, information is lacking about the effect of these nutrients on patients without malnutrition and with a minor surgical stress only.

Immunonutrients have been shown to upregulate host immune response, to control inflammatory response, and to modulate nitrogen balance and protein synthesis after injury. Zulfikaroglu et al. reported that enteral immunonutrition using glutamine or arginine, ω-3 fatty acids, and RNA supplemented enteral diet during both pre- and post-operative period decreased atrophy of intestinal mucosal villi and reduced bacterial translocation in rats with obstructive jaundice. Gurbuz et al. found improved mucosal morphometrics with supplemental dietary arginine. L-arginine increased villus height and crypt cell mitosis in the small intestinal mucosa and improved weight gain after massive small bowel resection in rats. However, Welters et al. demonstrated that parenteral arginine supplementation in rats with short bowel syndrome led to a slowing of the adaptive response. Immunonutrient enteral diets have also been shown to improve survival in animal models of gut-derived sepsis and have reduced infectious complications and/or hospital stay in several prospective, randomized clinical studies.

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It has been stated that diets enriched with a special formula enhance intestinal mucosal recovery after surgical stress. Our study demonstrated that, fibre or arginine enriched defined formula diets exerted a limited beneficial effect on mucosal morphometrics in rats that were well-nourished and undergone a minor surgical procedure. It was not possible to draw an association between the mortality rate and defined-formula enteral diets because of the limited number of rats in the study groups. However, autopsy revealed anastomotic breakdown or leakage in all of the dead animals which was thought to be mostly related to technique failure.

In conclusion, our study showed that the effect of defined-formula diets was not better than normal rat chow in terms of intestinal morphometry in the surgical stress model in rats, except an increase in the number of villi.
of villi on the 7th postoperative day. However, it is not obvious that number of villi is the leading determinant of mucosal recovery. We postulate that preoperative feeding with enriched diets does not improve mucosal reconditioning after minor surgical stress in rats without malnutrition.

Acknowledgment

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References


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