Use of serum leptin levels for determination of nutritional status and the effects of different enteral nutrients on intestinal mucosa after minor surgery: An experimental study

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Abstract

Background: We planned to evaluate the effects of different enteral nutrients on the levels of serum leptin, protein and albumin changes and also to compare their effects on mucosal morphology of small intestine.

Methods: Rats were randomly divided into 5 groups each including 10 animals. Group I rats were given rat chow and water. Group II rats were fed with standard enteral nutrient. Group III rats were fed with calorie enriched enteral nutrient. Group IV rats were given enteral nutrition supplemented with fiber. Group V rats were fed with immunonutrient. Serum albumin, protein, leptin levels were measured. Terminal ileum of each rat was scored.

Results: We found no difference in serum leptin, protein and albumin levels. The average mucosal atrophy of rats fed with standard chow was significantly different than that of rats fed with standard and calorie enriched enteral nutrient. Feeding with nutrients supplemented with fiber and immunonutrient did not cause significant distortion in mucosal integrity when compared with feeding with standard chow.

Conclusion: Low levels of leptin may show malnutrition but for determination of nutritional status of a patient receiving enteral nutrition, studies with long duration are required.

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Introduction

The beneficial effects of enteral nutrients have been investigated in many studies. Two decades ago, it was shown that early enteral nutritional support after abdominal surgery improved outcome of patients. In recent years, the researchers have been interested in the benefits of...
nutrients with different ingredients such as L-arginine, L-glutamine, ω-3 fatty acids, and nucleotide fragments. 2,3 These nutrients have effects on immunity, intestinal integrity and response to stress. 4-6 Although per operative usage of different enteral nutrients on malnourished patients have been studied extensively, the effects of these nutrients on patients without malnutrition after minor surgery are not well-documented. 7

Anorexia is commonly seen in postoperative patients. Multiple cytokines documented to induce anorexia were showed to increase leptin levels. The cytokine induction of leptin may play a significant role in the anorexia and cachexia of inflammatory diseases such as infections, collagen vascular disease, and cancer. 8 It was demonstrated that circulating levels of proteins such as insulin, growth hormone, leptin and cytokines were elevated by the factors that provoked acute stress response. After surgical and traumatic stress, because of changes in the levels of circulating leptin, anorexia and also negative nitrogen balance become evident. 9-11

Leptin is a hormone involved in regulation of food intake and energy metabolism. 12,13 This protein is specifically released from white adipose tissue and acts on the hypothalamic—hypophyseal axis. The glucocorticoids, endotoxins and cytokines have a regulatory effect on circulating levels of leptin. 8,14 Experimental studies suggest that leptin may be an important metabolic signal for energy regulation. Total parenteral nutrition (TPN) was reported to cause more than fourfold increase in serum leptin levels in 24 h after surgery in humans. 15 Hernandez et al. demonstrated in their study that surgical stress was associated with increase of serum leptin concentrations. They also concluded that total and hypo caloric parenteral nutrition produced quite different effects on serum leptin levels that could be related to distinct insulin response. 16 In another experimental study, the surgical interventions in young rats were shown to be accompanied by decreases in serum leptin levels. The lower leptin concentrations after surgery might be due to decreased postoperative food intake. 17 There was no study comparing the effects of enteral nutrients on serum leptin levels.

The aim of our study was to compare the effects of various defined formula diets containing special combinations of nutrients and normal rat chow on the levels of serum leptin, protein and albumin in rats that underwent minor surgical stress without previous malnutrition. We also designed this study to compare the effects of different enteral nutrients on mucosal morphology of small intestine and body weight changes in surgically stressed rats.

Materials and methods

The procedures in this experimental study were performed in accordance with the national guidelines for the use and care of laboratory animals and were approved by the Animal Ethics Committee of Ankara Training and Research Hospital.

Animals

A total of 50 female Wistar—Albino rats without malnutrition, weighing 235 ± 35 g were included in this study. The animals were allowed free access to water and an isocaloric and isonitrogenous diet (rat chow) 7 days preoperatively. The animals were deprived of food 12 h before surgery but were given free access to water two hours before receiving anesthesia. They were housed under constant temperature (21 ± 2°C) individually in wire cages with 12:12 h light/dark cycle.

Surgical procedure and study groups

All animals were anesthetized by intramuscular injection of 60 mg/kg ketamine hydrochloride (Ketalar®, Parke-Davis, Eczacibasi, Istanbul). The weight of each rat was recorded. The abdomens were shaved and prepared with povidone—iodine. Under sterile conditions, a 2-cm midline incision was made and exploratory laparotomy was performed in all groups as a model for minor surgery. The midline incisions were closed en-bloc with 2-0 silk sutures. The same surgeon performed all operations.

After surgical procedures the rats were randomly divided into 5 groups each including 10 animals. Group I rats were given rat chow and water. Group II rats were fed with standard enteral nutrient (Biosorb Standard®, Nutricia, the Netherlands). Group III rats were fed with calorie enriched enteral nutrient (Biosorb Energy Plus®, Nutricia). Group IV rats were given enteral nutrition supplemented with fiber (Biosorb Fibre®, Nutricia). Group V rats were fed with arginine, ω-3 fatty acids and m-RNA supplemented enteral nutrient (Impact®, Novartis, Switzerland) (Table 1).

Daily food and water intake

In the previous studies, with similar characteristics of rats, the calculated amount of food intake was 350 kcal/kg body weight per day. Therefore, the rats fed one of the four enteral diets received 350 kcal/kg per day. The calorie content was 1.5 kcal/ml for calorie enriched nutrient and 1 kcal/ml for others. These enteral diets were placed in calibrated feeding bottles with fitted pipes. Except for those in the rat chow-fed group, the animals were not allowed to drink water. The liquid diets were renewed every day at 08:00 h in order to avoid spoilage and to control consumption of each rat daily. If the rats did not consume the entire nutrient, the remnant of calculated amount per day was given by orogastric tube for once a day in order to give required calorie to each rat.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Contents of nutrients in 1000 ml</th>
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<tbody>
<tr>
<td></td>
<td>Biosorb Standard®</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>40</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>123</td>
</tr>
<tr>
<td>Lipid (g)</td>
<td>39</td>
</tr>
<tr>
<td>Osmolarity (mOsm)</td>
<td>265</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>1000</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>—</td>
</tr>
<tr>
<td>Arginine (g)</td>
<td>—</td>
</tr>
<tr>
<td>ω-3 fatty acids (g)</td>
<td>—</td>
</tr>
<tr>
<td>m-RNAs (g)</td>
<td>—</td>
</tr>
</tbody>
</table>
Serum leptin, albumin and total protein measurements

On day 7, after being weighed, animals in all groups were euthanized. Blood samples were taken from inferior vena cava at 08:00 and 09:00 h. The serum leptin levels were measured with a commercially available kit (Active® Murine Leptin Elisa DSL-10-24100, Diagnostic Systems Laboratories Inc.). Serum albumin and protein values were evaluated using standard biochemical procedures.

Histological examination

The terminal ileum was removed after the death of the animals and the specimens were fixed in 10% formalin in 0.15 M phosphate buffer (pH: 7.2) and embedded in paraffin. Then they were cut into 5-μm serial sections and stained by hematoxylin–eosin (H&E). They were examined under the light microscope with a magnification of 100×, by the same pathologist who had no knowledge about experimental design in a blinded fashion.

The changes in the terminal ileum were scored from 0 to 4 using the morphological scoring system where '0' represents villous or fingerlike (with no changes), '1' villous, leaf-like or tongue-like, '2' convoluted or cerebroid (Fig. 1), '3' mosaic (Fig. 2) and '4' represents flat (Fig. 3).

Statistical analysis

All results were expressed as mean ± standard deviation (SD). Differences between the groups were evaluated by Mann–Whitney U-test. Multiple comparisons between groups were performed with one way ANOVA and post-hoc tests. The correlations were evaluated in and between the groups by Pearson correlation test. Statistical analysis was done with SPSS 10.0 for Windows (SPSS Inc., Chicago, IL) and p values of less than 0.05 were considered to be significant.

Results

There was one death in control group as a complication of anesthesia and it was excluded from study. There was no other complication during the experimental period in groups. At the end of the experiment, the weight loss was most significant in rats that were given arginine, ω-fatty acids and m-RNA supplemented enteral nutrient (Group V) (p < 0.05; Table 2).

In this study, we found no difference in serum leptin, protein and albumin levels when enteral nutrients with different components were given to rats that underwent minor surgery (p > 0.05; Table 3). Terminal ileum changes of rats fed by standard (Group II) and energy enriched nutrients (Group III) were significantly different when compared with that of control group (p < 0.05; Table 4). There were lymphoid aggregates under muscularis mucosa of rats fed by energy enriched nutrient.

When terminal ileum changes of the rats fed by nutrient supplemented with fiber (Group IV) and arginine, ω-fatty acids and m-RNA supplemented enteral nutrient were compared with that of the control group, although minor morphologic alterations in the villi were observed, no significant difference was recorded (p > 0.05).

Discussion

This experimental study was designed to evaluate the effects of postoperative enteral nutrition with normal rat...
chow, standard, fiber enriched, calorie enriched and arginine, ω-fatty acids and m-RNA supplemented formula on the serum leptin levels and intestinal mucosa following minor surgical stress.

Pre- and postoperative nutritional support for major surgeries improves the outcome in patients with moderate to severe malnutrition. Several studies demonstrated the beneficial effects of defined formula diets on models of major surgeries, but there was only one study showing the effects of immunonutrients after minor surgery. In our study, enteral nutritional support was given after minor surgical stress to rats without previous malnutrition.

There are no certain rules for determination of the nutritional status, for selection of appropriate type of enteral nutrient and for follow-up of enteral nutrition. Furthermore, the effects of enteral nutrition on many organs are not yet clearly defined.

Enteral nutrition has an important role in clinical practice. It protects intestinal morphology and precludes jejunal and ileal mucosal atrophy. There are some studies that were planned to show the effects of different nutrients on body weight changes. One of them reported body weight gains of 100% in rats on enteral nutrition for 28 days with all animals in good nutritional status. In another study, it was shown that L-arginine enriched total enteral nutrition had no effect on body weight gain or tumor growth in rats inoculated with Walker tumor in the kidney. In our study, only arginine, ω-fatty acids and m-RNA supplemented enteral nutrient fed groups had a significant weight loss (p < 0.05), but there was no significant difference between the groups regarding total protein and albumin levels. Since the half life of albumin and protein is longer than our study period (7 days), no change was expected.

The relationship of leptin with nutrition has been researched in many experimental studies. In rodents, fasting results in a decrease in leptin secretion and eating results in an increase. Leptin injection causes a reduction in food intake within 30 min in lean and ob/ob mice and this effect persists for 24 h.

In an experimental study it was demonstrated that obstructive cholestasis in bile duct ligated rats caused significant anorexia for up to 7 days after surgery. Leptin production was found to be significantly increased early after biliary obstruction but it was reduced when obstruction was prolonged. The authors concluded that an increase in serum leptin levels might have contributed to the intense anorexia observed early after biliary obstruction.

Clinical studies showed elevated leptin levels when measured after abdominal and cardiopulmonary bypass surgery in children and adults and have been implicated to promote post surgical anorexia and negative nitrogen balance. It was found that the main increase of leptin occurred approximately 12 h after surgery. The mechanism of the increased leptin levels in the postoperative period is still unclear. Elevated secretion of stored leptin, stimulation of mRNA synthesis of leptin, or increased amount of protein-bound form are possible explanations.

It was reported that simultaneous measurements of serum IGF-I and leptin might provide information about the postoperative nutritional status of oral tumor patients. The authors explained that the effect of prolonged fasting on leptin concentration was stronger than that of patients who underwent resection surgeries. They suggested that serum leptin concentration might be a useful biomarker to reflect the change in body fat level. They found that leptin was the only protein that had a positive correlation with body mass index in healthy volunteers and oral tumor patients.

Major surgeries as well as other injuries have been shown to affect the gut function. Therefore, in order to prevent the effects of surgery we preferred to use a minor surgery model. In our study, no significant difference was observed between the serum leptin levels of all groups. Although the average weight loss in group V was significantly different from the other groups, there was no significant difference between the serum leptin levels of group V and the other groups.

Several factors may influence leptin gene expression and secretion during the postoperative inflammatory and metabolic process. If we evaluate the effectiveness of leptin as a single indicator of nutritional status, it seems that it has little clinical value. Leptin is an indicator of fasting or eating and its secretion is regulated by serum insulin levels. If serum leptin levels are low despite adequate feeding, it shows that there should be severe malnutrition. Although animals in our study had been fed by different nutrients, they had similar

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**Table 2** Changes in body weights of rats between groups (SD, g) mean ± SD, p Values

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SD</th>
<th>p Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (n = 9)</td>
<td>+2.2 ± 11.4</td>
<td>I vs. V: 0.003</td>
</tr>
<tr>
<td>Group II (n = 10)</td>
<td>+5.5 ± 13.0</td>
<td>II vs. V: 0.002</td>
</tr>
<tr>
<td>Group III (n = 10)</td>
<td>+1.0 ± 14.3</td>
<td>III vs. V: 0.009</td>
</tr>
<tr>
<td>Group IV (n = 10)</td>
<td>−1.0 ± 16.8</td>
<td>IV vs. V: 0.035</td>
</tr>
<tr>
<td>Group V (n = 10)</td>
<td>−17.5 ± 12.3</td>
<td>¬*</td>
</tr>
</tbody>
</table>

*Only significant p values are given in the table.

**Table 3** Average serum albumin, protein and leptin values of the groups (mean ± SD)

<table>
<thead>
<tr>
<th>Protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Leptin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>7.72 ± 0.36</td>
<td>3.72 ± 0.22</td>
</tr>
<tr>
<td>Group II</td>
<td>7.77 ± 0.41</td>
<td>3.93 ± 0.36</td>
</tr>
<tr>
<td>Group III</td>
<td>7.60 ± 0.35</td>
<td>3.59 ± 0.50</td>
</tr>
<tr>
<td>Group IV</td>
<td>7.79 ± 0.38</td>
<td>3.78 ± 0.35</td>
</tr>
<tr>
<td>Group V</td>
<td>7.68 ± 0.47</td>
<td>3.67 ± 0.42</td>
</tr>
</tbody>
</table>

For all groups p > 0.05.

**Table 4** Ileal scores of the groups mean ± SD, p Values

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean scores ± SD</th>
<th>p Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>1.22 ± 0.67</td>
<td>I vs. II: 0.017</td>
</tr>
<tr>
<td>Group II</td>
<td>2.50 ± 1.08</td>
<td>¬*</td>
</tr>
<tr>
<td>Group III</td>
<td>3.20 ± 0.98</td>
<td>I vs. III: 0.001</td>
</tr>
<tr>
<td>Group IV</td>
<td>2.20 ± 1.23</td>
<td>¬*</td>
</tr>
<tr>
<td>Group V</td>
<td>1.80 ± 1.03</td>
<td>III vs. IV: 0.009</td>
</tr>
</tbody>
</table>

*Only significant p values are given in the table.
leptin values when compared with each other. This could be explained by the equal amount of calories and almost similar amount of components that were given.

Immunonutrition has a better protective effect on atrophy of intestinal mucosal barrier when it is given both before and after operation than only in the postoperative period in an experimental model of obstructive jaundice.\(^5\) Feeding with nutrients supplemented with fiber and immunonutrients did not cause significant distortion in mucosal integrity when compared with standard chow. We surmised that the fiber and arginine in these nutrients might have a protective effect on intestinal villi.

In experimental studies the distal portion of the ileum, mostly the villi, was reported as the most vulnerable part of the gut to physical injury in obstructive jaundice.\(^2\) Blood flow to the distal gut was increased in rats fed with immunonutrient as compared to rats fed with standard rat chow.\(^24,25\) The mechanism for enterally stimulated mucosal blood flow to the distal gut was increased in rats fed with immunonutrient production and cellular immune function by in-\underline{munonutrition involves selective perfusion of the terminal ileum during immunonutrient absorption. Immunonutrients enhance immune competence noted both clinically and in cytokine production and cellular immune function by increasing ileal blood flow in rats.\(^25,26\)}

In our study, the average score of mucosal atrophy of rats fed with standard chow was significantly different from the rats fed with standard and calorie enriched nutrients. When we compared these two groups there was no significant difference but distortion of villi and flattening was more obscure in the calorie enriched group. The only difference between these groups was osmolarity of the nutrients. The nutrient components and rate of contribution were similar.

**Conclusion**

Although the weight loss in rats fed with arginine, \(\omega\)-fatty acids and m-RNA supplemented enteral nutrient was significant, preservation of intestinal continuity may be due to the protective effects of this nutrient. The preference of these nutrients should be limited to specific patients. In our study, no significant difference was detected between the serum leptin levels of all groups. In clinical studies, it was demonstrated that the main increase of leptin occurs 12 h after surgery. It was also suggested that simultaneous use of leptin and IGF-I might provide information with regard to the efficacy of nutritional support and nutritional status. Although low levels of leptin may show malnutrition, further clinical studies are required regarding the use of leptin for determination of nutritional status of patients receiving enteral nutrition.

**Conflicts of interest**

None.

**Funding**

None.

**Ethical approval**

Ethical approval was given by Animal Ethics Committee of Ankara Training and Research Hospital. Reference Number:06.10.2004/111.573.

**References**


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**Disclosure**

None.

**Funding**

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