Early Postoperative Oral Intake Accelerates Upper Gastrointestinal Anastomotic Healing in the Rat Model

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Early postoperative oral intake accelerates upper gastrointestinal anastomotic healing in the rat model

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A running title; Mechanical benefits of early postoperative oral intake

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Abstract

Background In our previous study, we reported that early postoperative oral feeding accelerated upper gastrointestinal anastomotic healing in rats. To investigate its underlying mechanism, we performed in vivo and in vitro experiments.

Materials and Methods Rats that received proximal jejunal anastomosis were divided into four groups: the enteral nutrition (EN) group were fed via gastrostomy, the total parental nutrition (TPN alone) group were fed via a venous catheter, the TPN + saline group received an additional administration of normal saline solution via gastrostomy, and the TPN + water group received an additional administration of distilled water via gastrostomy. The anastomotic bursting pressure (ABP) and the hydroxyproline content of the anastomotic tissue were measured 5 days postoperatively. In an in vitro setting, the rat gastrointestinal fibroblasts were subjected to uniaxial stretching for 60 min, and the expression of type I and type III collagen mRNA was evaluated.

Results The ABP and hydroxyproline content in the EN group, the TPN + saline group, and the TPN + water group were significantly higher than those in the TPN alone group (ABP; 214.6 ± 42, 199.4 ± 36 and 187.3 ± 29 vs. 149.5 ± 49mmHg; p<0.01, Hydroxyproline; 63.5 ± 10, 67.8 ± 13 and 64.1 ± 14 vs. 50.5 ± 12 micro-mol/g dry

2
tissue; $p<0.01$). The mRNA levels of type I and type III collagen were increased by stretch stimulation.

Conclusions These results suggest that mechanical loading plays a key role in anastomotic healing. Further investigations are necessary to confirm this suggestion.

Key words mechanical loading; early postoperative oral intake; upper gastrointestinal; anastomotic healing
INTRODUCTION

Early postoperative oral feeding is generally defined as a liquid diet on either postoperative day 1 or 2, with advancement of the diet as tolerated [1, 2]. Based on recent studies, there is a growing body of evidence to demonstrate the feasibility and effectiveness of early postoperative oral feeding in the field of lower gastrointestinal surgery [1-10]. Early postoperative oral feeding has not been attempted after upper gastrointestinal anastomosis because of a fear of possible anastomotic leakage due to mechanical stimulation and the greater intraluminal pressure increase caused by early oral feeding after upper gastrointestinal anastomosis [1]. Some recent clinical experiments have shown the benefits of early enteral feeding after upper gastrointestinal anastomosis [11, 12], but these studies were designed so that the anastomosis was protected by infusing the enteral formula distal to the anastomotic site. Hence, it is impossible to regard these series in the same light as early postoperative oral feeding. As might be expected, few animal experiments have evaluated the effect of early oral feeding after upper gastrointestinal anastomoses. So, we performed our previous study to determine whether early postoperative oral feeding after upper gastrointestinal resection has a definite advantage in terms of anastomotic healing compared to parenteral nutrition with a fasting period [13]. To examine this issue, we
designed a rat model of proximal jejunal anastomotic healing, in which nutrient solutions were administered via gastrostomy as a substitute for feeding by mouth; the nutrient solutions stream passed immediately through the anastomosis. This rat model allowed for the evaluation of the effect of early postoperative oral feeding on upper gastrointestinal anastomotic healing in the clinical setting. As a result, early enteral feeding via tube-gastrostomy enhanced anastomotic healing of the proximal jejunum in rats as compared with that after parenteral feeding with a fasting period; i.e., it was indicated that early oral feeding after upper gastrointestinal surgery led to prompt anastomotic healing. In the discussion of our previous study, we pointed out three possible explanations for the improved healing induced by early postoperative enteral nutrition via gastrostomy. The first hypothesis was that the enteral feeding itself had had a trophic effect on cells such as fibroblasts and smooth muscles cells or perhaps the nutrient solution had reduced collagen breakdown by controlling inflammation at the anastomotic site. The second option was that the enteral feeding via gastrostomy had induced a systemic effect, such as activation of a gut-derived hormone, that had impacted on anastomotic healing. The third possibility was that the mechanism was associated with an effect of mechanical loading on anastomotic fibroblasts. It is possible that continuous infusion of solutions activates intestinal fibroblasts cells,
leading to mechanical loading, e.g., early recovery of bowel peristalsis or shear stress, and accelerates the synthesis of new collagen. The aim of the present study is to clarify the underlying mechanism of upper gastrointestinal anastomotic healing accelerated by early postoperative oral feeding. To address this issue, we planned a two-step approach as follows. The first step was to test whether a trophic effect of enteral feeding on the anastomotic site is crucial to enhancing anastomotic wound healing through the use of the rat model mentioned above. The second approach was to explore the impact of mechanical loading on rat fibroblasts derived from the gastrointestinal tract in vitro.

MATERIALS AND METHODS

Animals

Eighty male, nine-week-old, Sprague-Dawley rats (Nippon Clea, Tokyo, Japan), weighing approximately 290 g, were used in the experiment. The animals were maintained at 21°C under 12 hour light / dark cycles and allowed free access to water and standard chow for 3 days. The experimental protocol were carried out in a humane manner after receiving approval from the Institutional Animal Experiment Committee of the University of Tsukuba and in accordance with the Regulations for Animal Experiments of our university and the Fundamental Guidelines for Proper Conduct in Animal Experiments and Related Activities in Academic Research Institutions under the
Operative procedure

Prior to surgery, all rats were fasted overnight. They were anesthetized by intraperitoneal injection of pentobarbital sodium (Nembutal®, Dai-Nippon Pharmaceutical Co., Tokyo) at a dose of 35mg/kg of body weight. The operative procedures were the same as those used in a previous report [13]: under aseptic conditions, jugular vein catheterization to a central venous position, gastrostomy, and jejunal anastomosis were carried out. The jejunal anastomoses were performed 2.0 cm distal from the duodenum. The rats were divided into two major groups, the enteral nutrition (EN) group (n=20), to which nutrient solutions were administered via gastrostomy, and the total parenteral nutrition (TPN) group (n=60), to which nutrient solutions were administered via a central venous catheter. The TPN groups were further divided equally into three subgroups: the TPN + saline group (n=20), which received a continuous infusion of normal saline solution via gastrostomy; the TPN + water group (n=20), which received a continuous infusion of distilled water via gastronomy; and the TPN alone group, which received no solution via gastrostomy. The TPN alone group underwent a fasting period; whereas, both the TPN + saline group and the TPN + water group underwent an enteral intake of either non-nutrient solution, the osmolarity of
which differed; normal saline solution as an isotonic solution; or distilled water as a hypotonic solution. Finally, the results were compared with those of the EN group and the two TPN subgroups that received administrations of non-nutritive solutions via gastrostomy (the TPN + saline group and the TPN + water group); whereas, the TPN alone group served as a control. Following these procedures, the rats were maintained in individual metabolic cages for 5 days.

**Nutrient solutions**

The nutrient solutions (Neoparen 2®, Otsuka Pharmaceutical Co., Ltd., Tokyo) contained glucose, amino acids, electrolytes, minerals, and vitamins (Table 1). Identical nutrient solutions were administrated to each group, via either gastrostomy or a central venous catheter. Continuous infusions using infusion pumps (SP-115, JMS Co., Ltd., Tokyo) were begun immediately after surgery. The full dose energy intake was 151 kcal/kg body weight per day, which was equal to an infusion rate of 9 ml/kg body weight per hour (approximately 2.6 ml per hour). This target value corresponds to about 21 kcal/kg body weight per day for humans [14], which is thought to be reasonable during the acute phase of surgical stress [15]. Twenty-four hours postoperatively, half of the target volume had been administered. Additionally, in the TPN + saline group and the TPN + water group, normal saline solution and distilled water, respectively,
were administrated via gastrostomy at an infusion rate of 1ml per hour immediately after surgery. The reason why an infusion rate of 1ml per hour for the non-nutrient solutions was designed is as follows. Volume overload not only has a harmful effect on circulation dynamics, but may have deleterious effects on anastomotic healing. So, we examined what was an appropriate infusion rate for the non-nutrient solutions that allowed for avoiding negative impacts on the circulation dynamics, including body weight gain induced by edema. After that, we reached a conclusion that an infusion rate of 1ml per hour seemed to be appropriate as it hardly strained the circulation dynamics.

**Nutritional parameters**

Urinary output and body weight were recorded. On postoperative day 5, the rats were deeply anesthetized by an intraperitoneal injection of pentobarbital sodium at a dose of 70 mg/kg of body weight, and the infusion of nutrients was stopped. Blood was drawn from the inferior vena cava to determine the serum levels of total protein, albumin, glucose, and blood urea nitrogen.

**Anastomotic bursting pressure**

After the animals had been anesthetized, the abdomen was opened, and the anastomotic bursting pressure (ABP) was measured in situ without interruption of the normal mesenteric blood supply or adhesions to the anastomosis. A 16-gauge silicone
rubber catheter was inserted 1.5 cm from the oral side of the anastomosis to the distal side, and two ligations were made, 1.5 cm proximal and distal from the anastomosis with 3-0 silk sutures. Normal saline solution was continuously infused through the catheter via a syringe pump at a rate of 1.0 ml/min. Intraluminal pressure was monitored continuously via a transducer (BLPR®, World Precision Instruments Inc., Saratosa, FL) and recorded on a chart recorder (PowerLab®, AD Instruments, Tokyo). ABP was defined as the peak pressure attained just before the rupture of the anastomosis.

**Collagen concentration**

Hydroxyproline is useful as an index of collagen concentration because it is the most frequently used amino acid in collagen formation [16]. After the bursting of the anastomosis, a 1 cm anastomotic segment, i.e., 0.5 cm proximal and distal from the anastomosis, was excised and dissected free of mesenteric fat and adherent omentum. The concentration of hydroxyproline was measured as described by Guzmán et al. [17]. First, the freeze-drying of the specimen and complete hydrolysis with 6 N hydrochloric acid were performed. Then, derivatization was carried out by the addition of fluorescamine solution to the sample, which was analyzed for hydroxyproline using capillary electrophoresis.
Isolation and culture of rat gastric fibroblasts

The gastric tissue obtained from male Sprague-Dawley rat was minced into small pieces and washed with serum free Dulbecco’s modified Eagle’s medium (DMEM) (Sigma-Aldrich, Taufkirchen, Germany). The tissue samples were then placed in culture dishes with DMEM containing 10% fetal bovine serum (Hyclone, Road Logan, UT), 1% penicillin, and streptomycin (Invitrogen, Grand Island, NY) at 37 °C in 5% CO2, whilst allowing the cells to migrate from the explants. Following explantation, the cells were maintained in DMEM supplemented with 10% fetal bovine serum. Daily observation with a phase contrast microscope revealed the migration and proliferation of spindle-shaped cells. These cells were subcultured. In immunohistochemistry, the spindle-shaped cells were found to be positive for prolyl 4-hydroxylase beta (Acris Antibodies GmbH, Herford, Germany), which is an enzyme critical to the production of collagen, and its presence was used as a fibroblast marker [18] (Fig. 1).

Stretch system

The cells were placed on silicone chambers (32 x 32 mm²) (STREX, Osaka, Japan) coated with bovine fibronectin (WAKO, Osaka) in DMEM supplemented with 10% fetal bovine serum for 36 h. The chambers were set on a pulse-motor-driven stretch machine (STREX) and a uniaxial stretch was applied at 37 °C in 5% CO2 (120% in
length, at a frequency of 30 cycle / min; normal bowel sounds were physiologically audible at the rate of about one per two to three seconds). The stretch was applied for 60 minutes.

Reverse transcriptase polymerase chain reaction for type I collagen and type III collagen

Total cellular RNA was isolated from silicon chambers using the Isogen reagent (Nippon Gene, Tokyo, Japan). First-strand cDNA was synthesized using reverse transcriptase, 2 µg of total RNA, and oligo (dT) primers. cDNA for type I collagen, type III collagen, and rat GAPDH was amplified by PCR. The primers for type I collagen, type III collagen and rat GAPDH were as follows: type I collagen: forward: 5’-TGGAGACAGGTCAGACCTG-3’, reverse: 5’-TA TTCGA TGACTGTCTTGCC-3’;
type III collagen: forward: 5’-TAAAGGGTGAACGGGGCAGT-3’, reverse: 5’-ACGTTCCCCATTATGGCCAC-3’; rat GAPDH: forward: 5’-TTCATTGACCTCAACTACAT-3’, reverse: 5’-GAGGGGCCATCCACAGTCTT-3’. The PCR products were separated on 2% agarose gels.

Statistics

All data are presented as the mean value ± standard deviation (SD). Statistical analyses were performed with one-way factorial ANOVA and Bonferroni's multiple
t-test; $P$ values less than 0.05 were considered statistically significant.

**RESULTS**

**In vivo**

**Nutritional parameters**

The initial body weight and body weight 5 days after the operation were similar in the four groups (Table 2). The total energy intake did not differ in the four groups. Total urine volume in the TPN + saline group and that in the TPN + water group were higher than those in the EN group and the TPN alone group because of the additional administration of normal saline solution or distilled water via gastrostomy. There were no significant differences among the four groups with respect to the serum levels of total protein, albumin, glucose, or blood urea nitrogen (Table 3).

**ABP**

There was no intraperitoneal abscess formation, and no anastomotic leakage was noted in any animal. All tested anastomoses burst at the suture line. The ABP values of the EN group, the TPN alone group, the TPN + saline group, and the TPN + water group were $214.6 \pm 42$, $149.5 \pm 49$, $199.4 \pm 36$, and $187.3 \pm 29$ mmHg, respectively (Fig. 2). In the EN group, the TPN + saline group, and the TPN + water group, the ABP values were significantly higher than that of the TPN alone group ($p<0.01$). No
significant difference was detected between the EN group and the TPN + saline group.

On the other hand, there was a significant difference between the EN group and the TPN + water group ($p < 0.01$).

**Collagen concentration in jejunal anastomotic tissue**

The hydroxyproline concentrations of the EN group, the TPN alone group, the TPN + saline group, and the TPN + water group were 63.5 ± 10, 50.5 ± 12, 67.8 ± 13, and 64.1 ± 14 micro-mol/g dry tissue, respectively (Fig. 3). The hydroxyproline concentrations of the EN group, the TPN + saline group, and the TPN + water group were significantly higher than that of the TPN alone group ($p < 0.01$).

**In vitro mRNA expression of type I and type III collagen**

The mRNA levels of type I and type III collagen were clearly increased at 60 min after the initiation of stretching (Fig. 4).

**DISCUSSION**

In our previous study, we demonstrated that early postoperative enteral feeding via gastrostomy accelerated jejunal anastomotic healing in comparison to parenteral feeding with a fasting period [13]. Regarding the underlying mechanism of enhanced anastomotic healing with early enteral nutrition via gastrostomy, we proposed three possible explanations: (1) a trophic effect originating from the enteral feeding itself, (2)
a systemic effect such as activation of a gut-derived hormone that has an impact on anastomotic healing, and (3) an effect of mechanical loading on anastomotic fibroblasts.

To elucidate the real cause, we planned a two-step approach. The first approach was to examine whether a trophic effect of enteral feeding on the anastomotic site played a crucial role in promoting anastomotic wound healing. This approach allowed us to rule out the first hypothesis as it was confirmed that parenteral feeding with the administration of non-nutrient solutions via gastrostomy promotes jejunal anastomotic healing as well as enteral feeding. Furthermore, the second hypothesis can probably be ruled out because non-nutrient solutions do not undergo digestive processes and so are unlikely to be associated with the activation of a gut-derived hormone. By a process of elimination, the effect of mechanical loading was identified as the probable mechanism behind the promotion of anastomotic healing. The results obtained from the first approach extend previous observations [13], and it might appear surprising that parenteral feeding with administration of non-nutrient solutions via gastrostomy has the same effect as enteral feeding on jejunal anastomotic healing. The ABP in the TPN + saline group and the TPN + water group were significantly higher than that in the TPN alone group. Moreover, the ABP in the TPN + saline group was equal to that in the EN group. The ABP in the TPN + water group suggested a lower trend than that in
the TPN + saline group. ABP was determined 5 days postoperatively in this study as well as in a previous study [13] because it has been reported that after 7 days the anastomoses that rupture predominantly do so outside the anastomosis [19-21], and anastomotic insufficiency usually occurs during the first postoperative week. The ABP is a more reliable measure for evaluating early postoperative anastomotic mechanical strength, especially within a week of the operation [22, 23]. Generally, the bursting pressure is considered to reflect the physiological strain in the intestine more accurately than the breaking strength [23]. In addition, the bursting pressure shows indirect collagen formation [24] and reflects the balance between collagen deposition and lysis. Therefore, the ABP might be regarded as not only an integrated measure of anastomotic wound healing but also the absolute outcome of gastrointestinal anastomoses. From the above-mentioned viewpoint, the results of the first approach showed that the administration of non-nutrient solutions via gastrostomy under TPN accelerated jejunal anastomotic healing in comparison to TPN alone with a fasting period and provided comparable effects to early enteral feeding. Needless to say, the data on collagen concentration at the anastomosis coincided with the ABP results and reinforced the notion mentioned above. To our knowledge, this paper is the first report demonstrating that an administration of non-nutrient solutions via gastrostomy has a positive impact
on upper gastrointestinal anastomotic healing. An administration of non-nutrient
solutions via gastrostomy is a suitable model of early oral intake of rehydration solution
or water in the clinical settings. As described previously, sequence-driven logic flow
allowed us to select the third hypothesis; i.e., the effect of mechanical loading on
anastomotic fibroblasts.

However, it remains unclear how non-nutrient solutions exert mechanical loading on
anastomotic fibroblasts. In general terms, mechanical loads are roughly divided into
two different types: stretch stimuli and shear stress. In the gastrointestinal tract, these
mechanical loads might include bowel peristalsis-induced stretch stimuli or enteric
flow-induced shear stress. In cases of very low enteric flow such as the continuous
infusion rate of 1ml per hour used in the above in vivo experiment, there seems to be
little possibility that the shear stress plays a part in anastomotic wound healing. So, we
focused attention on bowel peristalsis-induced stretch stimuli and designed a second
approach for the purpose of exploring the impact of mechanical loading on rat
fibroblasts derived from gastrointestinal tract in vitro. It seems likely that the
administration of non-nutrient solutions via gastrostomy facilitates resolution of the
postoperative ileus and active bowel peristalsis because early oral intake of rehydration
solutions after abdominal surgery shortens the time to the first flatus as well as early
enteral feeding. Using a pulse-motor-driven stretch machine, we evaluated the effect of stretch stimuli on collagen synthesis in rat gastric fibroblasts. The frequency of the stretch cycle was set to 30 cycles / min on the physiological basis that normal bowel sounds followed by bowel peristalsis are audible at the rate of about one per two to three seconds. In this in vitro setting, we clearly demonstrated that the expression of type I and type III collagen mRNA were increased by uniaxial stretching for 60 min in the rat gastric fibroblasts. These results imply that mechanical loading such as stretch stimuli contributes to the up-regulation of de novo type I and type III collagen synthesis by rat fibroblasts derived from the gastrointestinal tract. It is considered that collagen fibrils are ultimately responsible for anastomotic strength [23]. In the progress of intestinal anastomotic healing, type III collagen gene expression precedes that of type I collagen in the intestinal anastomosis [25]. The collagen types that are found in the intestinal wall and contribute to bowel strength are type I, type II, and type V. These make up 68%, 20%, and 12% of the collagen fractions, respectively [26]. Hence, the quantity of type I collagen secreted by fibroblasts after gastrointestinal anastomoses is crucial for developing the mechanical strength of the anastomotic site. The connective tissues in the body are constantly subjected to mechanical loading, and it is well known that mechanical loading plays a key role on the maintenance of tissue homeostasis. For
example, it has been demonstrated that the expression of type I and type III collagen mRNA was increased by stretch stimuli in human ligament fibroblasts [27, 28]. Human dermal fibroblasts also showed the increased induction of several extracellular matrix genes by cyclic and static stretching, including type I and type III collagen [29]. Likewise, up-regulation of collagen synthesis after stretching was demonstrated in rat cardiac fibroblasts [30] and human fetal pulmonary fibroblasts [31]. Moreover, mechanical loading delivers anti-apoptotic and proliferative stimuli to human gingival fibroblasts [32], which means that mechanical loading is necessary for the survival of fibroblasts. Thus, several fibroblast cellular processes are driven not only by molecular and biochemical cues, but also by their mechanical environment. Similarly, it is likely that gastrointestinal tract fibroblasts convert mechanical signals arising from bowel peristalsis into cellular biological events to maintain tissue homeostasis, including collagen synthesis. However, few reports have addressed the intimate relationship between mechanical loading and gastrointestinal fibroblasts.

Considering the results obtained from both the in vivo and in vitro experiments of the present study, mechanical loading is assumed to be a key factor for accelerating upper gastrointestinal anastomotic healing, but direct evidence for this idea is lacking.

The limitations of our study include several issues associated with the differences
between the in vivo and in vitro experiments. First, there is no definitive evidence that
the administration of non-nutrient solutions via gastrostomy promotes resolution of the
postoperative ileus and active bowel peristalsis. Secondly, it remains unclear how
different compositions and infusion rates of solutions administered via gastrostomy
affect mechanical loading such as bowel peristalsis. It is more likely that different
compositions of solutions—especially, osmolarity, viscosity value and electrolyte
concentration— influence mechanical loading because there was no significant
difference in ABP between the TPN + saline group and the EN group, in other words,
irrespective of whether the solutions administered via gastrostomy were rich or lacking
in nutrients. On the basis of osmolarity, solutions administered via gastrostomy are
categorized into three types: nutritional solutions are hypertonic, normal saline solution
is isotonic, and distilled water is hypotonic. The TPN + water group, in which the
solution was electrolyte-free and hypotonic, was associated with a lower ABP than that
in the TPN + saline group. Therefore, these data seems to suggest that the osmolarity
and electrolyte concentration of solutions play roles in promoting gastrointestinal
anastomotic healing. On the other hand, the different infusion rates of solutions via
gastrostomy appear not to be a key factor. This is because the ABP in the TPN + saline
group was not significantly different from that in the EN group, despite the fact that the
infusion rate of solutions via gastrostomy in the EN group was 2.6 times as much as that in the TPN + saline group. Third, it is necessary to analyze how bowel peristalsis stimulates gastrointestinal fibroblasts mechanically. We put bowel peristalsis into a simple mechanical loading such as stretch conditions, but it was likely to be simplified excessively. The process of wound healing is very complex, and there remains the possibility that other factors might have some impact on wound healing. Further investigations are necessary, including the above-mentioned questions.

Based on recent studies, including our previous study and this one, we deliver the following vision for clinical practice. Provided that anastomosis has been performed correctly and complies with the tension-free construction of the suture, adequate tissue perfusion, and oxygen delivery, early postoperative oral intake after upper gastrointestinal anastomosis does not have a negative impact on anastomotic wound healing. There is a possibility that early postoperative oral liquid intake accelerates upper gastrointestinal anastomotic healing when compared to parenteral nutrition with a fasting period. Again, we propose that it is time to reconsider imposing a period of starvation after upper gastrointestinal surgery.

In conclusion, the administration of non-nutrient solutions via gastrostomy under TPN accelerated jejunal anastomotic healing in comparison to TPN alone with a fasting
period and provided comparable effects to early enteral feeding. The mechanical loading such stretch conditions contributed to the up-regulation of de novo type I and type III collagen synthesis by rat fibroblasts derived from gastrointestinal tract. Further investigations are needed to bridge the gap between the two above-mentioned results.
References


Legends for Figures

Fig. 1

Immunohistochemistry for spindle-shaped cells isolated from the gastric tissue of rats using an antibody for prolyl 4-hydroxylase.

Fig. 2

Anastomotic bursting pressure (mmHg) 5 days postoperatively in the four groups. EN: fed via gastrostomy; TPN alone: fed via central venous catheter; TPN + saline: TPN with administration of normal saline solution via gastrostomy; TPN + water: TPN with administration of distilled water via gastrostomy. Each column represents the mean value ± standard deviation of rats in the four groups; *: $p < 0.01$ versus the TPN alone group, †: $p < 0.01$ versus the EN group (one way ANOVA and Bonferroni’s multiple t-test).

Fig. 3

The concentration of hydroxyproline in jejunal anastomotic tissue 5 days postoperatively in the four groups. EN: fed via gastrostomy; TPN alone: fed via central venous catheter; TPN + saline: TPN with administration of normal saline solution via
gastrostomy; TPN + water: TPN with administration of distilled water via gastrostomy.

Each column represents the mean value ± standard deviation of rats in the four groups.

*: $p < 0.01$ versus the TPN alone group (one way ANOVA and Bonferroni’s multiple t-test).

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Fig. 4

Type I collagen and type III collagen mRNA expression of rat gastric fibroblasts 0 min and 60 min after the initiation of stretching.

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<td>Amino acid</td>
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Table 2  Nutritional parameters in the four groups

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EN: fed via gastrostomy; TPN alone: fed via central venous catheter; TPN + saline: TPN with administration of normal saline solution via gastrostomy; TPN + water: TPN with administration of distilled water via gastrostomy.
Table 3  Biochemical nutritional parameters in the four groups

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<td>Blood urea nitrogen (mg/dl)</td>
<td>8.1 ± 2.4</td>
<td>11 ± 3.6</td>
<td>8.5 ± 2.2</td>
<td>11 ± 2.5</td>
</tr>
</tbody>
</table>

EN: fed via gastrostomy; TPN alone: fed via central venous catheter; TPN + saline: TPN with administration of normal saline solution via gastrostomy; TPN + water: TPN with administration of distilled water via gastrostomy.