Early enteral nutrition with arginine compensates for negative nitrogen balance in patients undergoing curative total gastrectomy

ORIGINAL

Early enteral nutrition with arginine compensates for negative nitrogen balance in patients undergoing curative total gastrectomy

Yasuko Okamoto[1,2], Takanori Sakaguchi[3], Yoshito Ikematsu[4], Toshikazu Kanai[4], Kazuhisa Hirayama[4,5], Hiroaki Tamura[4], Tadataka Hayashi[4], Yoshiro Nishiwaki[4], Hiroyuki Konno[6], and Katsunori Aoki[6]

[1]*Faculty of Home Economics, Aichi Gakusen University, Okazaki, Aichi, Japan,* [2]*Clinical Nutritional Management, Hamamatsu Medical Center, Hamamatsu, Shizuoka, Japan,* [3]*Division of Surgery, Iwata City Hospital, Iwata, Shizuoka, Japan,* [4]*Department of Gastroenterological Surgery, Hamamatsu Medical Center, Hamamatsu, Shizuoka, Japan,* [5]*Surgery, Shizuoka City Shimizu Hospital, Shizuoka, Japan,* [6]*Department of Emergency Medicine, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan*

Abstract : The effects of early enteral arginine-rich nutrition (EAN) were analyzed among patients undergoing curative-intent total gastrectomy for gastric cancer. There were 19 patients in this prospective study, all randomly assigned to either a parenteral nutrition (PN) group or an EAN group for the first seven days after surgery. The EAN group received 1.8-fold greater arginine (10.1 g/day) compared with the PN group, which was administered through an enteral tube inserted into the jejunal loop. Both groups were provided almost identical amounts of total amino acids (54 g/day), and the total energy was set at 65% of the total requirement (25 kcal/kg/day). No significant differences were observed between the two groups in postoperative complications, length of hospital stay, oral intake, nutritional status, or body weight. The serum arginine profile was similar in the two groups, as it decreased significantly on postoperative day (POD) 1, and gradually returned to preoperative levels by POD 7. The nitrogen balance remained negative until POD 7 in the PN group, but turned neutral at POD 7 in the EAN group. While we could not confirm body weight loss improvement, these results suggested that early arginine-rich enteral nutrition could improve the nitrogen balance after total gastrectomy. J. Med. Invest. 70 : ●-●, August, 2023

Keywords : *early enteral nutrition, arginine, nitrogen balance, gastric cancer*

INTRODUCTION

Despite advances in surgical techniques and perioperative management of total gastrectomy for gastric cancer, postoperative complications such as surgical site infection (SSI), anastomotic leakage, and postoperative intestinal paresis cannot always be avoided, and early postoperative oral or enteral nutritional support has been employed to reduce the incidence and the severity of these complications (1). Based on the Japanese Guidelines for Nutrition Support Therapy in Adult and Pediatric Critically Ill Patients (2), early enteral nutrition should start within 48 hours after an operation. Enteral nutrition support cannot be conducted for patients with milk allergies, and is likely to have gastrointestinal complications such as abdominal distensions and diarrhea. Even considering these disadvantages, enteral nutrition is more acceptable from a physiological standpoint, has fewer complications, and is less expensive, compared with parenteral nutrition. The European Society of Clinical Nutrition and Metabolism (ESPEN) guidelines on surgery recommend a total energy supply of 25 kcal/kg/day (3), but, in actual practice, it is hard to deliver this amount in the first seven days after surgery. Furthermore, the Canadian clinical practice guidelines underscore the importance of protein dosage based on the results of intentional underfeeding studies of hypocaloric enteral nutrition (4). Arginine is a conditional amino acid, along with cysteine, glutamine, tyrosine, glycine, ornithine, proline, and serine, and is a precursor for the synthesis of nitric oxide, polyamines and nucleic acids ; and the latter two are crucial for protein synthesis. It also enhances wound healing and increases hormone secretion with protein assimilation. In animal models, early postoperative arginine-rich enteral nutrition promoted wound healing (5), and suppressed bacterial translocation in the upper intestinal lumen (6, 7), indicating the clinical usefulness of early postoperative amino acid-rich enteral nutrition. Several reports have indicated that arginine is crucial for appropriate nitrogen balance recovery from starved conditions (8, 9). Based on these findings, the ESPEN and American Society for Parenteral and Enteral Nutrition (ASPEN) guidelines recommend arginine as a constituent of enteral nutrition formulations provided to patients during the early postoperative phase after injury or elective surgery (10, 11). However, only a few reports have assessed the effects of early postoperative arginine-rich enteral nutrition, without immunomodulating agents, among patients undergoing elective gastrointestinal surgery.

Abbreviations used :
ASPEN, American Society for Parenteral and Enteral Nutrition ; BCAAs, branched chain amino acids ; BMI, body mass index ; CRP, C-reactive protein ; DAO, diamine oxidase activity ; EAN, enteral arginine-rich nutrition ; EN, enteral nutrition ; ESPEN, European Society for Clinical Nutrition and Metabolism ; IL-6, interleukin-6 ; iNOS, nitric oxide synthase ; NO, nitric oxide ; PN, parenteral nutrition ; POD, postoperative day ; PPN, peripheral parenteral nutrition ; RE, retinol equivalents ; SGA, subjective global assessment ; SSI, surgical site infection

Received for publication October 4, 2022 ; accepted March 8, 2023.

Address correspondence and reprint requests to Yasuko Okamoto, Faculty of Home Economics, Aichi Gakusen University, 28 Kamikawanari, Hegoshi-cho Okazaki, Aichi 444-8520, Japan and Fax : +81-0564-34-1270. E-mail : yako.okamoto65@gmail.com

Therefore, the aim of this study was to investigate whether or not, compared to peripheral parental nutrition, early postoperative arginine-rich enteral nutrition improves nitrogen balance and suppresses weight loss in patients undergoing total gastrectomy in the first seven days after surgery. Specifically, the arginine-rich enteral nutrition (EAN) group was given 1.8-fold greater arginine, compared with the peripheral parenteral nutrition (PN) group. Due to a limitation in the number of available injection solutions, we could not match the total amount of amino acids and total energy of the two groups. Simanuki (12) reported that the nitrogen balance is proportional to total amount of nitrogen intake, while nitrogen balance does not depend on the non-protein energy intake. Therefore, the total amount of amino acids was set so that it was almost identical in both groups (54 g/day) at around 90% of the total requirement (60 g/day) (3), and total energy was set at around 65% of the total requirement (25 kcal/kg/day) in both groups.

MATERIALS AND METHODS

Patients and study design

This prospective study included 19 gastric cancer patients who underwent curative total gastrectomy between January 2010 and December 2011 at the Hamamatsu Medical Center. The age range of the patients was 56-82 years, with a median age of 71 years. The average values for age, height, and weight of the subjects were 69.6 ± 7.2 years, 166.2 ± 7.1 cm and 56.7 ± 13.3 kg for males, and 71.2 ± 10.7 years, 150.7 ± 3.9 cm and 45.0 ± 10.2 kg for females (means ± SD), respectively. The study protocol was approved by the Ethical Committee of the Hamamatsu Medical Center (No.21-10), and was registered in the UMIN-CTR as a clinical trial (UMIN000026770). All of the patients provided written informed consent. The inclusion criteria were as follows : 1) Patients with good performance, who did not have any other severe disease. 2) Patients who did not suffer from preoperative malnutrition, defined as unintentional weight loss of at least 10%-15% within the previous 3-6 months or a body mass index (BMI) of <18 kg/m^2. 3) Patients with clinical stages of gastric cancer that were less than Stage Ⅳ. 4) Patients who agreed to give their informed consent. Patients with milk allergies were excluded because enteral nutrition support was not appropriate.

All of the patients were randomly assigned using a sealed envelope system to one of 2 groups for the postoperative nutritional supplementation, specifically, parenteral nutrition (PN) alone (PN group) or those who received early enteral arginine-rich nutrition (EAN) along with parenteral nutrition (EAN group). A pair of opaque envelopes were prepared for each patient. The envelopes contained a sheet of paper with either PN or EAN written on it. After the patients had consented to the conditions of the study, each patient chose one of envelopes, and then they were offered nutritional treatment. We did not adjust the total number of patients in each group. As a result of the randomization by using the envelope system, 12 and 8 patients were assigned to the PN group and the EAN group, respectively. After the surgical operation, one of the patients in the EAN group was diagnosed as Stage IV. This patient was excluded from the EAN group, and so the total number of patients was 19. As shown in Table 1, all of the other patients satisfied the inclusion criteria. The distribution of the clinical stages of gastric cancer among the patients was as follows : 6 patients in Stage I, 7 in Stage II, and 6 in Stage III.

During the gastrectomy operations, perigastric lymph nodes were dissected and reconstruction was performed using the Roux-en-Y technique after total gastrectomy. In the EAN group alone, an enteral tube, 140 cm in length, was inserted into the jejunal loop and placed approximately 15-20 cm on the anal side of the lowest anastomosis. All of the other procedures were similar for the two groups.

Nutrition protocol and treatment schedule

Four types of injection solutions were used for the peripheral parenteral nutrition (PPN), viz., one pack of Lactec D Injection (100 kcal/500 mL) (Otsuka Pharmaceutical Factory. Inc., Tokushima, Japan) that contained 25 g glucose, 18 mEq lactate, 65 mEq Na, 2 mEq K, 1.5 mEq Ca, and 55 mEq Cl ; one pack of Lactec Injection (0 kcal/500 mL) (Otsuka Pharmaceutical Factory. Inc., Tokushima, Japan) that included 14 mEq lactate, 65 mEq Na, 2 mEq K, 1.5 mEq Ca, and 55 mEq Cl ; one pack of BFLUID Injection (420 kcal/1,000 mL) (Otsuka Pharmaceutical Factory. Inc., Tokushima, Japan) that included 75 g glucose, 35 mEq Na, 20 mEq K, 35 mEq Cl, 30.0 g free amino acids (including 3.15 g of arginine), and 1.5 mg vitamin (Vit) B_1 ; and one pack of SOLDEM 3AG Injection (150 kcal/500 mL) (TERUMO Inc., Tokyo, Japan) that provided 37.5 g glucose, 17.5 mEq Na, 10 mEq K, and 17.5 mEq Cl. Two types of supplements were used for the enteral nutrition (EN), specifically, one pack of arginine-rich nutritional supplement (100 kcal/125 mL) (Isocal Arginaid, Nestle Nutrition Company, Tokyo, Japan) that provided 5 g of protein (including 2.5 g L-arginine), 20 g carbohydrate, 500 mg Vit C, 125 μg retinol equivalents (RE) of Vit A, 2.4 μg Vit D, 5 mg Vit E, 0.9 mg Vit B_1, 0.8 mg Vit B_2, 10 mg zinc, and 100 μg folic acid ; and one pack of standard whole protein formula (300 kcal/300 mL) (Sanette-SA : Sanwa Kagaku Kenkyusho Co. Ltd., Nagoya. Japan), which contained 16.5 g protein (including 0.744 g L-arginine), 120 g carbohydrate, 45 mg Vit C, 225 μg RE of Vit A, 0.96 μg Vit D, 6 mg Vit E, 0.45 mg Vit B_1, 0.45 mg Vit B_2, 4.2 mg zinc, and 300 μg folic acid.

The details of the experimental protocols implemented in the EAN and the PN groups are shown in Fig. 1, and the amounts of the nutrition provided from POD 1 to POD 7 are summarized in Table 2. The nutrition solutions administered were identical in both groups on operation day (POD 0) and on POD 1. On POD 0, 1 pack of Lactec D and 1 pack of Lactec (0 kcal/500 mL) were injected intravenously, which provided 100 kcal of total energy and 1,000 mL of water, respectively. On POD 1, parenteral nutrition was supplied using 1 pack of BFLUID Injection and 2 packs of SOLDEM 3AG, which provided 720 kcal, 2,000 mL, and 3.15 g of total energy, water, and arginine, respectively.

In the PN group, only the peripheral parenteral nutritional (PPN) support was provided. As shown in the right column of Fig. 1, on POD1, this group received 1 and 2 packs of BFLUID Injection and a SOLDEM 3AG Injection, respectively, which provided 720 kcal, 2,000 mL, 3.15 g and 30.0 g of total energy, water, arginine and amino acids, respectively. On POD 2,1.5 and 1 packs of these solutions were applied, which provided 780 kcal, 2,000 mL, 4.73 g and 45.0 g of total energy, water, arginine and amino acids, respectively. From PODs 3-7, 2 packs of BFLUID Injection were provided daily, which provided 840 kcal, 2,000 mL, 6.30 g and 60.0 g of total energy, water, arginine and amino acids, respectively.

In the EAN group, water and electrolytes were provided by the PPN through 1 pack of BFLUID Injection, which was intravenously administered daily from PODs 2-7 until the patients could consume sufficient amounts of food orally. The enteral nutrition (EN) was started on POD 2, which was administered using an enteral feeding pump (Top enteral feeding TOP6100 ; Fuji Electric F-Tech Co., Ltd., Japan or Kangaroo Feeding Pump 624 Type ; Covidien Company, Japan). As shown in the left column of Fig. 1, on POD 2, 3 packs of arginine-rich nutritional supplement were applied, so that the total amounts were 720 kcal, 10.65 g and 45.0 g of total energy, and arginine

Table 1. Preoperative patient characteristics.

	PN group (n = 12)	EAN group (n = 7)	P value
Age median (range)	71.3 (56-82)	67.8 (61-82)	0.37
Gender male or female	9:3	6:1	0.58
Gastric cancer UICC-TMN stage			
I	3	3	0.71
II	5	2	
III	4	2	
IV	0	0	
ASA physical status			
Class I	3	1	1.00
Class II	9	6	
Prognostic Nutritional Index (PNI)	46.7 ± 6.7	48.9 ± 4.5	0.48
Subjective Global Assessment (SGA)			
Normal	2	0	0.25
Mild malnutrition	10	7	
Moderate malnutrition	0	0	
Severe malnutrition	0	0	
Height (cm)	161.6 ± 7.7	165.1 ± 10.6	0.21
Body weight (kg)	50.7 ± 11.2	59.8 ± 15.7	0.15
Body Mass Index (BMI) (kg/m^2)	19.3 ± 3.5	21.6 ± 2.8	0.17
Serum albumin (g/dL)	3.8 ± 0.4	3.9 ± 0.3	0.92
Serum prealbumin (mg/dL)	19.4 ± 3.8	21.6 ± 5.8	0.33
Fisher ratio	2.8 ± 0.3	3.2 ± 0.4	0.12
Branched chain amino acid (nmol/mL)	363.3 ± 75.9	405.1 ± 78.2	0.27
Serum arginine (nmol/mL)	91.9 ± 23.5	80.2 ± 14.8	0.26
White blood cell ($10^3/\mu L$)	6.7 ± 2.2	5.2 ± 1.2	0.13
C-reactive protein (mg/dL)	0.6 ± 0.9	0.7 ± 0.9	0.80
Interleukin-6 (pg/mL)	3.0 ± 1.5	3.3 ± 1.9	0.66
Total lymphocyte count (TLC) (/μL)	1508 ± 563	1471 ± 316	0.88

Data are presented as mean ± SD.

and amino acids, respectively. On POD 3, 3 and 2/3 packs of arginine-rich nutritional supplement and standard whole protein formula were applied, respectively, so that the total amounts were 920 kcal, 11.15 g and 56.0 g of total energy, and arginine and amino acids, respectively. On PODs 4-7, 3 packs and then 1 pack of arginine-rich nutritional supplement and standard whole protein formula were applied, respectively, so that the total amounts were 1,020 kcal, 11.39 g and 61.5 g of total energy, arginine and amino acids, respectively. Total amounts of water were adjusted as 2,000 mL by administration of water, and injection rates were 20 mL/h, 40 mL/h, 50 mL/h and 60 mL/h in the PODs 2, 3, 4 and 5-7, respectively.

Oral nutrition was started on POD 7, and the total energy given was adjusted to 25 kcal/kg/day to avoid unintentional weight loss and severe Protein-Energy Malnutrition (3).

Evaluation items and nutritional assessment

Nutritional screening was performed using the nutritional indexes : 1) subjective global assessment (SGA) (13, 14), 2) prognostic nutrition index (PNI) (15), and 3) total lymphocyte count (TLC). The performance status of the patients was evaluated according to the American Society of Anesthesiologists Physical Status (ASA-PS) standard (16). All of these assessments were also performed perioperatively.

In order to investigate the nitrogen balance, we measured the urinary excretion of urea nitrogen on PODs 1, 3, and 7. The nutritional biochemistry assessment used serum levels of albumin, prealbumin, amino acids including arginine, which were measured preoperatively and on PODs 1, 3, and 7. White blood cell counts, serum levels of C-reactive protein (CRP) and interleukin-6 (IL-6) as inflammatory markers, and diamine oxidase activity (DAO) as a marker of small intestinal damage, were also measured. These analyses were performed on routinely drawn blood samples and the data were retrieved from hospital records.

Statistical analyses

Male to female ratio and cancer stages between the groups were compared using Fisher's exact test. For other factors, we used Student's *t*-test. A P value less than 0.05 was defined as statistically significant. All statistical analyses used JAMP (SAS Institute Japan, JMP Japan, Tokyo, Japan). Sample sizes were estimated using Easy R Statistics software (EZR v2.7-1) with R (The R Foundation for Statistical Computing Platform, v.3.6.3) (17, 18). We expected a between group difference of 1.25 SD, which required a sample size of 8 to discriminate between the two groups with a one-sided α error of 0.05 and a power of 0.8.

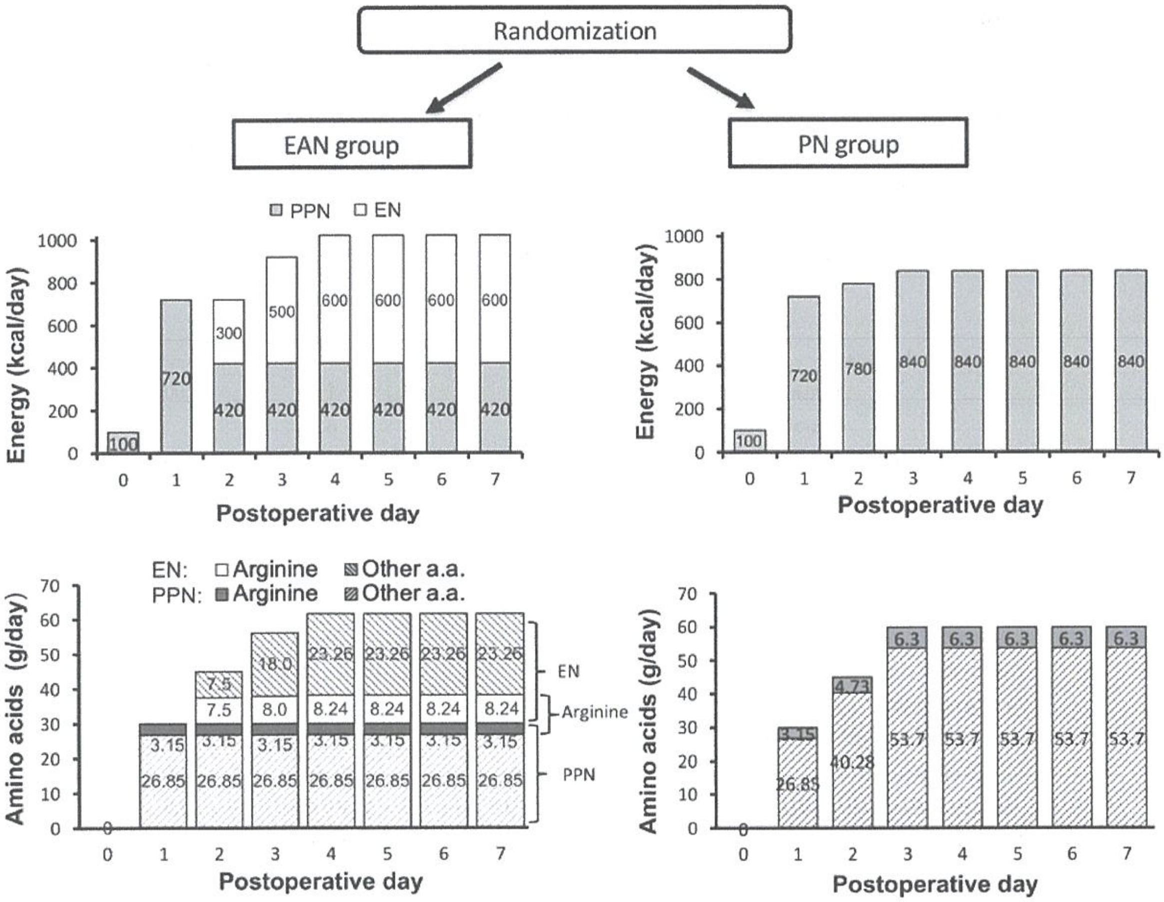

Figure 1. Study design and treatment schedule.
The left and right columns represent the contents of the enteral arginine-rich nutrition (EAN) and parenteral nutrition (PN) groups, respectively. The upper and lower rows represent the quantities of energy and amino acids applied per day, respectively. In the upper row, quantities of energy applied by the enteral nutrition (EN) and peripheral parenteral nutrition (PPN) are presented by white and grey blocks, respectively. The blocks in the figure represent the applied amounts of energy in kcal/day. In the lower row, the quantities of arginine applied by the EN and PPN are indicated by white and grey blocks. The blocks in the figure represent the applied amounts of amino acids in g/day. In the EAN group, the daily amounts of arginine were 3.2 g, 10.7 g, 11.1 g, 11.4 g on postoperative days of 1, 2, 3, and 4-7, respectively. Those values were almost twice that shown in the PN group. The other amino acids (Other a.a.) supplied by the EN and PPN are presented by upper-left and upper-right diagonal pattern blocks, respectively. For the EAN group, daily total amount of amino acids were 30 g, 45 g, 56 g, 61.5 g on postoperative days of 1, 2, 3, and 4-7, respectively. These values are almost the same as those in the PN group.

Table 2. Nutrition from POD 1 to POD 7

	PN group (n = 12)	EAN group (n = 7)	*P* value
Total amount for 7 days			
Energy (kcal)	5,700	6,440	
Total amino acids (g)	375	377	
Arginine (g)	39.38	70.52	
Average amount per day			
Energy (kcal/day)	814 ± 47	920 ± 141	0.09
Total amino acids (g/day)	53.6 ± 11.8	53.9 ± 12.2	0.97
Arginine (g/day)	5.63 ± 1.24	10.08 ± 3.07	0.004
Average amount per kg per day			
Energy (kcal/kg/day)	16.8 ± 3.9	16.1 ± 3.5	0.70
(% of 25 kcal/kg/day)	(67.4)	(64.5)	
Total amino acids (g/kg/day)	1.11 ± 0.26	0.94 ± 0.08	0.17
Arginine (g/kg/day)	0.116 ± 0.027	0.177 ± 0.038	0.008

Data are presented as mean ± SD.

RESULTS

As a result of the randomization using the envelope system, 12 patients and 7 patients were assigned to the PN group and the EAN group, respectively. Using sample size calculations, a pair of sample sizes of 12 and 7 was enough to discriminate 1.25 SD differences between the two groups with a one-sided α error of 0.05 and a power of 0.8.

There were no significant differences between the PN group and the EAN group in the preoperative factors, such as age, gender, cancer stage, PNI, SGA, BMI, ASA-PS, and nutritional and inflammatory markers (Table 1). The PNI were 46.7 ± 6.7 for the PN group and 48.9 ± 4.5 for the EAN group, so there were no statistical differences shown between the two groups. The TLC were 1,508 ± 563 for the PN group and 1,471 ± 316 for the EAN group, so both patient groups were in the mild malnutrition range without statistical differences. The SGA values also indicated the mild malnutrition condition in both groups. Average amounts of nutrition were calculated for 7 days from POD 1 to POD 7. The results are shown in Table 2. There were no significant differences between the PN group and the EAN group in the total energy and the total amino acids ($P > 0.05$), and only the amounts of arginine in the EAN group were significant larger than those in the PN group ($P < 0.01$). The lymphadenectomy scale, the surgical operation time and the blood loss are shown in Table 3, and there were no significant differences between the PN group and the EAN group. Postoperatively, no adverse events such as anastomotic leakage or severe infection occurred. No statistically significant differences were noted between the two groups in other factors, such as the occurrence of complications and the number of additional antibiotic treatments, or the length of the hospital stay. Initiation of oral nutrition tended to be later in the EAN group. (Table 3).

Postoperatively, inflammation markers, i.e., CRP and IL-6 levels were significantly higher on POD 1 compared to preoperative levels, but were not different between the two groups (Fig. 2 A, B, C). There was no difference in the DAO levels between the groups during the peri-operative period (Fig. 2D). Prealbumin, the most convenient nutritional marker, decreased from POD 1 to POD 3, but there was no difference between the two groups. On POD 1, serum albumin levels fell significantly, compared with preoperative values, but there was no difference between the two groups (Fig. 3A, B). BCAA increased on POD 3 in both groups, and while the Fisher ratio remained unchanged on POD 1, it improved on POD 3, but there was no difference between the two groups (Fig. 3C, D). As shown in Fig. 4A, arginine concentration decreased significantly on POD 1 in both groups compared with preoperative levels ($P < 0.001$), and returned to preoperative levels by POD 3 in both groups. Nitrogen balance became negative on POD 1 and remained so till POD 7 in the PN group, indicating protein hypermetabolism. In contrast, in the EAN group, it turned positive on POD 7 (Fig. 4 B). Regarding body weight, there was no decrease in body weight or BMI at POD 7 in either group ($P > 0.05$), and the balance of body weight tended to be positive in the EAN group, but these differences were not statistically significant (Fig. 4C). At the time of discharge, body weight and BMI decreased significantly, but there were no significant differences between the EAN and PN groups

Table 3. Postoperative factors.

	PN group (n = 12)	EAN group (n = 7)	*P* value
Oral nutrition start day (POD) mean ± SD	7.1 ± 0.3	10.1 ± 0.3	0.08
All complications, n (%)	12 (100.0)	7 (100.0)	0.50
Classification of surgical complications, n (%)			
Grade I	7 (58.3)	0 (0.0)	0.70
Grade II	2 (16.6)	3 (42.9)	
Grade IIIa	2 (16.6)	2 (28.5)	
Grade IIIb	0 (0.0)	2 (28.5)	
Grade IV	1 (8.3)	0 (0.0)	
Grade V	0 (0.0)	0 (0.0)	
Lymphadenectomy scale			
D1	3	0	0.26
D2	9	7	
Surgical operation time (h) mean ± SD	4.3 ± 0.5	4.5 ± 0.5	0.41
Surgical blood loss (mL) mean ± SD	490 ± 281	590 ± 353	0.53
Diarrhea, n (%)	5 (41.6)	4 (57.1)	0.54
Surgical site infection (SSI), n (%)	2 (16.7)	2 (28.5)	0.57
Others, n (%)	8 (66.7)	6 (85.6)	0.15
Number of additional antibiotics			
0	7	4	0.14
1	5	1	
2	0	1	
3	0	1	
Post-operative hospital stay (days) mean ± SD	32.7 ± 44.1	20.2 ± 8.1	0.36

*Classification of surgical complications were based on the Clavien-Dindo scale (37).

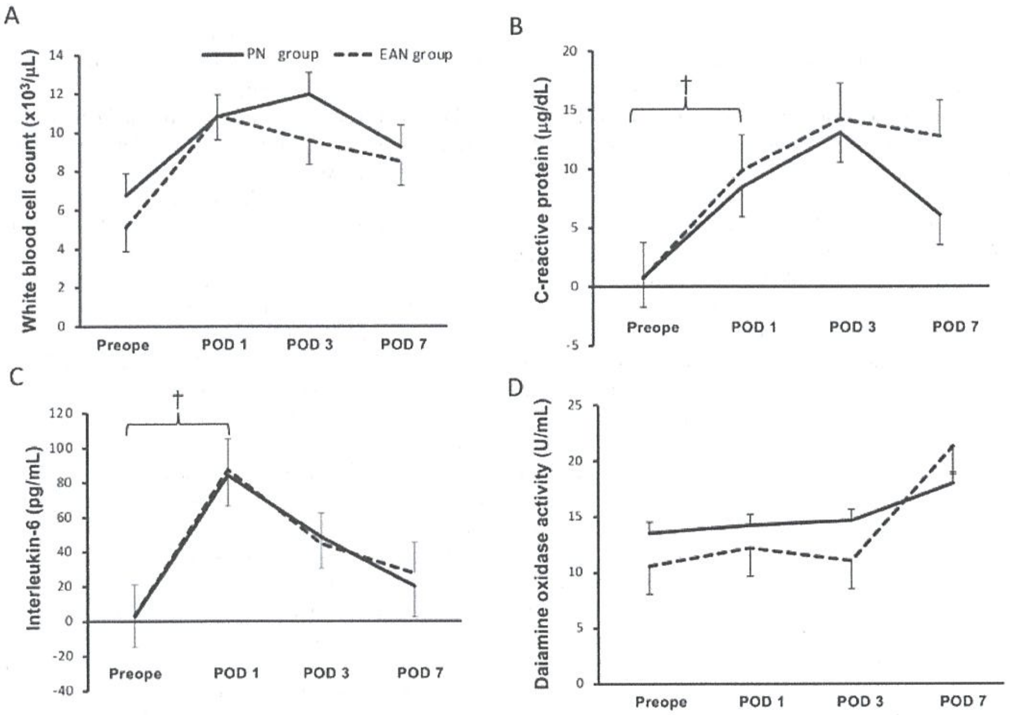

Figure 2. Changes in inflammatory markers.
This figure shows the means and standard error of the means obtained before the operation (Preope), and at postoperative days 1, 3 and 7 (POD 1, POD 3 and POD 7). The bold and dotted lines represent the results of the PN and EAN groups, respectively. A) Number of white blood cells. B) Serum concentration of C-reactive protein. C) Serum concentration of interleukin-6. D) Serum diamine oxidase activity. The symbols (†) represent statistically significant difference values between the Preope and POD 1 for the PN and the EAN groups ($P < 0.001$).

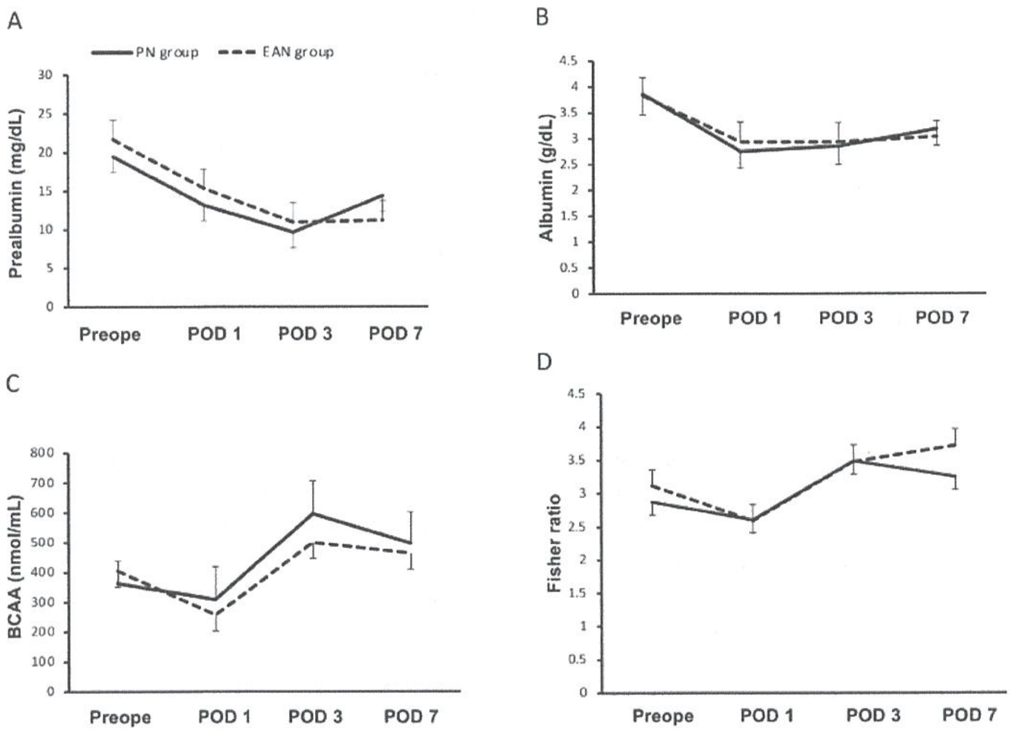

Figure 3. Changes in nutritional indices.
This figure shows the means and standard error of the means obtained before the operation (Preope), and at postoperative days 1, 3 and 7 (POD 1, POD 3 and POD 7). The bold and dotted lines represent the results obtained for the PN and EAN groups, respectively. Serum concentrations of A) prealbumin, B) albumin, and C) branched chain amino acid (BCAA). D) Fisher ratio.

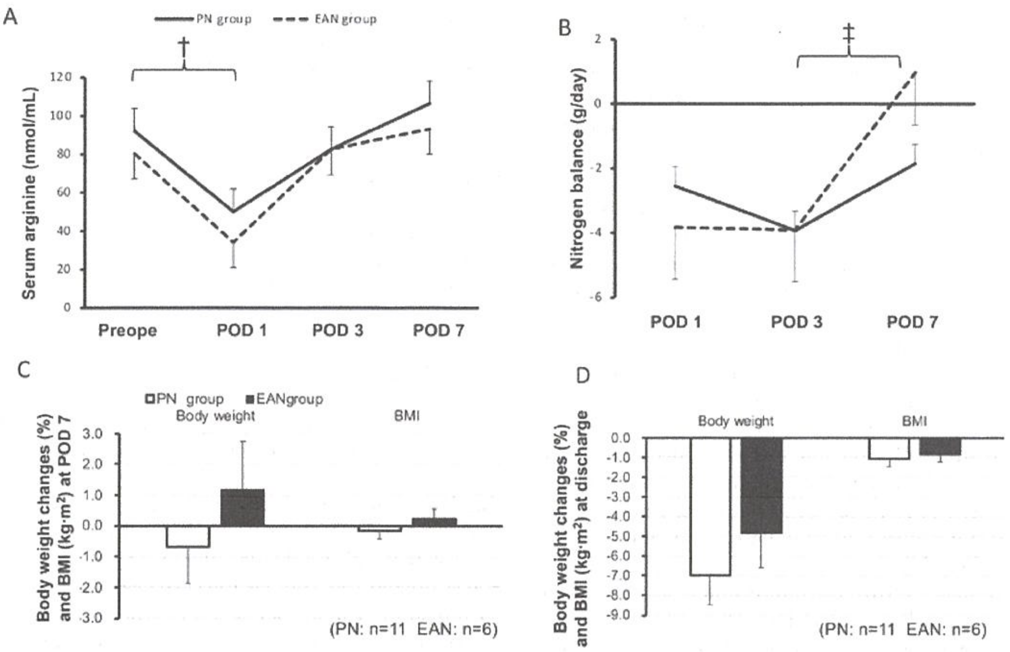

Figure 4. Changes in arginine, nitrogen balance, and body weight and body mass index (BMI).
A) Serum arginine concentration. This figure shows the means and standard error of the means obtained before the operation (Preope), and at postoperative days 1, 3 and 7 (POD 1, POD 3 and POD 7). The symbols (†) represent the statistically significant difference values between the Preope and POD 1 for the PN and the EAN groups ($P < 0.001$). B) Nitrogen balance. This shows the means and standard error of the means of POD 1, POD 3 and POD 7. The symbols (‡) represents statistically significant difference values between the POD 3 and POD 7 for the EAN group ($P < 0.05$). C) Changes in body weight and BMI at POD 7, compared with values obtained before the operation. D) Changes in body weight and BMI at discharge, compared with values obtained before the operation. The means and standard error of the means of body weight and BMI are shown. The bold and dotted lines in A and B represent the results for the PN and EAN groups, respectively. The white and black blocks in C and D are values obtained for the PN and EAN groups, respectively.

(Fig. 4D). Due to faulty communication with the patients, we could not get body weight or BMI for 1 patient in each group.

DISCUSSION

In this study we investigated whether early postoperative arginine-rich enteral nutrition improved nitrogen balance and suppressed weight loss in patients undergoing total gastrectomy. Under normal conditions, 60 g protein and 6 g arginine are required per day by a 50 kg adult persons (19), and the average dietary intake of arginine for all ages and genders has been estimated to be around 4.2 g per day (20) . Therefore, we provided 5.6 g/day of arginine on the average from POD1 to POD 7 in the PN group. Arginine can be synthesized from citrulline, but as its production is limited, serum concentration of arginine decreases when demand increases, i.e., such as during surgery, injury, or infection. Arginine is a precursor for nitric oxide (NO), a signaling molecule for both polyamines and nucleic acids, which are important for protein synthesis. It may also indirectly promote protein synthesis by acting on the pituitary gland to encourage the secretion of growth hormones and prolactin (21, 22). Arginine also enhances wound healing and increases hormone secretion with protein assimilation. Hence, we provided 10.1 g/day of arginine on the average from POD 1 to POD 7 in the EAN group. On POD 1, 3.15 g/day of arginine was administered to both groups, but this amount is expected to be too little for the patients. Indeed, serum arginine on POD 1 decreased to almost 50% of the preoperative levels in both groups, suggesting an increase in arginine consumption, which was restored after POD 3. Notably, despite a 1.8-fold enrichment of arginine, no significant differences in serum arginine were seen between the EAN and the PN groups, and this absence of elevated serum arginine levels in the EAN group indicates that the 10.1 g/day arginine might have been utilized for protein synthesis, which was necessary for healing. The early recovery of the nitrogen balance at POD 7 in the EAN group supports this hypothesis. Nitrogen balance, which represents the difference between administered nitrogen volume and excreted nitrogen volume, is an index that demonstrates the state of protein metabolism in living organisms. It is positive in a normal state, but remains negative during protein hypermetabolism. In animal models, arginine enriched feeding improved the nitrogen balance and body weight of protein-depleted rats (23), increased muscle creatine and improved the nitrogen balance of the rats after laparotomy (24). It also increased the protein turnover rate and the nitrogen balance significantly increased in burned rats (25). Even though only a small number of studies have been conducted using human patients, high doses of arginine (30 and 25 g/day) improved nitrogen balance and also decreased loss of body weight after cholecystectomy (26) and also in post-operated cancer patients (27). It is crucial that, even though body weight balance did not recover, the 1.8-fold enrichment of arginine recovered the nitrogen balance in the EAN group at POD 7 after the total gastrectomy. Compared with the high dosage of arginine (30-25 g/day), medium dosage (11 g/day) is more suitable to make formula, not only for EAN, but also PN.

During inflammation, NO is produced from arginine by the enzyme inducible nitric oxide synthase (iNOS) (28, 29). Highly intense inflammatory responses have been reported along with EAN, but these may not have been caused by arginine because those experiments did not involve the administration of arginine (29, 30). In the present study, no statistically significant differences in the inflammatory response were noted between the two groups, and similarly, no significant difference was noted in the frequency of postoperative complications, except diarrhea, which is a common gastrointestinal complication during enteral nutrition. Bliss *et al.* have reported that diarrhea occurred in 2%-26% of cases (31), and in our cohort, it was observed in around 50% of the patients in the EAN and PN groups, with no significant difference shown between the groups. Enteral nutrition administered via a jejunal fistula should proceed slowly and carefully using an enteral feeding pump, because nutrients administered directly into the intestines can, in many patients, lead to discomfort. Additionally, as patients may be unable to tolerate enteral nutrition after suffering diarrhea once, adequate caution is required. Moreover, as many cases of diarrhea are induced by water-soluble contrast agents used during postoperative gastric fluoroscopy, clinicians must confirm if enteral nutrition was the cause of the diarrhea (32).

As mentioned above, nitrogen balance was restored by POD 7 in the EAN group, whereas it remained negative in the PN group and nutritional indices tended to improve, but there was no significant difference between the two groups at POD 7, nor at discharge. This represents a major limitation of this study. We attribute the absence of statistical significance to the small sample size. For example, detecting a difference of approximately 0.5 SD in body weight, with a one-sided α error of 0.05 and a power of 0.8, requires a sample size of 64. Improving the accuracy of body weight measurement is also beneficial to reduce sample size. As far as we found by searching in the literature, there are only a few studies reporting on the long term effect of arginine-enriched nutrition (33, 34), it might be true that 7-days is too short to evaluate the body weight recovery, which suggested conducting a study in the future on the long term effect, for a period longer than a month. Despite the identical protein administration (54 g/day ; 1 g/kg/day) in both groups, the poorer recovery of body weight in the EAN group points toward a shortage in protein dosage, potentially due to the higher turnover during the postoperative period. In healthy elderly subjects consuming adequate protein (1.0-1.2 g/kg/day), the decrease in lean body weight during a period of 3 years was reduced by 40%, compared with those consuming less protein (0.8 g/kg·day) (35, 36). Additionally, maintaining identical protein intake probably ensured no adverse effects on mortality and length of stay between the two groups with a calorie intake at 42%-50% and 72%-75% by enteral nutrition. Importantly, the Canadian clinical practice guidelines agree on the need for maintaining protein intake (4). Despite an extensive literature search, we could not find studies that have evaluated the effect of protein and arginine enrichment in postoperative enteral nutrition. Hence, in the near future, we plan to assess the advantages of high protein and arginine intake to confirm these results.

CONCLUSION

The objective of early enteral nutrition is to minimize the reduction in body weight, and it is known that enteral nutritional supplements containing a high level of arginine can improve protein turnover. Patients in the EAN group were provided with an amount of arginine (10.1 g/day) that was 1.8-fold greater than that given to those in the PN group in the first seven days after total gastrectomy. The nitrogen balance turned neutral on POD 7 in the EAN group, indicating a quick restoration of protein metabolism in this group. Even though we could not confirm improvement in the body weight loss, these results suggested that early arginine-rich enteral nutrition could improve nitrogen balance after total gastrectomy.

CONFLICTS OF INTEREST

The authors of this research did not receive any specific grants from funding agencies in the public, commercial, or non-profit sectors. The authors all hereby declare no conflict of interest.

ACKNOWLEDGEMENTS

The authors would like to express their sincere thanks to the patients who agreed to participate in this study and also thank the surgical ward staff. Furthermore, we would also like to thank the companies that provided us with equipment employed in the study (Fuji Electric F-Tech Co., Ltd., and Covidien Company, Japan). We would also like to extend our thanks to Dr. Y. Seo (National Institute for Physiological Sciences : SEIRIKEN) for providing helpful comments. Furthermore, we thank Nestle Nutrition, Japan for supporting our research, and Mr. D. Vancil for correction of the English language text in the manuscript.

REFERENCES

1. Weimann A, Braga M, Carli F, Higashiguchi T, Hübner M, Klek S, Laviano A, Ljungqvist O, Lobo DN, Martindale R, Waitzberg DL, Bischoff SC, Singer P : ESPEN guideline : Clinical nutrition in surgery. Clin Nutr 36 : 623-650, 2017 doi.org/10.1016/j.clnu.2017.02.013.
2. The Committee on Japanese Guidelines for Nutrition Support Therapy in the Adult and Pediatric Critically Ill Patients, Japanese Society of Intensive Care Medicine : Japanese guidelines for nutrition support therapy in the adult and pediatric critically ill patients. J Jpn Soc Intensive Care Med 23 : 185-281, 2016
3. Braga M, Ljungqvist O, Soeters P, Fearon K, Weimann A, Bozzetti F, ESPEN : ESPEN guidelines on parenteral nutrition : surgery. Clin Nutr 28 : 378-386, 2009 doi.org/10.1016/j.clnu.2009.04.002
4. Canadian Clinical Practice Guidelines Committee : Highlights of the 2015 Canadian clinical practice guidelines. 3.3b. Intentional underfeeding : Hypocaloric enteral nutrition. 2015 Available at : https://www.criticalcarenutrition.com/docs/CPGs 202015/3.3b 202015.pdf (accessed May 2022)
5. Farreras N, Artigas V, Cardona D, Rius X, Trias M, González J A : Effect of early postoperative enteral immunonutrition on wound healing in patients undergoing surgery for gastric cancer. Clin Nutr 24 : 55-65, 2005 doi.org/10.1016/j.clnu.2004.07.002
6. Quirino IEP, Correia MITD, Cardosa VN : The impact of arginine on bacterial translocation in an intestinal obstruction model in rats. Clin Nutr 26 : 335-340, 2007 doi.org/10.1016/j.clnu.2006.12.007
7. Fan J, Meng Q, Guo G, Xie Y, Li X, Xiu Y, Li T, Ma L : Effects of early enteral nutrition supplemented with arginine on intestinal mucosal immunity in severely burned mice. Clin Nutr 29 : 124-130, 2010 doi.org/10.1016/j.clnu.2009.07.005
8. Iyama JO, Adamson I : Effect of supplemental arginine on rehabilitation of the starved rat. Nutr Reports Int 26:335-345, 1982

9. Ha YH, Milner JA : Arginine requirements in immature dogs. J Nutr 108 : 203-210, 1978
10. Valentini L, Schuetz T, Allison S, Howard P, Pichardand C : ESPEN guidelines on enteral nutrition. Clin Nutr 25 : 175-360, 2006 doi.org/10.1016/j.clnu.2006.02.004
11. McClave SA, Martindale RG, Vanek VW, McCarthy M, Roberts P, Taylor B, Ochoa JB, Napolitano L, Cresci G, A.S.P.E.N. Board of Directors ; American College of Critical Care Medicine ; Society of Critical Care Medicine : Guidelines for the provision and assessment of nutrition support therapy in the adult critically ill patient : Society of critical care medicine (SCCM) and american society for parenteral and enteral nutrition (A.S.P.E.N.). JPEN J Parenter Enteral Nutr 33 : 277-316, 2009 doi.org/10.1177/0148607109335234
12. Shimanuki M : Nitrogen balance and nitrogen utilization rate with special reference to protein-energy interactions. Eiyo To Shokuryo 31 : 41-48, 1978 (in Japanese)
13. Baker JP, Detsky AS, Wesson DE, Wolman SL, Stewart S, Whitewell J, Langer B, Jeejeebhoy KN : Nutritional assessment : a comparison of clinical judgment and objective measurements. N Engl J Med 306 : 969-972, 1982 doi.org/10.1056/NEJM198204223061606
14. Desky AS, McLaughlin JR, Baker JP, Johnston N, Whittaker S, Mendelson RA, Jeejeebhoy KN : What is subjective global assessment of nutritional status? JPEN J Parenter Enteral Nutr 11 : 8-13, 1987 doi.org/10.1177/014860718701100108
15. Onodera T, Goseki N, Kosaki G : Prognostic nutritional index in gastrointestinal surgery of malnourished cancer patients. J Jpn Surg Soc 85 : 1001-1005, 1984 (in Japanese)
16. Committee of Economics in American Society of Anesthesiologists : ASA physical status classification system. Last amended December 13, 2020. https://www.asahq.org/standards-and-guidelines/asa-physical-status-classification-system
17. Florey CD : Sample size for beginners. Brit Med J 306 : 1181-1184, 1993 doi.org/10.1136/bmj.306.6886.1181
18. Kanda Y : Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant 48 : 452-458, 2013 doi.org/10.1038/bmt.2012.24
19. WHO : Protein and amino acid requirements in human nutrition, in : W. H. Organization (Ed.), Report of a joint FAO/WHO/UNU expert consultation. World Health Organ Tech Rep Ser 2007, 935. Geneva, Switzerland. Available at http://apps.who.int/iris/bitstream/handle/10665/43411/WHO_TRS_935_eng.pdf (accessed May 2022)
20. VKM : Risk assessment of "other substances" L-arginine and arginine alpha-ketoglutarate. VKM Report 2016, 07. The Norwegian Scientific Committee for Food Safety. Available at https://vkm.no/download/18.645b840415d03a2fe8f26066/1502798687898/8b62ea4db0.pdf (accessed May 2022)
21. Witte M B, Barbul A : Arginine physiology and its implication for wound healing. Wound Repair Regen 11 : 419-423, 2003 doi.org/10.1046/j.1524-475x.2003.11605.x
22. Besset A, Bonardet A, Rondouin G, Descomps B, Passouant P : Increase in sleep related GH and Prl secretion after chronic arginine aspartate administration in man. Acta Endocrinol (Copenh) 99 : 18-23, 1982 doi.org/10.1530/acta.0.0990018
23. Steffee CH, Wissler RW, Humphreys EM, Benditt EP, Woolridge RW, Cannon RR : Studies in amino acid utilization : V. The determination of minimum daily essential amino acid requirements in protein-depleted adult male albino rats : One figure. J Nutr 40 : 483-497, 1950 doi.org/10.1093/jn/40.4.483
24. Minuskin ML, Lavine ME, Ulman EA, Fisher H : Nitrogen retention, muscle creatine and orotic acid excretion in traumatized rats fed arginine and glycine enriched diets. J Nutr 111 : 1265-1274, 1981 doi : 10.1093/jn/111.7.1265
25. Cui XL, Iwasa M, Iwasa Y, Ohmori Y, Yamamoto A, Maeda H, Kume M, Ogoshi S, Yokoyama A, Sugawara T, Funada T : Effects of dietary arginine supplementation on protein turnover and tissue protein synthesis in scald-burn rats. Nutrtion 15 : 563-569, 1999 doi : 10.1016/s0899-9007(99)00086-6
26. Elsair J, Poey J, Issad H, Reggabi M, Bekri T, Hattab F, Spinner C : Effect of arginine chlorhydrate on nitrogen balance during the three days following routine surgery in man. Biomed Express 29 : 312-317, 1978 (in French)
27. Daly JM, Reynolds J, Thom A, Kinsley L, Dietrick-Gallagher M, Shou J, Ruggieri B : Immune and metabolic effects of arginine in the surgical patient. Ann Surg 208 : 512-523, 1988
28. Elam RP, Hardin DH, Sutton RA, Hagen L : Effects of arginine and ornithine on strength, lean body mass and urinary hydroxyproline in adult males. J Sports Med Phys Fitness 29 : 52-56, 1989
29. Bertolini G, Iapichino G, Radrizzani D, Facchini R, Simini B, Bruzzone P, Zanforlin G, Tognoni G : Early enteral immunonutrition in patients with severe sepsis : results of an interim analysis of a randomized multicentre clinical. Intensive Care Med 29 : 834-840, 2003 doi.org/10.1007/s00134-003-1711-5
30. Dent DL : Immunonutrition may increase mortality in critically ill patients with pneumonia : results of a randomized trial. Crit Care Med 30 : A 17, 2002 doi.org/10.1097/00003246-200212001-00063
31. Bliss DZ, Guenter PA, Settle RG : Defining and reporting diarrhea in tube-fed patients--what a mess! Am J Clin Nutr 55 : 753-759, 1992 doi.org/10.1093/ajcn/55.3.753
32. Borthne AS, Dormagen JB, Gjesdal KI, Storaas T, Lygren I, Geitung JT : Bowel MR imaging with oral Gastrografin : an experimental study with healthy volunteers. Eur Radiol 13 : 100-106, 2003 doi : 10.1007/s00330-002-1528-6
33. Buijs N, van Bokhorst-de van der Schueren MA, Langius JA, Leemans CR, Kuik DJ, Vermeulen MA, van Leeuwen PA : Perioperative arginine-supplemented nutrition in malnourished patients with head and neck cancer improves long-term survival. Am J Clin Nutr 92 : 1151-1156, 2010
34. Kanekiyo S, Takeda S, Iida M, Nishiyama M, Kitahara M, Shindo Y, Tokumitsu Y, Tomochika S, Tsunedomi R, Suzuki N, Abe T, Yoshino S, Hazama S, Ueno T, Nagano H : Efficacy of perioperative immunonutrition in esophageal cancer patients undergoing esophagectomy. Nutrition 59 : 96-102, 2019
35. Paddon-Jones D, Rasmussen BB : Dietary protein recommendations and the prevention of sarcopenia. Curr Opin Clin Nutr Metab Care 12 : 86-90, 2009
36. Houston DK, Nicklas BJ, Ding J, Harris TB, Tylavsky FA, Newman AB, Lee JS, Sahyoun NR, Visser M, Kritchevsky SB : Health ABC Study. Dietary protein intake is associated with lean mass change in order, community-dwelling adults : the healthy aging, and body composition (Health ABC) study. Am J Clin Nutr 87 : 150-155, 2008 doi.org/10.1093/ajcn/87.1.150
37. Dindo D, Demartines N, Clavien PA : Classification of surgical complications : a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg 240 : 205-213, 2004