



A multicenter, randomized, controlled study of the use of nutritional supplements containing collagen peptides to facilitate the healing of pressure ulcers

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ABSTRACT

Since collagen peptide stimulates fibroblast growth in skin, it may accelerate the healing of pressure ulcers. Therefore, the purpose of our study was to verify whether the intake of collagen peptides in the form of a supplement drink facilitates the healing of pressure ulcers. We conducted a multicenter, randomized, controlled trial in patients with pressure ulcers. Patients were randomized into 3 groups at a 1:1:1 ratio; control group (usual care), collagen peptide-containing drink group and arginine-containing drink group. The DESIGN-R tool was used to assess healing of pressure ulcers, and nutritional status was measured for 4 weeks while consuming the usual daily meals. Out of 66 patients randomized, 51 patients were analyzed, since 15 patients were excluded from the final analysis. The total DESIGN-R score in patients who received the collagen peptide-containing drink ($n = 18$) was significantly lower than that in patients in the control group ($n = 16$) after 2 weeks as well as at the final value. However, the DESIGN-R score in the arginine-containing drink group ($n = 17$) did not show difference from the control group. There was no significant difference in nutrition status among the 3 groups through the study. Our results indicated that a supplemental addition of the collagen peptide-containing drink to the usual meal facilitated pressure ulcer healing, since the collagen peptide-containing drink led to more changes in the DESIGN-R scores.

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1. Introduction

Pressure ulcers commonly occur in thin patients with prominent bones who are bedridden and unable to reposition themselves and they are not easily cured when patients are malnourished. With sufficient nutrient intake, the proper distribution of body pressure and local treatment help heal pressure ulcers [1]. However, there is very little evidence that nutrients have positive effects on healing pressure ulcers. Wang et al. conducted a placebo-controlled trial to compare pressure ulcer healing rates in patients with a specialized amino acid mixture containing supplement versus patients with a placebo containing supplement. Assessment scores of pressure ulcer showed significant improvement in the experimental group,

however, wound area was not decrease significantly [2]. Langer and Fink performed systemic reviews using the Cochrane Library database to assess nutritional interventions for preventing and treating pressure ulcers [3]. They reported that there was no clear evidence of nutritional interventions for either the prevention or treatment of pressure ulcers, and further evaluations of high methodological quality are necessary.

Among nutrients, arginine (Arg) treatment shortened the duration required for the cure of pressure ulcers compared with control treatment without Arg [4]. Dermal collagen is associated with skin elasticity [5], and skin elasticity showed a close relation to collagen type I RNA expression and fibroblast growth after oral collagen peptide (CP) treatment [6]. It was also reported that treatment with CP supplementation (2.5 g or 5 g per day) for 4 weeks significantly increased skin elasticity in healthy women [7]. These findings indicate that Arg and CP may positively affect the healing of pressure ulcers.

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For this reason, we conducted a multicenter, randomized, controlled trial to determine whether the intake of CP or Arg facilitates healing of pressure ulcers. In this study, the DESIGN-R assessment tool consisting of 7 items (depth, exudates, size, inflammation/infection, granulation, necrosis, and pocket formation) [8] was used to compare the wound-healing process among the CP or Arg intake and no supplement groups. DESIGN-R was modified by weighting items of the original DESIGN tool developed in 2002 by the Scientific Education Committee of the Japanese Society of Pressure Ulcers [9].

2. Methods

2.1. Study design

This study was conducted at 22 facilities by specialists of the Japanese Society for Parenteral and Enteral Nutrition and Japan Society of Pressure Ulcers (Table 1) as a multicenter, randomized, controlled, open-label study. In order to reduce evaluation variability and maintain uniformed quality, institutes where treatment in pressure ulcer was conducted by specialists in pressure ulcers and medical team for nutrition therapy were involved in the study. This study protocol was approved by the Institutional Ethical Review Boards of the individual facilities. For facilities without such a review board, approval was received from the equivalent Ethical Review Committee.

2.2. Patients

Among patients with pressure ulcers who were receiving tube or oral feeding, those who met the following inclusion criteria were enrolled between March 2014 and April 2015 after written informed consent was obtained from them or their families in advance. The inclusion criteria were those receiving nasogastric tube, gastrostomy tube, or oral feeding who were able to consume $\geq 60\%$ of the caloric requirement calculated using the Harris-Benedict equation [10] prior to study initiation (i.e., patients whose intake content and quantity could be confirmed); and those who satisfied all the following items in the DESIGN-R assessment tool (Fig. 1) [8]: pressure ulcer depth, D3–D4; exudate, e0–E6; size, s3–S15; inflammation/infection, i0–i1 (local inflammation was

included, but local infection was excluded); granulation, g1–G5; necrotic tissue, n0–N3 (patients with necrotic tissue were included when yellow slough was $\leq 1/3$, and those that satisfied the selection criteria after debridement were included); and pocket formation, p0–P6 (those that satisfied the selection criteria after pockets were excised were included).

Patients with the following were excluded: a history of or current serious hepatic or renal dysfunction; current hemodialysis use; uncontrolled diabetes (hemoglobin A1c $\geq 8.0\%$ [National Glycohemoglobin Standardization Program value] or $\geq 7.6\%$ [Japan Diabetes Society value]); C-reactive protein level ≥ 3.0 mg/dL due to systemic infection (patients with local inflammation were not excluded); the onset of aspiration pneumonia within the previous 1-month period; cancerous cachexia (diagnosis as refractory cachexia based on the international consensus [11]); ulcers in the legs caused by venous insufficiency, arteriosclerosis obliterans, severe diabetes, etc.; pressure ulcers that could not be evaluated and untreatable pressure ulcers; and those determined to be inappropriate for study inclusion by the attending physicians.

2.3. Study methods

Patients were randomized to control group (usual care) or treatment groups (CP-containing drink group [CP group] or Arg-containing drink group [Arg group]) by stratified randomization at a 1:1:1 ratio at Epoca Marketing Co., Ltd. (Osaka, Japan) as the study center. For patient randomization, a central randomization system by using a stratified randomization method to minimize imbalance was employed through internet.

Daily meal intake was not restricted in the 3 groups throughout the 4-week study period. The CP group received a 125-mL bottle of V CRESC CP10 (CP-containing drink [12.0 g of protein containing 10.0 g of CP], Nutri Co., Ltd., Yokkaichi Japan) and the Arg group received a 125-mL bottle of Isocal Arginaid (Arg-containing drink [5.0 g of protein containing 2.5 g of Arg], Nestle Japan Ltd., Kobe, Japan) once daily by nasogastric tube, gastrostomy tube, or orally. Nutritional information on each product is shown in Table 2. CP is low molecular protein (molecular weight; several hundred to several thousand) produced by hydrolyzing collagen included in fishes, cattle and pig skin. Collagen molecular is dissoluble in water, however, CP possess a characteristic to be easily absorbed.

Standard (usual) nutritional care was provided prior to and during the study. The enteral nutrient products, calorie counts, and meal schedules provided prior to the initiation of the study were not changed. Supplement products similar to the test products were not consumed. When supplements containing vitamins, trace elements, and CP or Arg had been administered previously, they were switched to the test products without a washout period. CP or Arg supplemental drink was added to usual meals under the same way as the usual manner in the clinical setting. The amount of protein, total calorie and trace elements additionally supplied was not subtracted from usual meal in each patient in the supplemental drink groups. Therefore, the total amount of nutrition was not adjusted among patients.

Standard care for pressure ulcers was provided prior to and during the study. However, antimicrobial agents were administered if necessary, although fibroblast growth factor formulations were prohibited during the study period. The mattresses used prior to the study period were not changed. Patients were repositioned on the mattresses approximately every 2 h.

2.4. Assessment

For assessing the condition of pressure ulcers, DESIGN-R scores were recorded prior to study initiation and at weeks 1, 2, 3, and 4. In

Table 1
List of participating institutions and investigators overseeing the study.

| Institution | Investigator |
|--|--------------------|
| Nakajima Hospital | Tomokazu Hoshi |
| JCHO Noboribetsu Hospital | Masashi Nakamura |
| Muroran City General Hospital | Tsugufumi Nakagawa |
| Kiryu Kosei General Hospital | Katsuyuki Okada |
| Ageo Central General Hospital | Kenji Omura |
| Ageo Kousei Hospital | Kazuhiko Fujisawa |
| Tokatsu Clinic Hospital | Kazuhiro Akiyama |
| Mejiro Second General Hospital | Hideaki Mizuno |
| Eisei Hospital | Tatsuya Nomoto |
| Minami Yamato Hospital | Shin Fujii |
| Koyo Hospital | Hajime Nakase |
| Kanazawa Nishi Hospital | Tsutomu Kikuchi |
| Aiseikai Yamashina Hospital | Hideki Aragane |
| Kyoto Kujo Hospital | Kazutomo Kitagawa |
| Wakakusa-Daiichi Hospital | Hideharu Yamanaka |
| Wakakusa-Tatsuma Rehabilitation Hospital | Masataka Itoda |
| Kaneda Hospital | Takuji Mimura |
| Kenwakai Ohtemachi Hospital | Yoshiteru Ishii |
| Haradoi Hospital | Masako Shimoda |
| Fukuoka Rehabilitation Hospital | Kensuke Takeuchi |
| Oita Oka Hospital | Masahide Furukawa |
| Kumamoto Daiichi Hospital | Tetsushi Nogami |

| | | | | | |
|--|-----------|---|----------|---------------------------------------|---|
| Depth: this should be measured at the deepest point of the wound. If the wound becomes shallower, the decreased depth should be reflected in the assessment | | | | | |
| d | 0 | No particular skin lesion and no redness | D | 3 | Lesion extends into the subcutaneous tissue |
| | 1 | Persistent redness | | 4 | Lesion extends to muscle, tendon and bone |
| | 2 | Lesion extends into dermis | | 5 | Lesion extends into the articular or body cavity |
| | | | U | It is impossible to measure the depth | |
| Exudate: amount | | | | | |
| e | 0 | None | E | 6 | Heavy: requires dressing change more than twice a day |
| | 1 | Slight: does not require daily dressing change | | | |
| | 3 | Moderate: requires daily dressing change | | | |
| Size: the area of a skin injury (length x width). | | | | | |
| s | 0 | None | S | 15 | 100 cm ² or larger |
| | 3 | Smaller than 4 cm ² | | | |
| | 6 | 4 cm ² or larger, but smaller than 16 cm ² | | | |
| | 8 | 16 cm ² or larger, but smaller than 36 cm ² | | | |
| | 9 | 36 cm ² or larger, but smaller than 64 cm ² | | | |
| | 12 | 64 cm ² or larger, but smaller than 100 cm ² | | | |
| Inflammation/Infection: | | | | | |
| i | 0 | None | I | 3 | Clear signs of local infection (eg, inflammation, pus and foul smell) |
| | 1 | Signs of inflammation (fever, redness, swelling, and pain around the wound) | | 9 | Systemic impact, such as fever |
| Granulation tissue: percentage of healthy granulation | | | | | |
| g | 0 | Granulation cannot be assessed because the wound is healed or too shallow | G | 4 | Healthy granulation tissue occupies 10% or more, but less than 50% |
| | 1 | Healthy granulation tissue occupies 90% or more | | 5 | Healthy granulation tissue occupies less than 10% |
| | 3 | Healthy granulation tissue occupies 50% or more, but less than 90% | | 6 | No healthy granulation tissue exists |
| Necrotic tissue: when necrotic and non-necrotic tissues are mixed, the dominating condition should be used for assessment | | | | | |
| n | 0 | None | N | 3 | Soft necrotic tissue exists |
| | | | | 6 | Hard and thick necrotic tissue is attached to the wound |
| Pocket: the area obtained by subtracting the ulcer from the entire affected area, including the pocket | | | | | |
| p | 0 | None | P | 6 | Smaller than 4 cm ² |
| | | | | 9 | 4 cm ² or larger, but smaller than 16 cm ² |
| | | | | 12 | 16 cm ² or larger, but smaller than 36 cm ² |
| | | | | 24 | 36 cm ² or larger |

Fig. 1. Assessment table for the DESIGN-R tool. The table is reproduced from the DESIGN-R scoring manual by the Japanese Society of Pressure Ulcers.

order to maintain reliability among evaluators, DESIGN-R score was graded by a physician with a nurse who holds the Wound, Ostomy and Continence Nursing (WOCN) authorization. Blood samples were collected prior to study initiation and at weeks 2 and 4 to measure red blood cell count, white blood cell count, platelet count, lymphocyte subsets, and levels of hemoglobin, albumin, prealbumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, blood urea nitrogen (BUN), creatinine, C-reactive protein (CRP), sodium, potassium, chloride, phosphorus, and zinc. Body weight and Braden scale scores were recorded prior to study initiation and at weeks 1, 2, 3, and 4. Vital signs (body temperature, blood pressure, and pulse rate) and the occurrence of diarrhea and allergic responses were recorded daily. The primary endpoint was total DESIGN-R scores (summation of scores in 6 items in DESIGN-R), and the secondary endpoint was DESIGN-R subscore (depth, exudate, size, inflammation/infection, granulation, necrotic tissue and pocket formation).

2.5. Statistical analysis

Prior to statistical analysis, photographs of each study patient's pressure ulcers were submitted to the evaluation committee to reduce the variation in assessments of severity. For analysis of the DESIGN-R scores, all patients who met to eligibility criteria according to an intention-to-treat (ITT) analysis were subjected. The final scores were aggregated using the results at 4 weeks, however, for patients whose pressure ulcers completely healed, the final data was fulfilled by "0", and for patients who discontinued treatment during the intervention period, the final scores were fulfilled by using last observation carried forward (LOCF) method.

Based on 80% power to detect a significant difference with one-sided alpha level of 0.05 and 20% of loss to follow up, 35 patients were required for each group according to a preliminary study.

Mean values and standard deviations were calculated in each group unless otherwise specified. For the comparative analysis of patients' background characteristics, body weight, and Braden scale scores, the chi-square test or Kruskal-Wallis test was used.

Table 2
Nutritional composition of the test nutritional supplement drinks (per 125-mL bottle).

| CP group | | | |
|----------------------------------|--------------------|--------------------------|--------------------|
| Calories: 80 kcal | | | |
| Protein: 12.0 g | Fat: 0 g | Carbohydrates: 8.0 g | Water: 110 g |
| Collagen peptides included: 10 g | | | |
| Sodium: 42 mg | Potassium: 30 mg | Calcium: 75 mg | |
| Magnesium: 1.0 mg | Phosphorus: 4.0 mg | | |
| Iron: 5.0 mg | Zinc: 12 mg | Copper: 0 mg | Selenium: 50 µg |
| Vitamin A: 300 µg RAE | Vitamin B1: 3.0 mg | Vitamin B2: 3.0 mg | Vitamin B6: 5.0 mg |
| Vitamin B12: 10.0 µg | Vitamin C: 500 mg | Niacin: 15 mg | Folate: 550 µg |
| Vitamin D: 5.5 µg | Vitamin E: 20 mg | Pantothenic acid: 10 mg | Biotin: 50 µg |
| CoQ10: 15 mg | | | |
| Arg group | | | |
| Calories: 100 kcal | | | |
| Protein: 5.0 g | Fat: 0 g | Carbohydrates: 20.0 g | Water: 107 g |
| Arginine included: 2.5 g | | | |
| Sodium: 55 mg | Potassium: 30 mg | Calcium: 20 mg | |
| Magnesium: 3.5 mg | Phosphorus: 630 mg | | |
| Iron: 7.0 mg | Zinc: 10 mg | Copper: 1.0 mg | Selenium: 50 µg |
| Vitamin A: 150 µg RAE | Vitamin B1: 0.9 mg | Vitamin B2: 0.8 mg | Vitamin B6: 1.0 mg |
| Vitamin B12: 0 µg | Vitamin C: 500 mg | Niacin: 10 mg | Folate: 100 µg |
| Vitamin D: 2.4 µg | Vitamin E: 5.0 mg | Pantothenic acid: 5.0 mg | Biotin: 0 µg |
| CoQ10: 0 mg | | | |

RAE = retinol activity equivalents.

Differences in DESIGN-R scores among the control, CP, and Arg groups were analyzed using Steel's multiple-comparison test at each week. To analyze differences in the laboratory test measurements, the paired *t*-test was used compared with prior to the initiation of supplement intake), and multiplicity was corrected by the Bonferroni method for comparison. Since pressure ulcers were given standard care at all facilities, we performed a one-sided test to analyze DESIGN-R scores to assess the improvement of pressure ulcers.

The data were recorded and processed by Epoca Marketing Co., Ltd., and statistical analysis was performed by Esumi Co., Ltd. (Tokyo, Japan). A *p* value of less than 0.05 was considered to represent a statistically significant difference.

3. Results

3.1. Patient demographics

Sixty-six patients were enrolled in this study, with 22 assigned to each group. However, because of protocol violations, 6 (nonfulfillment of inclusion criteria, *n* = 5; exclusion criteria applicable, *n* = 1) in the control group, 4 (nonfulfillment of inclusion criteria, *n* = 3; exclusion criteria applicable, *n* = 1) in the CP group, and 5 (nonfulfillment of inclusion criteria, *n* = 3; use of concomitant medications, *n* = 2) in the Arg group were excluded from the final analysis based on the photograph evaluations, and 51 patients were included in the final analysis (control group, *n* = 16; CP group, *n* = 18; and Arg group, *n* = 17) (Fig. 2).

3.2. Patient characteristics

Patient background characteristics are shown in Table 3. There were no significant differences in gender, age, body mass index, basal energy expenditure, caloric intake, total DESIGN-R scores prior to the initiation of supplement intake, total Braden scale scores, and albumin levels among the 3 groups. Since the mean caloric intake rate to basal energy expenditure was 1.5-fold, 1.5-fold, and 1.4-fold in the control group, CP group, and Arg group, respectively, patients enrolled in this study were receiving

sufficient total daily calories.

3.3. Safety

Skin redness and heat, swelling of both femurs, and diarrhea occurred in 1 patient in the Arg group, and therefore administration of the supplement was discontinued. The symptoms improved thereafter. No other adverse events were observed during the study.

3.4. Assessment of the healing of pressure ulcers

The measured values and variables of the DESIGN-R scores are shown in Fig. 3. The final scores decreased compared with those prior to the initiation of intake in all groups. In the CP group, total scores after 2 weeks of supplement intake and the final scores were significantly lower than those in the control group at the same times. In the Arg group, the scores decreased until week 2 but did not decrease thereafter. There was no difference in the scores in the Arg group compared with those in the control group. The changes in the total scores at week 4 from the supplement initiation and the final scores were significantly lower in the CP group compared with that in the control group.

The variables for each item of the DESIGN-R scores are shown in Fig. 4. The scores for each item changed, and there were no significant differences among groups. The scores for granulation was tended to be lower at week 4 (*p* = 0.084) and the final scores (*p* = 0.092) in the CP group compared with those in the control group. There was a decrease in the scores for exudate at week 4 (*p* = 0.071) and in the final exudate scores (*p* = 0.068) in the CP group compared with the control group. For necrotic tissue, the final scores (*p* = 0.092) in the CP group, and the scores at week 4 (*p* = 0.063) and the final scores (*p* = 0.093) in the Arg group, decreased compared with those in the control group.

3.5. Changes in laboratory test results

Laboratory test results are shown in Table 4. There were no significant changes in the levels of nutritional indicator proteins,

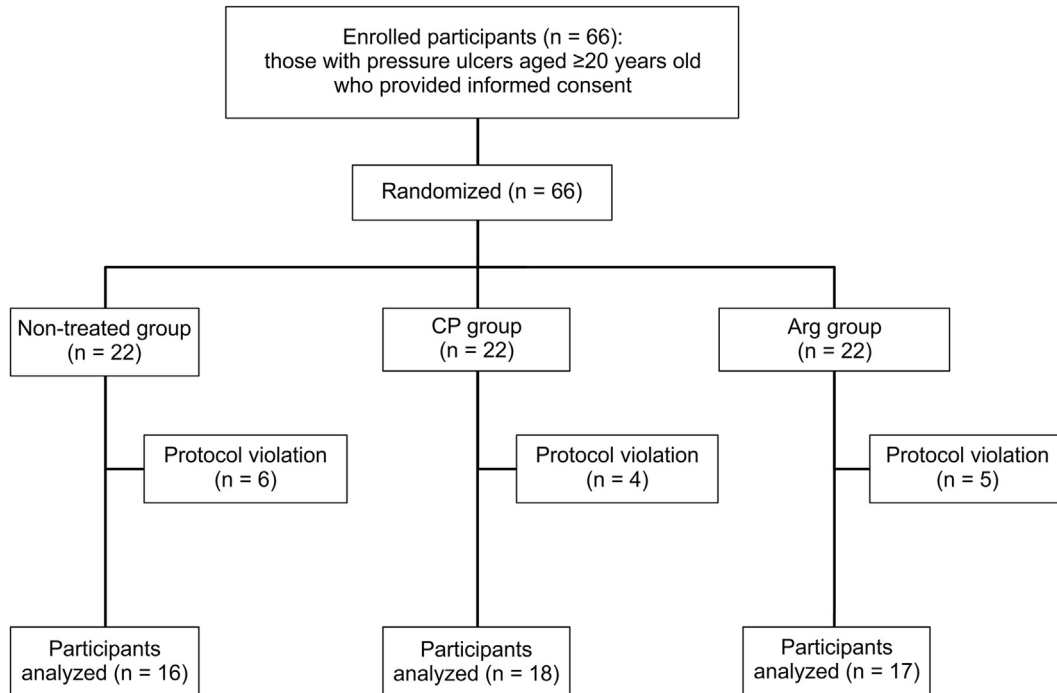


Fig. 2. Flow chart of patients included in the analyses.

Table 3

Background characteristics of patients analyzed in each group (all values mean \pm SD unless otherwise indicated).

| | Control group | CP group | Arg group | p value |
|---|--------------------|--------------------|--------------------|--------------------|
| Gender | | | | |
| Male | 5 | 9 | 7 | 0.54 ¹⁾ |
| Female | 11 | 9 | 10 | |
| Age (y) | 79.9 \pm 7.7 | 76.8 \pm 13.9 | 79.6 \pm 12 | 0.9 ²⁾ |
| BMI (kg/m ²) | 18.5 \pm 2.8 | 18.8 \pm 2.1 | 18.2 \pm 2.7 | 0.58 ²⁾ |
| BEE (kcal/day) | 937.5 \pm 101.4 | 995.2 \pm 153.3 | 952.0 \pm 119.7 | 0.61 ²⁾ |
| Caloric intake (kcal/day) | 1394.8 \pm 333.1 | 1472.4 \pm 307.3 | 1336.0 \pm 288.1 | 0.27 ²⁾ |
| Total DESIGN-R scores prior to initiation of intake | 15.9 \pm 5.7 | 14.1 \pm 4.3 | 14.1 \pm 5.6 | 0.46 ²⁾ |
| Total Braden scale scores | 12.4 \pm 2.8 | 14.1 \pm 3.8 | 12.9 \pm 2.4 | 0.56 ²⁾ |
| Alb (g/dL) | 2.89 \pm 0.71 | 3.21 \pm 0.39 | 3.02 \pm 0.54 | 0.22 ²⁾ |

SD, standard deviation; BMI, body mass index; BEE, basal energy expenditure; Alb, albumin; CP-containing drink group [CP group]; Arg-containing drink group [Arg group]. BEE was calculated by Harris-Benedict Equation. There were no significant differences among groups. 1) Assessed by the chi-square test; 2) Assessed by the Kruskal-Wallis test.

including serum total protein, albumin, and prealbumin in any group. The levels of trace elements including zinc in all groups satisfied the nutritional requirements for Japanese during the study period. In the Arg group, there were significant increases in BUN and serum phosphorus levels at week 2 compared with those prior to the initiation of supplement intake, but the levels remained within the normal range.

4. Discussion

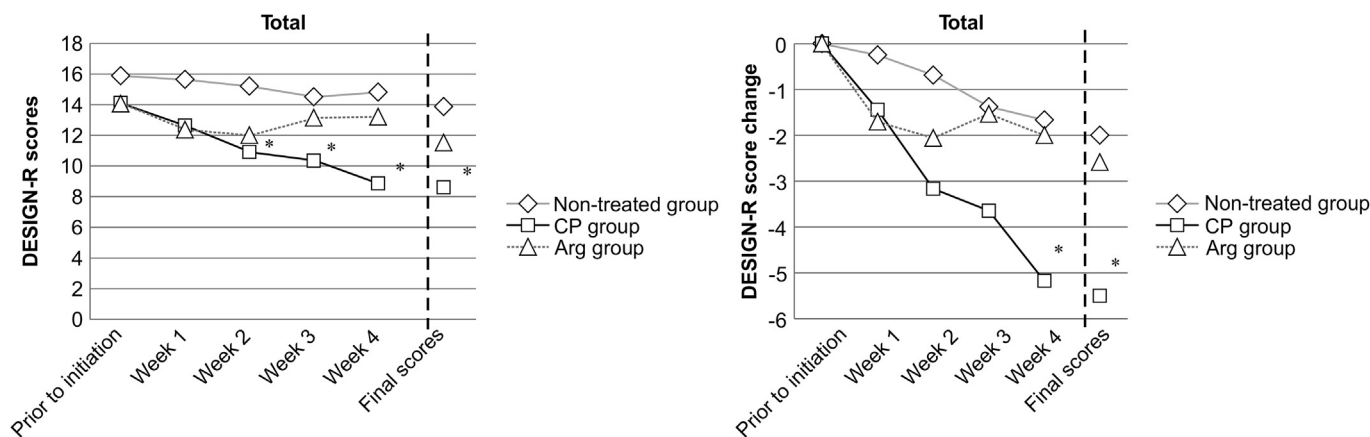
Our results showed that the final total DESIGN-R scores decreased in all groups. However, the scores were significantly lower 2 weeks after the initiation of supplement intake in the CP group compared with those in the control group, which clearly indicates the facilitation of healing.

Supplement drinks employed in this study contain several nutrition (zinc, Arg, CP, etc.) related to pressure ulcer healing. Among them, it was reported that Arg shortened the duration until cure in patients with pressure ulcers [12], since Arg enhances polyamine synthesis, which is important for cell growth, and utilizes proline synthesis [13]. On the other hand, dipeptides including

hydroxyproline, which are metabolites of CP, stimulate fibroblast proliferation and migration, enhancing the synthesis of collagen, hyaluronic acid, and elastin. It was reported that CP shortened the duration of skin wound healing in a rat model of pressure ulcers [14]. The 4th guideline for prevention and management of pressure ulcer issued from Japanese Society of Pressure Ulcer in 2015 newly added CP as nutrition to be possibly supplied to patients with pressure ulcer under consideration level as C1 (limited data, but recommended). These findings may support the suggestion that CP and Arg have different mechanisms of action in patients with pressure ulcers in this study.

The usefulness of DESIGN-R for predicting healing was reported [15]. Another study also reported that DESIGN-R score prior to treatment was discriminative for prediction wound healing duration of pressure ulcer up to 90 days. Total DESIGN-R score of 9 or less and that of 18 or less were predictive values for healing within 30 days and from 30 to 90 days, respectively [16]. These findings suggested that patients with total DESIGN-R score of around 9 at 4 weeks in the CP group would show the pressure ulcer healing within 30 days with high possibility.

The analysis of each item of DESIGN-R provided interesting



| DESIGN-R scores | Group | Prior to initiation of intake | | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Final scores | | |
|-----------------|-------------------|-------------------------------|-------|------|--------|-------|------|--------|--------|-------------------|--------|--------|-------------------|--------|--------|-------------------|--------------|--------|-------------------|
| | | Mean | SD | (n) | Mean | SD | (n) | Mean | SD | (n) | Mean | SD | (n) | Mean | SD | (n) | Mean | SD | (n) |
| Total | Non-treated group | 15.9 | ± 5.7 | (16) | 15.6 | ± 6.2 | (16) | 15.2 | ± 6.5 | (16) | 14.5 | ± 6.9 | (16) | 14.8 | ± 7.3 | (15) | 13.9 | ± 7.9 | (16) |
| | CP group | 14.1 | ± 4.3 | (18) | 12.6 | ± 4.7 | (18) | 10.9 | ± 4.5 | (18) ^a | 10.4 | ± 4.9 | (17) ^b | 8.8 | ± 6.3 | (17) ^c | 8.6 | ± 6.2 | (18) ^d |
| | Arg group | 14.1 | ± 5.6 | (17) | 12.4 | ± 6.7 | (17) | 12.0 | ± 11.5 | (17) | 13.1 | ± 13.1 | (15) | 13.2 | ± 13.4 | (14) | 11.5 | ± 12.9 | (17) |

| DESIGN-R score change | Group | Prior to initiation of intake | | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Final scores | | |
|-----------------------|-------------------|-------------------------------|-------|------|--------|-------|------|--------|-------|------|--------|--------|------|--------|--------|-------------------|--------------|--------|-------------------|
| | | Mean | SD | (n) | Mean | SD | (n) | Mean | SD | (n) | Mean | SD | (n) | Mean | SD | (n) | Mean | SD | (n) |
| Total | Non-treated group | 0.0 | ± 0.0 | (16) | -0.3 | ± 3.6 | (16) | -0.7 | ± 4.3 | (16) | -1.4 | ± 4.1 | (16) | -1.7 | ± 4.7 | (15) | -2.0 | ± 4.7 | (16) |
| | CP group | 0.0 | ± 0.0 | (18) | -1.4 | ± 2.5 | (18) | -3.2 | ± 3.1 | (18) | -3.6 | ± 3.2 | (17) | -5.2 | ± 4.9 | (17) ^e | -5.5 | ± 5.0 | (18) ^f |
| | Arg group | 0.0 | ± 0.0 | (17) | -1.7 | ± 2.8 | (17) | -2.1 | ± 8.0 | (17) | -1.5 | ± 10.1 | (15) | -2.0 | ± 10.9 | (14) | -2.6 | ± 10.1 | (17) |

^a $p = 0.022$, ^b $p = 0.029$, ^c $p = 0.012$, ^d $p = 0.027$, ^e $p = 0.042$, ^f $p = 0.042$

Fig. 3. Changes in the total DESIGN-R scores by group (including measured values and differences). CP-containing drink group [CP group]; Arg-containing drink group [Arg group]; SD, standard deviation. The graph shows the mean values of patients analyzed in each group. The table shows the mean \pm SD and number of participants. * $p < 0.05$, Steel's multiple-comparison test (one-sided) compared with the control group.

results, suggesting the possibility that granulation formation was facilitated by the intake of CP-containing drink, and necrotic tissue was reduced by the immune/inflammatory response due to the intake of Arg-containing drink.

Total protein intake is related to the healing of pressure ulcers [17]. However, the levels of the nutritional indicator proteins albumin, prealbumin, and total protein did not change, as shown in Table 4, and body weight remained unchanged, indicating that nutritional status in the patients enrolled in this study remained the same among the 3 groups.

Since this study was conducted under a usual care condition, CP and Arg preparations were utilized in accordance with a usage way in clinical setting. Since both preparations were added to usual meals in each patient like a usual way, the adjustment of contents of protein, total calorie and trace elements among patients was not conducted. This study, however, revealed that CP-containing drink has an additive effect on pressure ulcer healing compared to usual care.

Although significant difference in DESIGN-R score was detected in this study, the sample size was not adequate. Therefore, this significant difference may be overlooked in several endpoints. We initially estimated that 35 patients were required in each group to detect a significant effect of CP- or Arg-containing drinks pressure ulcer healing, however, only 22 patients in each group were randomized and 15 patients were excluded for the final analysis. To conduct assessment in patients with homogenous severity of pressure ulcers among patients to be enrolled, the inclusion and

exclusion criteria were strictly set. However, the strict criteria might be a limitation for patient reenrollment. For future study, it would be more important to reduce evaluation variability among investigators and severity variability among patients. however, it had better to enroll larger numbers of patients with pressure ulcer.

5. Conclusions

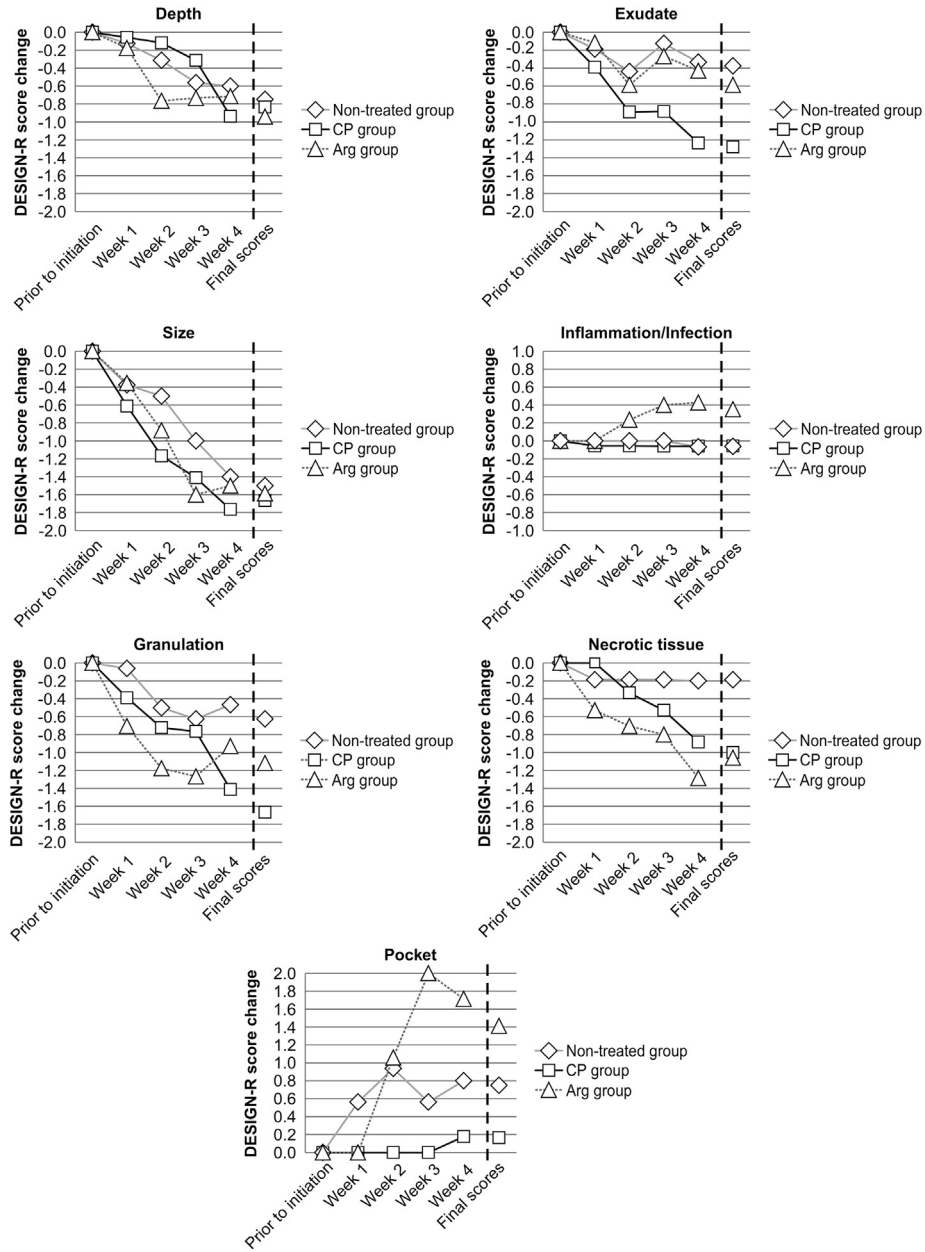
We compared the supplemental CP- or Arg-containing drinks with no additional intervention under following a usual manner to provide supplemental drinks by a multicenter, randomized, controlled trial in patients with pressure ulcers. The CP-containing drink facilitated the healing of pressure ulcers since the total DESIGN-R score was significantly lower in the CP group compared with those in the control group.

Funding source

The test drinks, laboratory testing, enrollment center sourcing expenses, and aggregate analysis sourcing expenses were paid for by Nutri Co., Ltd.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper. However, HY has received honoraria for lectures from Nutri Co., Ltd. (Yokkaichi, Japan).



| DESIGN-R score change | Group | Prior to initiation of intake | | Week 1 | | Week 2 | | Week 3 | | Week 4 | | Final scores | |
|------------------------|-------------------|-------------------------------|--------|-----------------|--------|-----------------|--------|-----------------|--------|-----------------|--------|-----------------|--------|
| | | Mean | SD (n) | Mean | SD (n) | Mean | SD (n) | Mean | SD (n) | Mean | SD (n) | Mean | SD (n) |
| Depth | Non-treated group | 0.0 ± 0.0 (16) | | -0.1 ± 0.3 (16) | | -0.3 ± 0.6 (16) | | -0.6 ± 0.9 (16) | | -0.6 ± 1.1 (15) | | -0.8 ± 1.2 (16) | |
| | CP group | 0.0 ± 0.0 (18) | | -0.1 ± 0.2 (17) | | -0.1 ± 0.3 (17) | | -0.3 ± 0.5 (16) | | -0.9 ± 1.0 (16) | | -0.8 ± 1.0 (18) | |
| | Arg group | 0.0 ± 0.0 (17) | | -0.2 ± 0.4 (17) | | -0.8 ± 1.0 (17) | | -0.7 ± 1.0 (15) | | -0.7 ± 0.7 (14) | | -0.9 ± 1.0 (17) | |
| Exudate | Non-treated group | 0.0 ± 0.0 (16) | | -0.2 ± 0.8 (16) | | -0.4 ± 1.0 (16) | | -0.1 ± 1.2 (16) | | -0.3 ± 1.2 (15) | | -0.4 ± 1.2 (16) | |
| | CP group | 0.0 ± 0.0 (18) | | -0.4 ± 0.9 (18) | | -0.9 ± 1.2 (18) | | -0.9 ± 1.3 (17) | | -1.2 ± 1.4 (17) | | -1.3 ± 1.4 (18) | |
| | Arg group | 0.0 ± 0.0 (17) | | -0.1 ± 0.5 (17) | | -0.6 ± 1.3 (17) | | -0.3 ± 1.8 (15) | | -0.4 ± 1.9 (14) | | -0.6 ± 1.8 (17) | |
| Size | Non-treated group | 0.0 ± 0.0 (16) | | -0.4 ± 1.4 (16) | | -0.5 ± 1.5 (16) | | -1.0 ± 1.7 (16) | | -1.4 ± 1.8 (15) | | -1.5 ± 1.8 (16) | |
| | CP group | 0.0 ± 0.0 (18) | | -0.6 ± 1.2 (18) | | -1.2 ± 1.4 (18) | | -1.4 ± 1.7 (17) | | -1.8 ± 1.6 (17) | | -1.7 ± 1.6 (18) | |
| | Arg group | 0.0 ± 0.0 (17) | | -0.4 ± 1.5 (17) | | -0.9 ± 2.0 (17) | | -1.6 ± 2.2 (15) | | -1.5 ± 2.2 (14) | | -1.6 ± 2.1 (17) | |
| Inflammation/Infection | Non-treated group | 0.0 ± 0.0 (16) | | 0.0 ± 0.0 (16) | | 0.0 ± 0.0 (16) | | 0.0 ± 0.0 (16) | | -0.1 ± 0.3 (15) | | -0.1 ± 0.3 (16) | |
| | CP group | 0.0 ± 0.0 (18) | | -0.1 ± 0.2 (18) | | -0.1 ± 0.2 (18) | | -0.1 ± 0.2 (17) | | -0.1 ± 0.2 (17) | | -0.1 ± 0.2 (18) | |
| | Arg group | 0.0 ± 0.0 (17) | | 0.0 ± 0.0 (17) | | 0.2 ± 0.8 (17) | | 0.4 ± 1.1 (15) | | 0.4 ± 1.1 (14) | | 0.4 ± 1.0 (17) | |
| Granulation | Non-treated group | 0.0 ± 0.0 (16) | | -0.1 ± 1.1 (16) | | -0.5 ± 1.0 (16) | | -0.6 ± 1.1 (16) | | -0.5 ± 1.0 (15) | | -0.6 ± 1.1 (16) | |
| | CP group | 0.0 ± 0.0 (18) | | -0.4 ± 1.9 (18) | | -0.7 ± 1.8 (18) | | -0.8 ± 1.6 (17) | | -1.4 ± 1.6 (17) | | -1.7 ± 1.9 (18) | |
| | Arg group | 0.0 ± 0.0 (17) | | -0.7 ± 1.0 (17) | | -1.2 ± 1.7 (17) | | -1.3 ± 1.8 (15) | | -0.9 ± 2.5 (14) | | -1.1 ± 2.4 (17) | |
| Necrotic tissue | Non-treated group | 0.0 ± 0.0 (16) | | -0.2 ± 1.3 (16) | | -0.2 ± 1.3 (16) | | -0.2 ± 1.3 (16) | | -0.2 ± 1.4 (15) | | -0.2 ± 1.3 (16) | |
| | CP group | 0.0 ± 0.0 (18) | | 0.0 ± 1.0 (18) | | -0.3 ± 1.0 (18) | | -0.5 ± 1.2 (17) | | -0.9 ± 1.4 (17) | | -1.0 ± 1.5 (18) | |
| | Arg group | 0.0 ± 0.0 (17) | | -0.5 ± 1.2 (17) | | -0.7 ± 1.3 (17) | | -0.8 ± 1.8 (15) | | -1.3 ± 1.9 (14) | | -1.1 ± 1.8 (17) | |
| Pocket | Non-treated group | 0.0 ± 0.0 (16) | | 0.6 ± 2.3 (16) | | 0.9 ± 2.4 (16) | | 0.6 ± 2.3 (16) | | 0.8 ± 2.4 (15) | | 0.8 ± 2.3 (16) | |
| | CP group | 0.0 ± 0.0 (18) | | 0.0 ± 0.0 (18) | | 0.0 ± 0.0 (18) | | 0.0 ± 0.0 (17) | | 0.2 ± 2.7 (17) | | 0.2 ± 2.6 (18) | |
| | Arg group | 0.0 ± 0.0 (17) | | 0.0 ± 0.0 (17) | | 1.1 ± 4.4 (17) | | 2.0 ± 5.4 (15) | | 1.7 ± 6.0 (14) | | 1.4 ± 5.4 (17) | |

Fig. 4. Changes in the DESIGN-R subscores by depth, exudate, size, inflammation/infection, granulation, necrotic tissue, and pocket formation (including differences). CP-containing drink group [CP group]; Arg-containing drink group [Arg group]; SD, standard deviation. The graph shows the mean values of patients analyzed in each group. The table shows the mean ± SD and number of patients. **p* < 0.05, Steel's multiple-comparison test (one-sided) compared with the control group.

Table 4
Changes in laboratory test results.

| | Reference | Group | Prior to initiation | | Week 2 | | Week 4 | |
|--|----------------------------------|---------|---------------------|-------------|---------|----------------------|---------|-------------|
| | value | | Mean | SD | Mean | SD | Mean | SD |
| Total protein (g/dL) | (6.7–8.3) | Control | 6.43 | ± 0.73 | 6.53 | ± 0.58 | 6.45 | ± 0.57 |
| | | CP | 6.68 | ± 0.52 | 6.64 | ± 0.51 | 6.80 | ± 0.63 |
| | | Arg | 6.64 | ± 0.78 | 6.72 | ± 0.87 | 6.68 | ± 0.76 |
| Albumin (g/dL) | (3.9–4.9) | Control | 2.89 | ± 0.71 | 2.91 | ± 0.74 | 2.89 | ± 0.68 |
| | | CP | 3.21 | ± 0.39 | 3.14 | ± 0.37 | 3.25 | ± 0.40 |
| | | Arg | 3.02 | ± 0.54 | 3.11 | ± 0.76 | 3.18 | ± 0.63 |
| Prealbumin (mg/dL) | (22.0–40.0) | Control | 15.79 | ± 5.83 | 15.88 | ± 6.23 | 16.13 | ± 6.07 |
| | | CP | 16.67 | ± 5.98 | 15.80 | ± 5.54 | 15.84 | ± 5.40 |
| | | Arg | 17.61 | ± 5.24 | 17.89 | ± 6.69 | 17.23 | ± 5.56 |
| Hemoglobin (g/dL) | (M: 13.5–17.6) (F: 11.3–15.2) | Control | 10.37 | ± 1.08 | 10.61 | ± 1.34 | 10.40 | ± 1.31 |
| | | CP | 11.34 | ± 1.65 | 10.86 | ± 1.56 | 11.17 | ± 1.68 |
| | | Arg | 11.13 | ± 1.84 | 11.34 | ± 2.29 | 11.51 | ± 2.21 |
| Lymphocytes (%) | (18.0–59.0) | Control | 28.88 | ± 6.99 | 27.96 | ± 8.86 | 26.89 | ± 5.81 |
| | | CP | 27.23 | ± 10.43 | 24.70 | ± 6.85 | 25.76 | ± 6.76 |
| | | Arg | 28.22 | ± 9.08 | 26.72 | ± 10.90 | 26.09 | ± 10.00 |
| Red blood cell count ($\times 10^4/\mu\text{L}$) | (M: 427–570) (F: 376–500) | Control | 342.06 | ± 42.53 | 351.19 | ± 44.96 | 344.60 | ± 44.72 |
| | | CP | 380.00 | ± 60.20 | 367.06 | ± 55.82 | 377.88 | ± 56.60 |
| | | Arg | 361.76 | ± 60.87 | 369.47 | ± 76.73 | 369.14 | ± 74.55 |
| White blood cell count (/ μL) | (M: 3900–9800) (F: 3500–9100) | Control | 6518.75 | ± 1494.09 | 6356.25 | ± 2414.67 | 6513.33 | ± 2026.91 |
| | | CP | 6850.00 | ± 1944.00 | 7329.41 | ± 2174.21 | 7270.59 | ± 2269.30 |
| | | Arg | 6982.35 | ± 2602.94 | 7782.35 | ± 3519.63 | 7000.00 | ± 2973.73 |
| Platelet count ($\times 10^4/\mu\text{L}$) | (M: 13.1–36.2) (F: 13.0–36.9) | Control | 30.99 | ± 9.01 | 30.21 | ± 11.86 | 28.52 | ± 10.35 |
| | | CP | 24.58 | ± 12.92 | 28.22 | ± 13.03 | 28.37 | ± 14.42 |
| | | Arg | 27.00 | ± 7.10 | 26.60 | ± 8.17 | 27.35 | ± 9.39 |
| AST (IU/L) | (10–40) | Control | 20.56 | ± 6.67 | 20.25 | ± 7.70 | 21.07 | ± 6.93 |
| | | CP | 20.17 | ± 8.21 | 23.59 | ± 9.23 | 23.76 | ± 10.41 |
| | | Arg | 29.00 | ± 26.08 | 20.41 | ± 4.57 | 23.21 | ± 8.73 |
| ALT (IU/L) | (5–40) | Control | 15.0 | [11.3–21.5] | 13.0 | [10.3–22.8] | 16.0 | [10.0–23.0] |
| | | CP | 13.5 | [10.8–23.0] | 16.0 | [11.0–25.5] | 18.0 | [10.5–29.5] |
| | | Arg | 15.0 | [11.0–34.5] | 18.0 | [10.5–24.5] | 19.0 | [10.8–29.0] |
| ALP (IU/L) | (115–359) | Control | 271.31 | ± 109.64 | 274.31 | ± 114.75 | 276.60 | ± 100.60 |
| | | CP | 268.06 | ± 51.88 | 271.00 | ± 72.96 | 287.65 | ± 71.56 |
| | | Arg | 323.94 | ± 138.87 | 322.29 | ± 95.87 | 331.57 | ± 195.26 |
| BUN (mg/dL) | (8.0–22.0) | Control | 21.13 | ± 9.22 | 21.24 | ± 8.17 | 21.53 | ± 9.48 |
| | | CP | 17.55 | ± 6.36 | 19.21 | ± 7.18 | 18.88 | ± 6.40 |
| | | Arg | 23.62 | ± 13.54 | 26.30 | ± 14.31 ^a | 25.44 | ± 14.92 |
| Creatinine (mg/dL) | (M: 0.61–1.04) (F: 0.47–0.79) | Control | 0.57 | ± 0.26 | 0.57 | ± 0.28 | 0.59 | ± 0.29 |
| | | CP | 0.59 | ± 0.27 | 0.63 | ± 0.30 | 0.61 | ± 0.29 |
| | | Arg | 0.64 | ± 0.37 | 0.62 | ± 0.37 | 0.65 | ± 0.38 |
| CRP (mg/dL) | (0–0.30) | Control | 0.81 | [0.27–2.19] | 0.81 | [0.36–1.50] | 0.51 | [0.19–1.35] |
| | | CP | 0.61 | [0.23–1.68] | 0.99 | [0.20–3.01] | 0.84 | [0.28–2.94] |
| | | Arg | 0.69 | [0.23–1.54] | 1.05 | [0.34–1.92] | 1.05 | [0.29–2.42] |
| Na (mEq/L) | (136–147) | Control | 140.13 | ± 5.84 | 140.44 | ± 6.62 | 138.53 | ± 4.91 |
| | | CP | 138.22 | ± 7.17 | 137.65 | ± 6.45 | 137.65 | ± 5.70 |
| | | Arg | 139.41 | ± 3.97 | 139.94 | ± 3.44 | 138.71 | ± 3.05 |
| K (mEq/L) | (3.6–5.0) | Control | 4.24 | ± 0.52 | 4.14 | ± 0.62 | 4.05 | ± 0.57 |
| | | CP | 4.09 | ± 0.55 | 4.08 | ± 0.38 | 4.19 | ± 0.42 |
| | | Arg | 4.35 | ± 0.53 | 4.25 | ± 0.71 | 4.14 | ± 0.67 |
| Cl (mEq/L) | (98–109) | Control | 103.44 | ± 6.27 | 103.56 | ± 6.42 | 102.53 | ± 5.66 |
| | | CP | 101.50 | ± 6.20 | 100.94 | ± 6.09 | 100.94 | ± 5.38 |
| | | Arg | 104.41 | ± 3.66 | 105.47 | ± 3.20 | 103.71 | ± 2.81 |
| P (mg/dL) | (2.4–4.3) | Control | 3.42 | ± 0.62 | 3.46 | ± 0.70 | 3.45 | ± 0.66 |
| | | CP | 3.58 | ± 0.49 | 3.51 | ± 0.51 | 3.57 | ± 0.58 |
| | | Arg | 3.69 | ± 0.55 | 4.01 | ± 0.72 ^b | 3.88 | ± 0.60 |
| Zn ($\mu\text{g/dL}$) | (65–110) | Control | 55.94 | ± 14.52 | 52.13 | ± 14.05 | 49.33 | ± 10.73 |
| | | CP | 66.89 | ± 16.56 | 70.88 | ± 16.25 | 72.00 | ± 15.76 |
| | | Arg | 64.12 | ± 23.75 | 57.65 | ± 19.76 | 56.21 | ± 16.26 |

A significant difference by the paired *t*-test compared with prior to the initiation of intake. Data indicate mean \pm standard deviation except ALT and CRP described as median and quantile. CP-containing drink group [CP group]; Arg-containing drink group [Arg group]. M, male; F, female; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; CRP, C-reactive protein; Na, sodium; K, potassium; Cl, chloride; P, phosphorus; Zn, zinc.

^a *p* = 0.012.

^b *p* = 0.014.

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this study.

Abbreviations

ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; Arg = arginine; CP = collagen peptide; CRP = C-reactive protein.

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