Journal of Nutrition & Intermediary Metabolism 8 (2017) 51–59

Contents lists available at ScienceDirect

Journal of Nutrition & Intermediary Metabolism

journal homepage: http://www.jnimonline.com/

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

Hideharu Yamanaka, MD, PhD [a, *], Shingo Okada, MD [b], Hiromi Sanada, RN, PhD [c]

[a] Department of Surgery, Wakakusa Daiichi Hospital, Osaka, Japan
[b] Department of Surgery, Kitamihara Clinic, Hakodate, Japan
[c] Department of Gerontological Nursing/Wound Care Management, Division of Health Sciences and Nursing, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

ARTICLE INFO

Article history:
Received 20 December 2016
Received in revised form
6 May 2017
Accepted 9 May 2017
Available online 10 May 2017

Keywords:
Pressure ulcer
Wound healing
Collagen peptide
Arginine
Nutrition therapy

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.

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.

* Corresponding author. Department of Surgery, Wakakusa-Daiichi Hospital, 1-6 Wakakusa-cho, Higashi Osaka 579-8056, Japan. Tel.: +81 72 988 1409; fax: +81 72 985 6731.
E-mail address: wakakusa@wakakoukai.or.jp (H. Yamanaka).

http://dx.doi.org/10.1016/j.jnim.2017.05.001

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 ≥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 ≤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 ≥ 8.0% [National Glycohemoglobin Standardization Program value] or ≥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.

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

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

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 Cotinence 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,

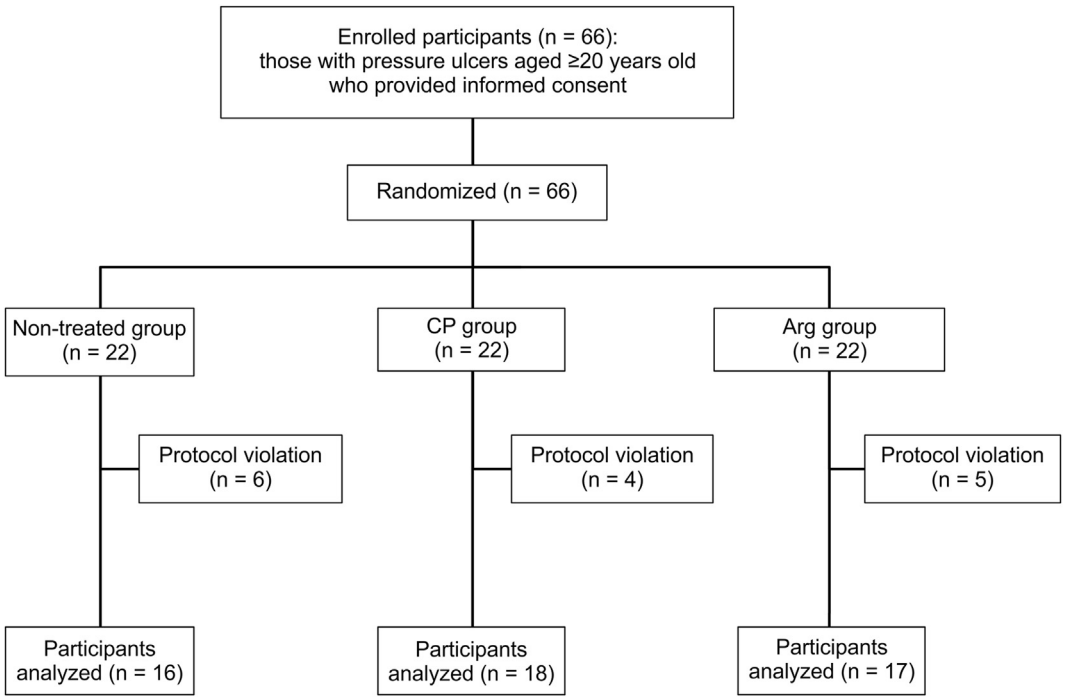


Fig. 2. Flow chart of patients included in the analyses.

Table 3
Background characteristics of patients analyzed in each group (all values mean ± 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 ± 7.7	76.8 ± 13.9	79.6 ± 12	0.9[2)]
BMI (kg/m^2)	18.5 ± 2.8	18.8 ± 2.1	18.2 ± 2.7	0.58[2)]
BEE (kcal/day)	937.5 ± 101.4	995.2 ± 153.3	952.0 ± 119.7	0.61[2)]
Caloric intake (kcal/day)	1394.8 ± 333.1	1472.4 ± 307.3	1336.0 ± 288.1	0.27[2)]
Total DESIGN-R scores prior to initiation of intake	15.9 ± 5.7	14.1 ± 4.3	14.1 ± 5.6	0.46[2)]
Total Braden scale scores	12.4 ± 2.8	14.1 ± 3.8	12.9 ± 2.4	0.56[2)]
Alb (g/dL)	2.89 ± 0.71	3.21 ± 0.39	3.02 ± 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.

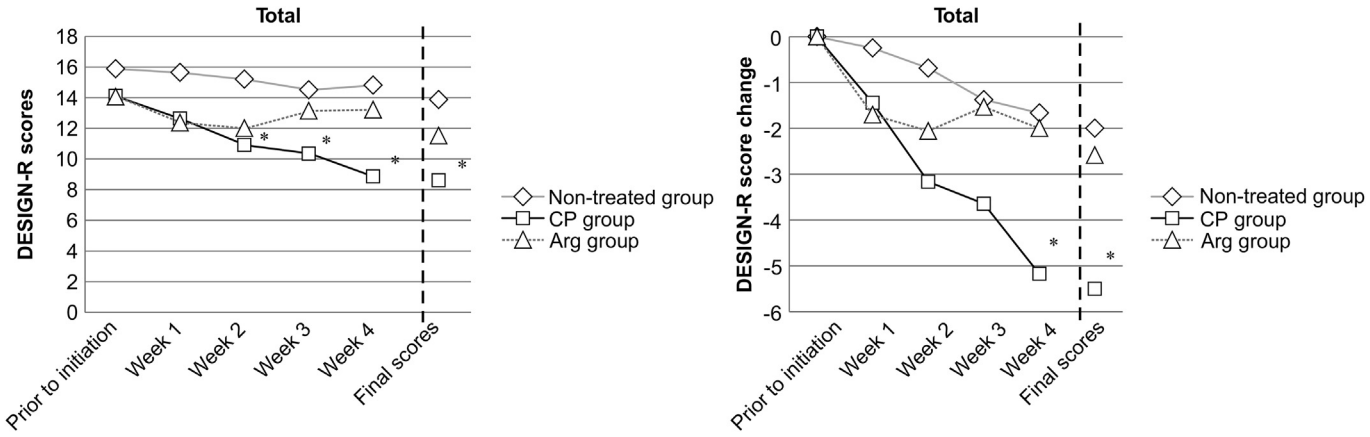
4. Discussion

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 predicative 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 ± 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

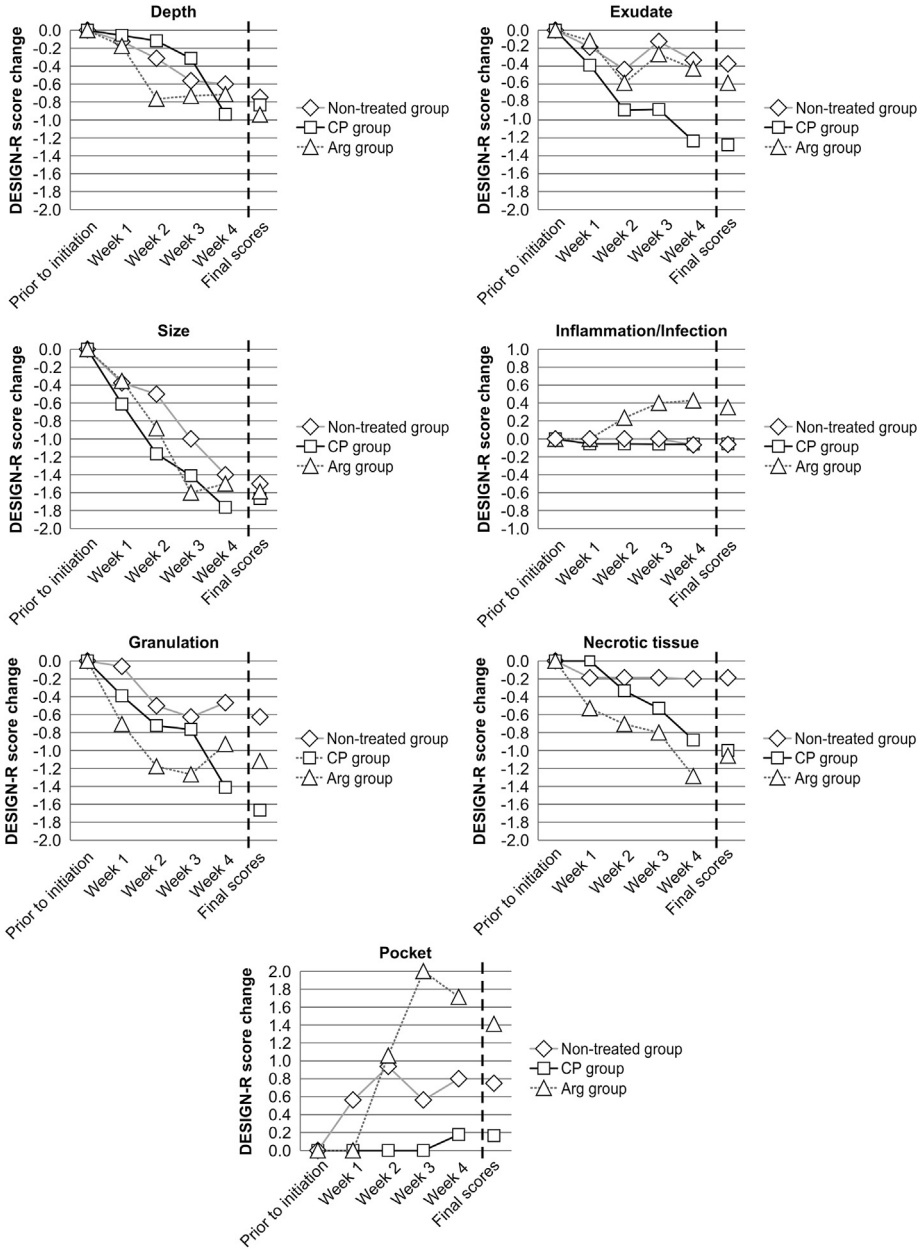
We compared the supplemental CP- or Arg-containing drinks with no additional intervention under following a usual manner to provide supplemental drinks by a multicenter, randomized, controlled trial in patients with pressure ulcers. The CP-containing drink facilitated the healing of pressure ulcers since 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 Mean ± SD (n)	Week 1 Mean ± SD (n)	Week 2 Mean ± SD (n)	Week 3 Mean ± SD (n)	Week 4 Mean ± SD (n)	Final scores 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	(6.7–8.3)	Control	6.43	±	0.73	6.53	±	0.58	6.45	±	0.57
(g/dL)		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	(3.9–4.9)	Control	2.89	±	0.71	2.91	±	0.74	2.89	±	0.68
(g/dL)		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	(22.0–40.0)	Control	15.79	±	5.83	15.88	±	6.23	16.13	±	6.07
(mg/dL)		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	(M: 13.5–17.6)	Control	10.37	±	1.08	10.61	±	1.34	10.40	±	1.31
(g/dL)	(F: 11.3–15.2)	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	(M: 427–570)	Control	342.06	±	42.53	351.19	±	44.96	344.60	±	44.72
cell count	(F: 376–500)	CP	380.00	±	60.20	367.06	±	55.82	377.88	±	56.60
($\times 10^4$/μL)		Arg	361.76	±	60.87	369.47	±	76.73	369.14	±	74.55
White blood	(M: 3900–9800)	Control	6518.75	±	1494.09	6356.25	±	2414.67	6513.33	±	2026.91
cell count	(F: 3500–9100)	CP	6850.00	±	1944.00	7329.41	±	2174.21	7270.59	±	2269.30
(/μL)		Arg	6982.35	±	2602.94	7782.35	±	3519.63	7000.00	±	2973.73
Platelet count	(M: 13.1–36.2)	Control	30.99	±	9.01	30.21	±	11.86	28.52	±	10.35
($\times 10^4$/μL)	(F: 13.0–36.9)	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	(8.0–22.0)	Control	21.13	±	9.22	21.24	±	8.17	21.53	±	9.48
(mg/dL)		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	(M: 0.61–1.04)	Control	0.57	±	0.26	0.57	±	0.28	0.59	±	0.29
(mg/dL)	(F: 0.47–0.79)	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 (μg/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 ± 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] $p = 0.014$.

Acknowledgements

We are deeply grateful to Dr. Shinji Iizaka of the Department of Gerontological Nursing/Wound Care Management, Division of Health Sciences and Nursing, The University of Tokyo Graduate School of Medicine, for providing advice about the assessment of pressure ulcers for the appropriate performance of 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.

References

[1] Ohura T, Nakajo T, Okada S, Omura K, Adachi K. Evaluation of effects of nutrition intervention on healing of pressure ulcers and nutritional states (randomized controlled trial). Wound Repair Regen 2011;19:330–6.

[2] Wong A, Chew A, Wang CM, Ong L, Zhang SH, Young S. The use of a specialized amino acid mixture for pressure ulcers: a placebo-controlled trial. J Wound Care 2014;23:259–60. 262-4, 266-9.

[3] Langer G, Fink A. Nutritional interventions for preventing and treating pressure ulcers. Cochrane Database Syst Rev 2014 Jun;12(6):CD003216.

[4] Kirk SJ, Hurson M, Regan MC, Holt DR, Wasserkrug HL, Barbul A. Arginine stimulates wound healing and immune function in elderly human beings. Surgery 1993;114:155–60.

[5] Varani J, Warner RL, Gharaee-Kermani M, Phan SH, Kang S, Chung JH, et al. Vitamin A antagonizes decreased cell growth and elevated collagen-degrading matrix metalloproteinases and stimulates collagen accumulation in naturally aged human skin. J Invest Dermatol 2000;114:480–6.

[6] Marini A, Grether-Beck S, Jaenicke T, Weber M, Burki C, Formann P, et al. Pycnogenol® effects on skin elasticity and hydration coincide with increased gene expressions of collagen type I and hyaluronic acid synthase in women. Skin Pharmacol Physiol 2012;25:86–92.

[7] Proksch E, Segger D, Degwert J, Schunck M, Zague V, Oesser S. Oral supplementation of specific collagen peptides has beneficial effects on human skin physiology: a double-blind, placebo-controlled study. Skin Pharmacol Physiol 2014;27:47–55.

[8] Japanese Society of Pressure Ulcers. DESIGN-R scoring manual. Available from:: http://www.jspu.org/pdf/DESIGN-R_manual_eng.pdf.

[9] Matsui Y, Furue M, Sanada H, Tachibana T, Nakayama T, Sugama J, et al. Development of the DESIGN-R with an observational study: an absolute evaluation tool for monitoring pressure ulcer wound healing. Wound Repair Regen 2011;19:309–15.

[10] Harris JA, Benedict FG. A biometric study of human basal metabolism. Proc Natl Acad Sci U. S. A 1918;4:370–3.

[11] Fearon K, Strasser F, Anker SD, Bosaeus I, Bruera E, Fainsinger RL, et al. Definition and classification of cancer cachexia: an international consensus. Lancet Oncol 2011;12:489–95.

[12] Brewer S, Desneves K, Pearce L, Mills K, Dunn L, Brown D, et al. Effect of an arginine-containing nutritional supplement on pressure ulcer healing in community spinal patients. J Wound Care 2010;19:311–6.

[13] Wu G, Morris Jr SM. Arginine metabolism: nitric oxide and beyond. Biochem J 1998;336:1–17.

[14] Nakao K, Kusubata M, Hara K, Igarashi M, Yamazaki N, Koyama Y. Effects of collagen peptide ingestion on healing of skin wound in a rat model of pressure ulcer. Jpn Pharmacol Ther 2013;41:587–96.

[15] Iizaka S, Sanada H, Matsui Y, Furue M, Tachibana T, Nakayama T, et al. Predictive validity of weekly monitoring of wound status using DESIGN-R score change for pressure ulcer healing: a multicenter prospective cohort study. Wound Repair Regen 2012;20:473–81.

[16] Sanada H, Iizaka S, Matsui Y, Furue M, Tachibana T, Nakayama T, et al. Clinical wound assessment using DESIGN-R total score can predict pressure ulcer healing: pooled analysis from two multicenter cohort studies. Wound Repair Regen 2011;19:559–67.

[17] Breslow RA, Hallfrisch J, Guy DG, Crawley B, Goldberg AP. The importance of dietary protein in healing pressure ulcers. J Am Geriatr Soc 1993;41:357–62.