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ORIGINAL ARTICLE

Investigating the Nutrient Content of Enteral Diets in Mashhad Hospitals and Designing A Blenderized Tube Feeding for Hospitals

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ARTICLE INFO	ABSTRACT		
<i>Keywords:</i> Nutrient content Enteral nutrition Blenderized tube feeding Hospital Iran	Background: Some patients cannot feed orally and use enteral nutrition to meet their daily food needs. This study aimed to investigate the calories and macronutrients of enteral formulas prepared in Mashhad hospitals and to design blenderized tube feeding for hospitals.		
	 Methods: Fifteen samples were taken from each hospital to measure the energy and macronutrients of enteral formulas of Mashhad hospitals. Fresh and natural foods were also used to design blenderized tube feeding. Results: The present study showed that the calories of blenderized tube feeding prepared in the hospitals were between 81-97 kcal/100 mL and in commercial enteral formula between 80-96 kcal/100 mL. In addition, protein, 		
	fat, and carbohydrates of blenderized tube feeding prepared in hospitals were between 8-16%, 31-39, and 51-56%, respectively. Also, commercial enteral formula's protein, fat, and carbohydrates in hospitals were between 13-15%,		
*Corresponding author: Mitra Rezaie, PhD; Department of Nutrition, Faculty of Medicine, Mashhad University of Medical	30-35, and 49-55%, respectively. Energy, protein, fat and carbohydrate of blenderized tube feeding designed from fresh and natural foods were between 102-112 kcal/100 mL, 16-23%, 31-34%, and 45-51%, respectively. Conclusion: The blenderized tube feeding prepared in hospitals and the		
Sciences, Mashhad, Iran. Tel: +98-51-38002362 Email: rezaeigm@mums.ac.ir Received: November 14, 2023 Revised: January 15, 2024 Accepted: January 20, 2024	commercial enteral formula did not meet the energy requirement of 1 kcal/mL. In addition, the energy and macronutrients of the blenderized tube feeding were used in the present study to design fresh and natural foods and were within the recommended range for patients.		

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Introduction

Enteral nutrition is a route of nutritional support and the provision of enteral food for patients who cannot meet their needs orally and are at risk of malnutrition (1). Lew *et al.* showed that malnutrition in the intensive care unit (ICU) is between 38 and 78%, which can increase the duration of hospitalization, illness, and death, the risk of infection, and increase hospital costs (2). Therefore, the initiation of nutritional support (enteral and parenteral nutrition) in critically ill patients and adequate intake of nutrients and energy can help

prevent the consequences of malnutrition and play an important role in modulating the patient's condition (3). Several studies showed that enteral nutrition was preferable to parenteral nutrition for critically ill patients, because it supports immune system function, enhances the nutritional effect on the intestinal mucosa, inhibits mucosal atrophy, and reduces the risk of intestinal bacterial overgrowth and bacterial translocation (4-6).

Although commercial enteral formulas have been used in hospitals for more than 30 years, they may contain processed carbohydrates and highly saturated fats, artificial flavors, and additives (such as emulsifiers) that can be associated with inflammatory diseases (3, 7). In recent years, the use of blenderized tube feeding has increased due to the following reasons: They contain antioxidants, polyphenols, and carotenoids and play an essential role in human health, including stimulating the immune system, reducing platelet aggregation, regulating hormone metabolism, and lowering blood pressure. Also, organic foods can be used to design formulas, increase food variety, and prevent the patient's food allergy (3, 8, 9). Many studies have shown that the use of mixed foods reduced tube feeding intolerance (gagging, retching, constipation, diarrhea, and abdominal pain), hospitalization, and the need for digestive medications (10-12). Nevertheless, nutritionists still have concerns about the nutrient content of blenderized tube feeding, because some countries still need a specific formula for blenderized tube feeding. For example, Vieira et al. examined 33 noncommercial enteral diet samples and showed that macronutrients and energy were prescribed less than 50% (13). There is still concern about the lack of nutrient content for blenderized tube feeding, which is why the purpose of this article was to investigate the energy and macronutrients of blenderized tube feeding and commercial enteral formulas in hospitals and also the design of blenderized tube feeding for hospitals, where the calories and macronutrients were not within the prescribed limits for patients.

Materials and Methods

In order to check the energy and macronutrients of blenderized tube feeding and commercial enteral formula, samples were taken from 6 hospitals. Fifteen samples were taken from 3 hospitals that used blenderized tube feeding for patients. Three hospitals used commercial enteral formulas for patients, and 15 samples were taken from each of these three hospitals. In order to measure energy and macronutrients, all the samples were transferred to the nutrition laboratory of the Mashhad Faculty of Medical Sciences, Mashhad, Iran. Foods selected using the blenderized tube feeding food pyramid were (i) Step 1: Milk group because some patients were lactose intolerant and lactose-free milk powder was used at this stage. (ii) Step 2 as fruits and vegetables group that compounds such as apples, pears, carrots, and broccoli were used based on their vitamins, minerals, flavonoids, antioxidants, and anti-inflammatory activity. (iii) Step 3 as meat and legumes group that ingredients such as eggs, chicken, and turkey were used, because they could provide all the amino acids needed to make the protein the human body needs. (iv) Step 4 as cereals group that rice flour was used because of its fiber and carbohydrate content. (v) Step 5 as oils such as olive, canola, and medium-chain triglyceride (MCT) oil that were used in blenderized tube feeding. Finally (vi) Step 6 as simple sugar, while sugar and honey were used in this stage to provide carbohydrates for blenderized tube feeding.

The steps for preparing blenderized tube feeding 1 were (i) Step 1 as in this step, raw foods such as carrots and chicken breast were boiled in a pot and eggs were boiled in a separate pot. (ii) Step 2 that in this step, all the food ingredients (raw, cooked food, rice flour, apple, sugar, walnut, milk powder, and olive oil) were mixed by a blender. (iii) Step 3 as in this step, the required amount of cooked chicken broth and vegetables was added to the blender (Table 1). Finally (iv) Step 4 that in this step all the ingredients added to the mixer were homogenized for 5 minutes, then placed in a sterile plastic container and stored in the refrigerator.

The steps for preparing blenderized tube feeding 2 were (i) Step 1 that in this step, raw foods such as carrots, broccoli, and chicken thighs were boiled together in a pot. (ii) Step 2 as in this step, all the food ingredients (raw, cooked food, rice flour, pear, honey, sugar, milk powder, and olive oil) were mixed by a blender. (iii) Step 3 that in this step, the required amount of cooked chicken broth and vegetables was added to the blender (Table 1). Finally (iv) Step 4 as in this step, all the ingredients added to the mixer were homogenized for 5 minutes, then placed in a sterile plastic container and stored in the refrigerator.

The steps for preparing blenderized tube feeding 3 were (i) Step 1 that in this step, raw foods such as carrots and turkey breast were boiled in a pot. (ii) Step 2 as in this step, all the ingredients (raw, cooked food, rice flour, pear, sugar, milk powder, MCT oil, and canola oil) were mixed by a blender. (iii) Step 3 that in this step, the required amount of cooked turkey broth and vegetables was added to the blender (Table 1). Finally (iv) Step 4 that in this step, all the ingredients added to the mixer were homogenized for 5 minutes, then placed in a sterile plastic container and stored in the refrigerator.

Nutrients	nts Blenderized tube feeding 1		Blenderized tube feeding 2		Blenderized tube feeding 3	
	Food	Weight	Food	Weight	Food	Weight
	ingredients		ingredients		ingredients	
Protein sources	Cooked	65	Cooked chicken	65	Turkey breast	70
(g)	chicken breast		thighs		Milk powder	29
	Boiled egg	40	Milk powder	33		
	Milk powder	25				
Carbohydrate	Rice flour	25	Rice flour	30	Rice flour	37
sources (g)	Sugar	10	Honey	7	Sugar	9
			Sugar	10		
Lipid sources	Walnut	2	Olive oil	4	MCT oil	3
(g)	Olive oil	8			Canola oil	8
Fruit and	Apple	45	Cooked broccoli	15	Pear	34
vegetable	Cooked carrots	30	Cooked carrots	15	Cooked carrots	25
sources (g)			Pear	30		
Water (mL)	Chicken and	250	Water	291	Turkey and	285
	vegetable				vegetable	
	juices				juices	
Total	-	500		500	-	500

For physicochemical analysis and energy measurement, Atwater's factors was used, while energy density was obtained by multiplying protein and total carbohydrates by 4 kcal and fat by 9 kcal. For moisture measurement, 50 g of the sample was weighed and left in the oven for 24 hours to dry; then, the moisture content was measured by weight difference. Regarding protein measurement, Kjeldahl method was applied. Briefly, one g of dry sample, along with 4.5 g of potassium sulfate and 0.5 g of copper sulfate were poured into a cell and placed in a digesting flask, and this process was continued until the content inside the cell became bright green. Then, 40 mL of 4% boric acid, along with a few drops of methyl red were poured into the cell and placed in the Kjeldahl apparatus, and then the sample, which turned bright green, was placed on the other side of the Kjeldahl. Then, 70 mL of distilled water and 75 mL of sodium hydroxide were added to the tube that contained the bright green sample, and the device was turned on. This work was continued until the sample turned yellow and the sample nitrogen was extracted entirely. Then Arlene was removed and titrated with 2/normal sulfuric acid. The amount of protein was calculated using the nitrogen conversion factor of 6.25.

For fat measurement, Soxhlet method was applied. In summary, 5 g of the sample was wrapped in filter paper, and its weight was recorded along with the filter paper. Then it was placed inside the tube with hexane inside the Soxhlet apparatus for 10 hours. The sample was further removed from the device with the filter paper and dried in the oven; and finally, the weight was recorded after placing it in the oven. The difference in weight determined the amount of fat. Ash measurement was undertaken by electric furnace method, while 1 g of the sample was weighed, placed in an electric furnace at 700°C for 5 hours, and them the amount of ash was evaluated by weight difference. Carbohydrate content was determined as carbohydrates g/100 g=100–(fat+ash+moisture+protein). Statistical analyses were carried out using SPPS software (Version 22, Chicago, IL, USA). The variance analysis and the means were compared with the Tukey post hoc test ($p \le 0.05$).

Results

Table 2 shows the analytical results of macronutrients and energy of BTFs prepared in the hospital. The energy of blenderized tube feeding prepared in the hospital (except hospital A) was lower than recommended for patients. However, macronutrients were within the recommended range for patients. Table 3 shows the energy and macronutrients of commercial enteral formula. Hospital commercial enteral formula energy was almost according to the guideline, but the other hospital was below the recommended level. However, the macronutrients were commercial enteral formulas according to the guidelines. Table 4 shows the macronutrients and energy of blenderized tube feeding prepared from fresh and natural ingredients with a unique formulation. The energy and macronutrients of the hospitalprepared blenderized tube feeding reached the recommended levels. Table 5 shows the emptying time of 60 mL of the formula prepared with natural and fresh ingredients. Formulas designed with natural ingredients were quickly drained without clogging the feeding tube.

Table 2: Energy and macronutrients of hospital-prepared blenderized tube feeding.			
Factor formula	Hospital A	Hospital B	Hospital C
	(n=15)	(n=15)	(n=15)
Moisture content, g/100 mL	80.22±1.8ª	82.65±0.01b	83.08±0.04 ^b
Protein, g/100 mL	2.09±0.19ª	$3.4{\pm}0.19^{\text{b}}$	$2.10{\pm}0.12^{a}$
Fat, g/100 mL	$4.30{\pm}0.14^{a}$	$3.70 {\pm} 0.05^{b}$	3.20±0.4°
Carbohydrate, g/100 mL	12.71±1.45 ^a	10.55±1.77 ^b	11.41 ± 0.96^{ab}
Ash, g/100 mL	0.68±0.12ª	$0.42{\pm}0.08^{b}$	$0.38 {\pm} 0.03^{\text{b}}$
Energy, kcal/100 mL	97.93±9.06ª	81.59±7.23 ^b	81.24±1.10 ^b
%Calorie from protein	8.68±1.53ª	16.96±2.02b	10.35±0.52°
%Calorie from fat	39.50±1.88ª	31.66±2.37 ^b	33.41±4.81 ^b
%Calorie from carbohydrate	51.84±1.43ª	51.37±3.96ª	56.22±4.98 ^b

The data were presented as mean \pm standard deviation. Any two means in the same row followed by the same letter were not significantly different from Tukey post-hoc test (p>0.05).

Table 3: Energy and macronutrients of commercial enteral formulas.			
Factor formula	Hospital D	Hospital E	Hospital F
	(n=15)	(n=15)	(n=15)
Moisture content, g/100 mL	$80.06{\pm}0.04^{a}$	79.52±0.52ª	83.22 ± 0.88^{b}
Protein, g/100 mL	3.24±0.19 ^a	3.73 ± 0.01^{b}	3.11±0.10°
Fat, g/100 mL	$3.24{\pm}0.00^{a}$	3.31 ± 0.03^{b}	$3.14{\pm}0.07^{\circ}$
Carbohydrate, g/100 mL	12.91±0.07 ^a	13±0.49ª	$9.90 {\pm} 0.88^{b}$
Ash, g/100 mL	$0.54{\pm}0.00^{a}$	$0.41 {\pm} 0.00^{b}$	$0.55{\pm}0.00^{a}$
Energy, kcal/100 mL	93.63±0.26ª	96.77±1.95 ^b	80.59±3.45°
%Calorie from protein	13.84±0.03ª	15.44 ± 0.29^{b}	15.77±0.69 ^b
%Calorie from fat	30.98±0.10ª	30.83±0.60ª	35.15±1.52 ^b
%Calorie from carbohydrate	55.17±0.14ª	53.71±0.90 ^b	49.07±2.21°

The data were presented as mean \pm standard deviation. Any two means in the same row followed by the same letter were not significantly different from Tukey post-hoc test (p>0.05).

Table 4: Macronutrients and energy according to the type of the blenderized tube feeding diet.			
Factor formula	Blenderized tube feeding	Blenderized tube feeding	Blenderized tube feeding
	1, mean±SD	2, mean±SD	3, mean±SD
	(n=3)	(n=3)	(n=3)
Moisture content, g/100 mL	78.87±0.03ª	78.02 ± 0.02^{b}	76.37±0.38°
Protein, g/100 mL	4.93±0.19ª	$4.44{\pm}0.09^{a}$	6.65 ± 0.40^{b}
Fat, g/100 mL	3.95±0.02ª	3.70 ± 0.05^{b}	$3.90{\pm}0.09^{a}$
Carbohydrate, g/100 mL	11.83±0.25 ^a	13.61±0.12 ^b	12.66±0.11°
Ash, g/100 mL	0.41 ± 0.00^{a}	0.21 ± 0.00^{b}	0.41±0.01ª
Energy, kcal/100 mL	102.61±0.14 ^a	105.60±0.33 ^b	112.38±1.15°
%Calorie from protein	19.21±0.77 ^a	16.83±0.33 ^b	23.67±1.22°
%Calorie from fat	34.64±0.21ª	31.59±0.32 ^b	31.26±1.22 ^b
%Calorie from carbohydrate	46.13±1ª	51.59±0.60 ^b	45.06±0.22ª

The data were presented as mean \pm standard deviation. Any two means in the same row followed by the same letter were not significantly different from Tukey post-hoc test (p>0.05).

Table 5: Time needed to finalize 60 mL of blenderized tube feeding through feeding syringe.		
Blenderized tube feeding Time needed to discharge 60 mL of blend		
	feeding through feeding syringe, s, mean±SD	
1	151±10 ^a	
2	70±7 ^b	
3	125±5°	

The data were presented as mean \pm standard deviation. Any two means in the same row followed by the same letter were not significantly different from Tukey post-hoc test (p>0.05).

Discussion

According to the guidelines, the acceptable range of macronutrients from total calories for carbohydrates is 45-65%, for protein is 5-20% regarding age of 1-3 years and 10-30% in age of four years and older and finally for fat is 30-40% in age of 1-3 years and 25-35% in age of four years and older. Table 3 shows that the energy in blenderized tube feeding in a hospital was 97 kcal/100 mL. It could almost provide the daily calories of the patients; but in other two hospitals, the calories were 80 kcal/100 mL, which did not provide the daily calories of the patients. Table 4 demonstrates that the energy of the commercial enteral formula of two hospitals was 96 and 93 kcal/100 mL, which could provide almost daily calories of the patients. Nevertheless, in the other hospital, the calorie was 80 kcal/100 mL, which did not provide daily calories of the patients. These findings are very worrying because it can lead to malnutrition among patients. Many researchers have reported that malnutrition increases the length of hospitalization, delay the recovery, lower quality of life, increases hospital costs, and rises mortality (14).

In another study, the researchers investigated the nutrients and energy content of blenderized tube feeding and commercial diets. They found that the energy of blenderized tube feeding was 40 kcal/100 mL, but for the commercial enteral diet was 1 kcal/mL (13). Several studies have reported that the prevalence of protein-energy malnutrition in hospitalized populations is between 40% and 60%, worldwide (15-17). One of the reasons for malnutrition in hospitals has been the lack of attention to medical foods regarding both blenderized tube feeding and commercial diets. Our findings have also shown that enteral nutrition formulas prepared in hospitals could not supply the patient's energy. In addition, the blenderized tube feeding diets designed in the present study utilizing fresh and natural ingredients, energy, and macronutrients were according to the guidelines. Bahramian et al. who designed blenderized tube feeding, showed that the calories of blenderized tube feeding were between 103 and 112 kcal/100 mL, similar to the present study (18). In another identical study, the designed blenderized tube feeding could provide an energy density of 1 kcal/mL (19).

According to the guidelines, protein should be 5-20% for ages 1-3 years and 10-30% for ages four years and older. In the current study, the protein percentage of total calories in the blenderized tube feeding diet was between 8 and 16%, and in the commercial enteral formula, between 13% and 15%, which was under the guidelines. Vieira *et al.* who evaluated the macronutrients of blenderized tube

feeding and commercial enteral formula, displayed that blenderized tube feeding had significantly lower values for protein. However, in the commercial enteral formula, the protein was about 20% more than the prescribed amount (13). In the design of blenderized tube feeding in the present study, the protein was between 16% and 23% based on the guidelines. In the design of blenderized tube feeding, the first important step is to choose the right source of protein. Our study used animal proteins due to their higher protein efficiency ratio, biological value, net protein utilization, and modified amino acid score with higher protein digestibility (20). In the present study, chicken, egg, and turkey were the main sources of blenderized tube feeding protein, which did not contain trans fatty acids, but beef included 2-5%, and mutton contained 8% trans fatty acids, which were not utilized in this study (21).

Eating red meat has been shown to cause inflammation. We need to push the blenderized tube feeding to use the compounds recommended in the Mediterranean diet; for this reason, it does not recommend red meat in the Mediterranean diet, and it suggests use of chicken meat because of their less saturated fatty acids (22). In white meat, half of monounsaturated fats and one third of saturated fats are important sources of essential fatty acids, especially ω -3 (23). Consuming meat or yogurt alone as a protein source in blenderized tube feeding design can increase the viscosity and thicken the blenderized tube feeding, which can result in the blockage of the feeding tube (24). In the study of Bahramian et al. who designed several blenderized tube feeding, the protein source was chicken meat, turkey, and milk powder, revealing that the protein range was between 16% and 22% of the total calories (18). In another similar study, the protein percentage of total calories was reported 17.7% (19).

According to the guidelines, fat should be 30-40% for ages of 1-3 years and 25-35% for ages of four years and older. In the current study, the fat percentage in the total calories in the blenderized tube feeding diet was between 31% and 39%, and in the commercial enteral formula, was between 30% and 35%, which was under the guidelines base. Including fat in blenderized tube feeding can provide calories and essential fatty acids. Usually, 25-40% of the total calories in a diet are related to fat content, depending on age. In the design of blenderized tube feeding in the present study (Table 4), 31-34% of total calories were allocated to fat content. In Bahramian et al.'s study, they designed blenderized tube feeding, while the percentage of fat in the total calories was defined 28-34% by employing oils such as canola, olive, and MCT as a fat source, and these findings are similar to our study (18). In another identical study, 30.8% of the total calories were related to fat by use of soybean and canola oils as fat sources (19). Because poultry breasts have less fat than thighs, more oil has been added to blenderized tube feeding. In the design of blenderized tube feeding, we observed when chicken thigh juice was consumed, the blenderized tube feeding formula became very viscous and blocked the feeding tube due to presence of chicken thigh fat.

When chicken and turkey breast juices were administered in blenderized tube feeding, while this was not observed, the formula was quickly drained. In the study of Bahramian et al., they also mentioned that chicken thigh juices increased the viscosity of blenderized tube feeding (18). To provide essential fatty acids, oils such as safflower, sunflower, soybean, and corn should not be used in blenderized tube feeding. These oils are the primary source of ω -6, and their excessive consumption can cause pro-inflammatory and suppressive effects on the immune system (25). So anti-inflammatory oils such as canola, olive, and MCT oils that were employed in the present study could provide calories and essential fatty acids. Also, canola and olive oils have less saturated fat, while the Mediterranean diet has recommended these oils too. The reason to use MCT oil in the design of blenderized tube feeding has been their good water permeability, fast hydrolysis, better absorption into intestinal mucosal cells, and transfer of medium-chain fatty acids to portal circulation when compared to long-chain triglycerides (26).

According to the guidelines, 45-65% of calories should be allocated to carbohydrates. In the current study, the percentage of carbohydrates in the total calories in the blenderized tube feeding diet was between 51% and 56%, and in the commercial enteral formula, between 49% and 55%, which was under the guidelines. The designed blenderized tube feeding carbohydrate was between 45% and 51%, which was in line with the guideline. Sources such as fruits, vegetables, cereals, and grains were shown to provide fiber and carbohydrates for blenderized tube feeding. Some studies have demonstrated that grains and cereals used in blenderized tube feeding can reduce the risk of airway inflammation when compared to commercial diets (27, 28). Apples and pears were used in this study because they are rich in carbohydrates, fiber vitamins and also because they are available in all seasons. Also, vegetables such as carrots and broccoli were used in the design of BTFs due to their anti-inflammatory properties. Bahramian et al. reported that corn, potato, and rice flours could make blenderized tube feeding viscous and increase the feeding tube blockage (18); therefore, we did not use these compounds in our study. Rice flour has been used as a carbohydrate source in the design of blenderized tube feeding, and as it does not contain gluten and some patients have celiac disease, it can be an excellent alternative source for celiac patients. To cover a wide range of nutrients, you can choose fruits and vegetables from two groups of vitamins A and C. Squash, carrots, sweet potato, peaches, and apricots were reported as good sources of vitamin A and bioactive compounds, carbohydrates, and calories. Among the sources that can be used to supply vitamin C, applesauce, pears, green peas, green beans, and spinach can be mentioned (29). Researchers have shown that fruits and vegetables can protect against allergic diseases such as asthma by reducing Th2 immune responses, airway inflammation, and oxidative stress (30). Another source of carbohydrates used in commercial enteral formulas as the main source of carbohydrates has been maltodextrin, and some hospitals have used it in blenderized tube feeding. But we did not utilize it in our study, because maltodextrin disrupts cellular antibacterial responses, suppresses intestinal antimicrobial defense mechanisms, and provides harmful bacteria such as salmonella to grow (31, 32). Another source used for carbohydrates in this study was honey, which has positive functions such as wound healing, anti-cancer, anti-tumor, antioxidant, antimicrobial and anti-inflammatory properties (33, 34). Of course, it should be noted that if the target group of blenderized tube feeding is children under one year of age, honey should not be used; because the intestinal microbial flora of children under one year of age has not been developed and they cannot eliminate *Clostridium botulinum* (35). In the present study, sugar was used as a source of carbohydrate, and according to the instructions, it should be less than 10% of the total calories (36). The present study used a maximum of 10 g (in 500 mL) that constituted 8% of the total calories. According to the guidelines, 45-60% of the total calories are related to carbohydrates, and in the present study, carbohydrate content of blenderized tube feeding was under this guideline. In the study by Bahramian et al. who designed blenderized tube feeding, they showed that 48-51% of the total calories of blenderized tube feeding were related to carbohydrates (18), similar to our study. Bento et al.'s study revealed that designed blenderized tube feeding was consisted of 35.5% total calories provided from carbohydrates, and they could not support the daily needed carbohydrates (19), contrary to our research.

Conclusion

The blenderized tube feeding diets in two hospitals

and the commercial enteral formula of one hospital did not provide an energy level of 1 kcal/mL. In addition, in blenderized tube feeding made with fresh and natural foods, the energy and macronutrients of all formulas were within the recommended range for patients. A planned blenderized tube feeding diet can be an excellent choice for patients using enteral nutrition, especially when made from fresh, natural foods like the recipes in our study.

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Authors' Contribution

M. Rezaie designed research; S. Toranj conducted research; B. Bahramian analyzed data; and S. Toranj and B. Bahramian wrote the paper. M. Rezaie had primary responsibility for final content. All authors read and approved the final manuscript.

Conflict of Interest

None declared

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