The Relationship Between Dietary Intakes of Amino Acids and Bone Mineral Density Among Individuals with Spinal Cord Injury

Hadis Sabour[1]*, Maryam Nazari [2], Sahar Latifi[1], Zahra Soltani[1], Hania Shakeri[3], Bagher Larijani[4], Seyed-Mohammad Ghodsi[1] and Seyed-Hassan Emami Razavi[1]

[1]Brain and Spinal Cord Injury Research Center, Tehran University of Medical Sciences, Tehran, Iran
[2]Nutrition and Metabolic Diseases Research Center, Ahvaz Jundishapour University of Medical Sciences, Ahwaz, Iran
[3]Department of Pathology, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[4]Endocrinology and Metabolism Research Institute, Tehran University of Medical Sciences, Tehran, Iran

ABSTRACT

Objectives: The effect of dietary protein intake on bone mineral density (BMD) has not been explained in patients with spinal cord injury (SCI). In this study, we looked at the relationship between BMD and higher protein intake in patients with SCI while controlling for possible confounders. *Methods*: Patients with SCI, who were referred to the Brain and Spinal Cord Injury Research Center between November 2010 and April 2012, were included in the study. In total, the dietary intakes of 103 patients were assessed by 24–hour dietary recall interviews. We used dual-energy X-ray absorptiometry to measure BMD in the femoral neck, trochanter, intertrochanteric zone, hip, and lumbar vertebras. *Results:* Eighty-six men and 17 women participated in this study. Protein intake was negatively associated with the BMD of lumbar vertebrae ($p = 0.001$, r = −0.37 for T-score and $p = 0.030$, r = −0.24 for Z-score). The BMD of lumbar vertebrae were negatively associated with intake of tryptophan, isoleucine, lysine, cysteine, and tyrosine ($p = 0.007$, 0.005, 0.009, 0.008, and 0.008 for T-score, respectively). Higher intakes of threonine, leucine, methionine, phenylalanine, valine, and histidine were related to a lower BMD of lumbar vertebrae ($p = 0.006$, 0.010, 0.009, 0.010, 0.009, and 0.008 respectively for T-scores). *Conclusions:* We found that high protein intake led to a lower BMD of lumbar vertebrae in patients with SCI after controlling for confounders including demographic and injury-related characteristics and calcium intake. No relationship between higher amino acids intake and BMD of the femur and hip was detected. Intake of alanine, arginine, and aspartic acid were not related to BMD.

lthough proteins are known to be a major constituent of bone,[1] there are controversial reports about the association between protein intake and bone mineral density (BMD). Previously, Pernow et al,[2] showed a possible correlation between some amino acids (including tryptophan) and osteoblast function. Sellmeyer et al,[3] reported the stimulation of osteoclastic bone resorption after protein digestion due to the release of cysteine and methionine. Similarly, the increased urinary calcium observed in patients with high-protein diets have been proposed to be associated with lower BMD.[4,5] However, the negative correlation between high-protein intake and BMD has only been confirmed among individuals with insufficient dietary intake of calcium.[6] Since evidence exists to support the correlation between amino acids and osteoblastic

and osteoclastic functions,[2,3] we hypothesized that the background altered activity of osteoblasts and osteoclasts observed among individuals with immobility[7,8] may affect the association between amino acids intake and BMD.

Spinal cord injury (SCI) is one the most important etiologies of immobility and is associated with bone loss, which starts immediately after occurrence of the injury.[9–11] Coincidental increased activities of osteoblastic and osteoclastic functions were shown after SCI.[8] Previously it has been shown that reduced vegetable protein intake was associated with lower BMD in young women.[12] However, this correlation has not yet been described in other populations including people with immobility. To date, no study has investigated the association between dietary protein intake and BMD among individuals with SCI. In our previous study we identified the most

common dietary pattern among Iranian people with SCI,[13] and here we have tried to determine whether there is any relationship between BMD and diet in patients with SCI.

METHODS

This cross-sectional investigation was designed to determine the correlation between dietary intake of protein and BMD among individuals with SCI. Participants were patients with SCI, who were referred to the Brain and Spinal Cord Injury Research Center between November 2010 and April 2012. The ethics committee of Tehran University of Medical Sciences approved the study.

Individuals with SCI were invited to participate in the investigation based on the following inclusion criteria; traumatic SCI and post injury duration longer than one year. We excluded patients with an injury less than one year due to depressive, mood-induced dietary changes that may occur mostly during this time.[14] Since depressive mood may influence nutritional intake and subsequently affect body composition, we evaluated only those who were medically stable after SCI. Exclusion criteria included pregnant or lactating women, amputees, and patients with non-traumatic SCI etiology. Individuals with a history of smoking, diabetes, cancer, endocrinology disease, acute infection, use of special medications such as glucocorticoid, hormones, thyroid hormones, anticonvulsive drugs, heparin, aluminum containing antacids, lithium, blood glucose reducing agents, atorvastatin, gemfibrozil (serum lipid-reducing medications), omega 3 fatty acids, or other nutrients supplements were also excluded.

Dietary intake was assessed by recording consumed foods in 24-hour dietary recall interviews with participants. The data was entered into the Nutritionist IV 3.5.3. (N-Squared Computing, Oregon, US) software program modified for Iranian foods.[15] This software enables the user to analyze single foods, recipes, and meals for nutrient values. Crawford et al,[16] reported that a three-day dietary record is an appropriate and reliable choice for dietary measurements and the agreement between observed and reported intakes is admissible.

Dual-energy X-ray absorptiometry was used to measure BMD. Calibration of bone densitometer Lunar DPXMD device (Lunar Corporation, Wisconsin, US) was performed weekly using appropriate phantoms. The precision error for BMD measurements was 2–3 in the femoral, and 1–1.5 in the lumbar regions. All scans were performed according to the manufacturer's guidelines. The T- and Z-scores of femur neck, trochanters, intertrochanteric zone, and lumbar vertebrae (L1–L4) were investigated. In patients with a spinal implant, the involved lumbar vertebras were excluded, and the mean bone density of noninvolved vertebrae was entered into the analysis. Assessment of femur BMD was conducted using the mentioned three points (neck, trochanters, and intertrochanteric zone), which seemed to be adequate as an indicator of long bones. We also measured total hip BMD. According to the World Health Organization/ Osteoporosis Foundation[17] diagnostic categories, we defined osteoporosis as BMD T-score of –2.5 or less standard deviation (SD), and osteopenia as BMD T-score between –2.5 SD and –1 SD. A BMD T-score above –1 SD the young adult mean was considered normal.

The age, gender, and time since injury of participants were indexed in pre-prepared forms. Body weight was measured using a digital wheelchair scale, and height was obtained by measuring the supine length. Body mass index (BMI) was calculated as body weight (kg) divided by height squared (m).

The level of spinal injury was determined by magnetic resonance imaging (MRI) and a neurologist's confirmation. Completeness was classified as either complete (no preserved sensory or motor function) or incomplete (variable motor function preserved below the neurological level of injury).[18,19] Patients were classified according to the American Spinal Cord Injury Association Scale (ASIA).[20] ASIA-A describes complete injury with no preserved motor or sensory function below the neurological level, and ASIA-B describes incomplete injury with only sensory function preserved. ASIA-C illustrates preserved motor function in which more than half of key muscles below the neurological level have a muscle grade of three or less, and ASIA-D indicates preserved motor function in which at least half of key muscles below the neurological level have a muscle grade of three or more. Only ASIA-A represents complete spinal cord injury.

All statistical analysis was performed using SPSS Statistics (SPSS Inc., Chicago, US) version 21. Categorical variables were described as numbers and percentages, and mean ± SD was used to

describe continuous variables. Comparison of means between groups was done by *t*-test and one-way analysis of variance (ANOVA). The correlation between continuous variables was assessed by partial correlation test controlling for injury related confounders (time since injury, injury level, completeness, and ASIA score) and demographic characteristics (age, gender, and BMI). Previously, the adverse effect of high protein intake on bone was observed only among individuals with insufficient calcium intake.[6] Therefore, we performed the analysis with a partial correlation test controlling for calcium intake. A *p*-value <0.050 was considered statistically significant.

RESULTS

A total of 103 patients with SCI participated in this study. The majority of participants were men (83.5% men and 16.5% women). The patients mean age was 39.5±12.7 years, and their mean BMI was 23.9±4.4 kg/m², respectively. The most common injury level was at thoracic sections (61.2%). Most individuals had complete injury (73.8%) and were paraplegic (85.4%). As expected, ASIA-A, which represented complete injury, was the most common detected score (73.8%). Table 1 illustrates the baseline characteristics of participants along with measured dietary intakes of protein and amino acids. Mean daily protein intake was 78.1±25.3 g. Leucine, lysine, valine, and isoleucine were the major constituents of total protein intake (5126.5±2055.8 mg, 4707.4±1898.3 mg, 3667.8±1511.2 mg, and 3191.8±1270.1 mg, respectively). Arginine, alanine, and aspartic acid had the lowest daily intake (158.6±47.35 mg, 141.1±98.3 mg and 247.6±186.6 mg, respectively). The mean values of BMD in femur and hip showed osteopenia (−2.5<T-score<−1). However, the measured BMD was higher in spinal vertebrae [Table 1]. Mean calcium intake was 742.7±406.2 mg daily. Previously, Kerstetter et al,[6] showed that high protein intake may have adverse effects on bone only among individuals with insufficient calcium intake. Therefore, in the next step of the analysis, we used adjusted partial correlation with controlling for calcium intake.

BMD was higher among females in all measured points except the femoral neck. This finding indicates that gender can be a confounder that affects BMD among individuals with SCI. Further analysis

Table 1: Baseline characteristics of participants and dietary intakes of protein and amino acids among individuals with spinal cord injury.

Category	Frequency n (%)	Mean ± SD
Gender		
Male	86 (83.5)	-
Female	17 (16.5)	-
Injury level		
Cervical	23 (22.3)	-
Thoracic	63 (61.2)	-
Lumbar	17 (16.5)	-
Completeness of injury		
Complete	76 (73.8)	-
Incomplete	27 (26.2)	-
Plegia		
Paraplegic	88 (85.4)	-
Tetraplegic	15 (14.6)	-
ASIA score		
A	76 (73.8)	-
B	13 (12.6)	-
C	4 (3.9)	-
D	10 (9.7)	-
Age	-	39.5±12.7
Weight (kg)	-	68.7±14.5
BMI (kg/m²)	-	23.9±4.4
Total energy intake (kcal)	-	1756.1±542.8
Carbohydrate intake (g)	-	224.9±72.2
Protein intake (g)	-	78.1±25.3
Tryptophan (mg)	-	722.5±294.9
Isoleucine (mg)	-	3191.8±1270.1
Lysine (mg)	-	4707.4±1898.3
Cysteine (mg)	-	837.7±332.1
Tyrosine (mg)	-	2284.4±916.5
Arginine (mg)	-	158.6±47.35
Alanine (mg)	-	141.1±98.3
Glutamic acid (mg)	-	764.5±549.9
Tyrosine (mg)	-	2656.9±1059.4
Leucine (mg)	-	5126.5±2055.8
Methionine (mg)	-	1508.6±603.6
Phenylalanine (mg)	-	2875.8±1160.2
Valine (mg)	-	3667.8±1511.2
Histidine (mg)	-	1841.5±744.4
Aspartic acid (mg)	-	247.6±186.6
Calcium intake (mg)		742.7±406.2
Femoral neck BMD		
T-score	-	−2.1±1.1
Z-score	-	−1.7±1.0
Femoral trochanter BMD		
T-score	-	−1.9±0.9
Z-score	-	−1.8±0.9
Femoral intertrochanteric zone BMD		
T-score	-	−2.2±1.0
Z-score	-	−2.1±1.0
Hip BMD		
T-score	-	−2.2±0.9
Z-score	-	−2.1±1.0
Lumbar vertebrae BMD		
T-score	-	−0.4±1.6
Z-score	-	−0.3±1.6

ASIA: American Spinal cord Injury Association; BMD: bone mineral density; BMI: body mass index.

Table 2: The relationships between demographic and injury-related variables and bone mineral density among participants with spinal cord injury.

Category	Femoral neck		Femoral trochanter		Femoral intertrochanteric zone		Hip		Lumbar vertebrae	
	T-score	Z-score	T-score	Z-score	T-score	Z-score	T-score	Z-score	T-score	Z-score
Gender[a]	0.31	0.74	<0.0001**	<0.0001**	0.04*	0.04*	0.01*	0.02*	<0.0001**	0.005**
Injury level	0.71	0.98	0.27	0.43	0.76	0.74	0.68	0.88	0.06	0.12
Completeness of injury[b]	0.18	0.29	0.82	0.93	0.05	0.08	0.16	0.34	0.03*	0.01*
Plegia[c] (tetraplegic vs. paraplegic	0.60	0.76	0.52	0.59	0.89	0.98	0.68	0.81	0.20*	0.03*
ASIA score[d]	0.38	0.36	0.45	0.35	0.01*	0.04*	0.13	0.23	0.18	0.10
Age	0.07	0.08	0.47	0.59	0.07	0.89	0.08	0.45	0.22	0.09
BMI[e]	<0.0001 (r = 0.36)**	<0.0001 (r = 0.42)**	0.01 (r = 0.26)*	0.005 (r = 0.28)**	<0.0001 (r = 0.40)**	<0.0001 (r = 0.46)**	<0.0001 (r = 0.44)**	<0.0001 (r = 0.49)**	0.01 (r = 0.26)*	0.01 (r = 0.25)*

ASIA: American Spinal cord Injury Association; BMD: bone mineral density; BMI: body mass index.
*Significance at the level of $p<0.050$
**Significance at the level of $p<0.010$
[a]BMD was higher among females in all measured points except the femoral neck.
[b]Lumbar vertebras had significantly higher BMD in patients with incomplete injury.
[c]Participants with paraplegia had higher BMD in lumbar spinal sections compared with individuals with tetraplegia.
[d]BMD of femoral intertrochanteric zone was significantly higher among people with ASIA-D.
[e]BMI was positively correlated with BMD in all measured points.

was performed controlling for participants' gender. Patients with incomplete injury had a significantly higher BMD in lumbar vertebras only ($p = 0.030$ and $p = 0.010$ for T- and Z-scores, respectively). Similarly, participants with paraplegia had higher vertebral BMD compared to people with tetraplegia. However, the level of injury was not related with the BMD among individuals with SCI. Furthermore, higher BMD of the femoral intertrochanteric zone was detected among patients with ASIA-D [Table 2]. All these findings indicate that injury-related variables can affect BMD and, therefore, must be considered as confounders when determining the influence of other variables. The only factor that was positively correlated with BMD of the femur, hip, and lumbar vertebras was BMI [Table 2]. Age was not associated with BMD in any measured points.

The correlation between protein and amino acid intakes and BMD was determined after controlling for demographic characteristics (including gender and BMI) and injury-related variables (including completeness of injury, plegia type, and ASIA-score).

Total energy intake was not correlated with BMD. However, protein intake was negatively associated with BMD of lumbar vertebrae ($p = 0.001$, r = −0.37 for T-score and $p = 0.030$, r = −0.24 for Z-score). T-scores of lumbar vertebrae were negatively associated with intake of tryptophan, isoleucine, lysine, cysteine, and tyrosine ($p = 0.007$, 0.005, 0.009, 0.008 and 0.008, respectively). Similar results were detected when measuring the Z-scores of lumbar vertebrae ($p = 0.010$, 0.040, 0.040, 0.030, and 0.010, respectively). Higher intakes of threonine, leucine, methionine, phenylalanine, valine, and histidine were also related with lower BMD values in lumbar vertebrae ($p = 0.006$, 0.010, 0.009, 0.010, 0.009, and 0.008, respectively, for T-scores and $p = 0.040$, 0.040, 0.030, 0.030, 0.020, and 0.040 for Z-scores, respectively).

Altogether, intake of most amino acids (except alanine, arginine, glutamic acid, and aspartic acid) were associated with lower BMD in lumbar vertebrae [Table 3]. Protein and amino acid intake were not related with BMD of femur and hip.

DISCUSSION

Our study results suggest a relationship between higher total protein intake and amino acids, except alanine, arginine, glutamic acid, and aspartic acid, and lower BMD in the lumbar vertebrae of individuals with SCI. Previously, Sellmeyer et al,[3] reported that diets rich in protein from animal sources (such as cysteine and methionine) increase

Table 3: The associations between dietary intakes of protein and amino acids and bone minerals density after controlling for confounders including demographic and injury-related characteristics.

Dietary intake	Femoral neck		Femoral trochanter		Femoral intertrochanteric zone		Hip		Lumbar vertebrae	
	T-score	Z-score	T-score	Z-score	T-score	Z-score	T-score	Z-score	T-score	Z-score
Energy (kcal)	0.86	0.81	0.13	0.12	0.21	0.18	0.17	0.13	0.17	0.06
Protein	0.78	0.80	0.12	0.13	0.28	0.29	0.20	0.19	0.001 ($r = 0.37$)	0.03 ($r = -0.24$)
Tryptophan	0.73	0.70	0.14	0.13	0.27	0.27	0.20	0.18	0.007 ($r = -0.31$)	0.01 ($r = -0.22$)
Isoleucine	0.61	0.61	0.10	0.11	0.25	0.26	0.17	0.17	0.005 ($r = -0.32$)	0.04 ($r = -0.20$)
Lysine	0.84	0.61	0.26	0.26	0.61	0.65	0.45	0.46	0.009 ($r = -0.31$)	0.04 ($r = -0.13$)
Cysteine	0.45	0.41	0.03 ($r = -0.25$)	0.02 ($r = -0.26$)	0.12	0.10	0.07	0.06	0.008 ($r = -0.31$)	0.03 (-0.11)
Tyrosine	0.71	0.74	0.12	0.13	0.27	0.30	0.22	0.21	0.008 ($r = -30$)	0.01 ($r = -0.19$)
Arginine	0.98	0.95	0.76	0.77	0.68	0.73	0.81	0.82	0.54	0.76
Alanine	0.94	0.89	0.79	0.80	0.65	0.70	0.76	0.79	0.50	0.78
Glutamic acid	0.16	0.17	0.06	0.05	0.10	0.07	0.06	0.05	0.10	0.71
Threonine	0.70	0.68	0.12	0.13	0.29	0.32	0.21	0.20	0.006 ($r = -0.32$)	0.04 ($r = -0.15$)
Leucine	0.75	0.60	0.15	0.13	0.39	0.38	0.25	0.23	0.01 ($r = -0.29$)	0.04 ($r = -0.20$)
Methionine	0.83	0.82	0.13	0.13	0.49	0.50	0.31	0.29	0.009 ($r = -0.30$)	0.03 ($r = -0.16$)
Phenylalanine	0.62	0.60	0.08	0.08	0.26	0.25	0.16	0.14	0.01 ($r = -0.28$)	0.03 ($r = -0.18$)
Valine	0.69	0.70	0.12	0.12	0.35	0.37	0.23	0.22	0.009 ($r = -0.30$)	0.02 ($r = -0.28$)
Histidine	0.95	0.91	0.25	0.23	0.71	0.70	0.49	0.46	0.008 ($r = -0.30$)	0.04 ($r = -0.14$)
Aspartic Acid	0.85	0.20	0.86	0.83	0.30	0.35	0.55	0.06	0.90	0.95

the risk of osteoporosis since protein from animal sources contains acidifying amino acids. Here, we observed similar outcomes among people with SCI.

Other investigations have shown higher BMD with a higher intake of animal protein in adults older than 55 years.[21] This suggests that age may affect the association between dietary protein intake and BMD. To understand the role of age, further investigation with assessment of osteoclasts and osteoblasts in different age groups are required. We performed the analysis controlling for age and found a negative association between protein intake and lumbar vertebrae BMD. However, protein intake did not influence long bone BMD (e.g., femur).

A large cohort investigation of postmenopausal women, the European Prospective Investigation into Cancer and Nutrition, Potsdam (EPIC) study, found that increased animal protein intake was negatively correlated with BMD.[22] People with SCI and postmenopausal women are two known populations at risk of osteopenia and subsequent fractures. We concluded that animal protein intake is adversely correlated with BMD among populations with background increased risk of osteopenia and osteoporosis. Moreover, Beasley et al,[12] showed no adverse effect of higher protein intake on bone in premenopausal women, which is in line with our findings in long bones.

SCI causes significant reduced BMD in long bones due to mechanical unloading whereas the reduction of vertebral BMD is significantly less severe,[23] which shows dominant osteoclastic activities and bone resorption in long bones after SCI. It seems that the adverse effect of high animal protein intake on BMD can be camouflaged when high osteoclastic activity exists in these bone sites.

Intake of alanine, arginine, and aspartic acid were not correlated with BMD of femur, hip, and lumbar vertebrae among people with SCI. These amino acids are considered non-essential, which means they can be manufactured by the human body and do not need to be obtained through diet. Previously, Dargent-Molina et al,[24] showed no association between protein intake and BMD among postmenopausal women, which is in line with our findings on long bones and our results on intakes of alanine, arginine, aspartic acid and glutamic acid. However, the authors also reported a trend toward increased fracture risk with an increased intake of animal protein. Here, we did not follow patients for the occurrence of fractures. Further cohort studies with long-term follow-up are required to assess the relationship between fracture risk and protein intake among people with SCI.

The role of calcium on the association between BMD and protein intake has been described. Some investigations suggest that increased urinary calcium excretion due to higher protein intake is compensated by increased intestinal absorption of calcium.[5] Moreover, Kerstetter et al,[6] observed an adverse effect of high protein intake only among individuals with low calcium intake. Until now, no study has investigated these correlations with adjustment for calcium intake. Here, we detected that higher protein intake leads to lower vertebral BMD after controlling for calcium intake among individuals with SCI.

This study demonstrated the adverse effect of higher total protein intake and amino acids (except for alanine, arginine, glutamic acid, and aspartic acid) on BMD of lumbar vertebrae among individuals with SCI. We did not find any relationship between protein intake and BMD of femur and hip. However, clinical trials with intervention of special diets are required to confirm the findings of this study.

CONCLUSION

Higher protein intake led to lower BMD of the lumbar vertebrae among individuals with SCI after controlling for demographic, injury-related, and calcium intake confounders. There was no relationship between higher amino acids intake and BMD of femur and hip. Alanine, arginine, aspartic acid, and glutamic acid were not related to BMD.

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