Bone mineral density in short bowel syndrome: correlation with BMI and serum vitamins C, E and K

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ABSTRACT

Objective: Bone loss has been established as a major extra-intestinal complication of short bowel syndrome (SBS). The purpose of this study was to correlate bone mineral density (BMD) with body mass index (BMI), serum vitamin and mineral levels in patients with SBS. **Material and methods:** The study was conducted on 13 patients (8 male and 5 female, 54.7 ± 11.4 years) with SBS (residual small bowel length of 10 to 100 cm). We determined the food ingestion, anthropometry, serum levels of vitamins C, A, D, E and K, as well as serum and urinary levels of phosphorus and calcium. BMD was measured by dual-energy x-ray absorptiometry (DXA). **Results:** Osteopenia and osteoporosis was diagnosed in all but one SBS patient. Serum levels of vitamin D were low in all volunteers. Sixty-one percent of patients had vitamin E deficiency; hypovitaminosis A and C occurred in one subject. BMI and C, E and K vitamin serum levels correlated with T-score of BMD. **Conclusions:** Osteopenia and osteoporosis were common in SBS patients. There was a correlation between BMD and the serum levels of vitamins C, E and K, an indicative that such vitamins may influence bone health. Arch Endocrinol Metab. 2015;59(3):252-8

Keywords
Short bowel syndrome; bone density; vitamins; minerals

INTRODUCTION

Short bowel syndrome (SBS) may result from a state of malabsorption secondary to extensive intestinal resection, causing disturbances in the homeostasis of fluids and electrolytes, chronic diarrhea and weight loss (1). The nutritional therapy aims to replace nutrients and provide intestinal rehabilitation and their return to social activities, with improved quality of life (2). Although there are several degrees of anatomical and functional adaptation in the remaining bowel, some patients are unable to keep a satisfactory nutritional status by oral route, thus becoming dependent on parenteral nutrition (PN) (1). Recently, we showed that SBS patients present deficiencies in vitamins E, D (3,4) and K(3), regardless of venous infusion. Within this context, efforts are primarily devoted to intestinal adaptation to oral route nutrition (5). Patients with severe malabsorption need home PN (5), but it is not a therapeutic option provided by the Brazilian public health system. In our service, these patients receive intermittent PN in periodic hospital admissions (3,4).

In the last decades, specialized nutrition therapy techniques resulted in a greater lifetime after extensive intestinal resection, enabling the onset of complications that were usually undiagnosed. In this context, bone health should be remarked, once bone pain and fractures decrease the quality of life in SBS patients. It is widely known that chronic and severe vitamin D deficiency impairs calcium and phosphorus absorption leading to disturbances in bone mineralization. Slight vitamin D insufficiency has been associated with inability to reach a proper peak bone mass and/or to maintain skeletal homeostasis (6). The impact of other nutritional deficiencies on bone health is not well established. Vitamins K and C, phosphorus, magnesium and protein are nutrients essential to bone health (7), although the role of such nutrients has been scarcely documented in SBS patients. Therefore, the present study was designed to evaluate the correlations between bone mineral density and markers of nutritional status in patients with SBS.

MATERIAL AND METHODS

This transversal study was conducted in a public university hospital after approval by the institutional Ethics Committee (Protocol #29/2007) and all subjects signed an informed consent. Thirteen adults with SBS were included in this study, with clinical characterization showed in table 1. Hemodynamically unstable subjects and individuals with intestinal inflammatory diseases, neoplasia, hepatic failure, diagnosis of active infectious diseases and use of any medications that may impact bone health were excluded from the study.

Among the 13 SBS patients, seven were fed orally. They had received PN during immediate post-operative, but this approach was interrupted after 2 to 6 months due to satisfactory evolution. Six patients were PN dependent at the time of the study and they were admitted to the hospital to receive intermittent PN, according to the schedule established by the Medical Nutrition Division. During each admission cycle, the patients received PN for 5-8 days, with an interval of 10-40 days between hospitalizations, depending on diarrhea, dehydration, and nutritional status. PN was infused through a totally implantable central venous catheter. The PN mixture contained dextrose (Hiplex®, Fresenius Kabi, Campinas, Sao Paulo, Brazil), lipid emulsion (Lipofundin® MCT/LCT, B. Braun, Melsungen, Germany), crystalline amino acid solutions (Soramin®, Darrow, Rio de Janeiro, Brazil), electrolytes, vitamins (Cerne®, Baxter, Florida, USA), and trace elements (Ad-element®, Darrow, Rio de Janeiro, Brazil), according to the current recommendations (8). All patients were followed by a specialized outpatient service, where they were advised to go on an oral diet at home and to take tablets (Centrum®, Wyeth, Richmond, VA, USA) containing vitamins and minerals on a daily basis. The composition of the vitamin, mineral and electrolytes prescribed for SBS patients is presented in table 2.

Table 2. Supply of micronutrients in oral and venous route

	Daily oral supply	Venous supply in intermitent PN
Ascorbic acid (mg)	45	100
Folic acid (µg)	240	400
Biotin (µg)	30	60
Cyanocobalamin (µg)	2.4	5
Pantothenic acid (mg)	5	15
Riboflavin (mg)	1.3	3.6
Nicotinamide (mg)	16	40
Pyridoxine (mg)	1.3	4
Thiamin (mg)	1.2	3
Retinol (UI)	1467	3300
Tocopherol (UI)	10	10
Cholecalciferol (UI)	200	200
Vitamin K_1 (µg)	65	-
Zinc (mg)	7.0	2.5
Copper (mg)	0.45	0.8
Manganese (mg)	1.2	0.4
Chromium (µg)	18	10
Magnesium (mg)	100	9-27*
Phosphorus (mg)	125	310-930*
Calcium (mg)	250	198-297*

* According to the individual requirement.

Table 1. Clinical characteristics of SBS patients

	Age (years)	Gender	Diagnosis	Residual small bowel (cm)	Time on PN (months)	Exclusive oral feeding	Ileocecal valve	Time between hospitalizations (days)	Length of hospitalization (days)
1	34	F	Mesenteric ischemia	15	16	No	No	10	8
2	49	F	Mesenteric ischemia	20	84	No	No	40	5
3	44	M	Mesenteric ischemia	180	31	No	Yes	20	5
4	63	M	Mesenteric ischemia	10	88	No	No	40	8
5	62	F	Mesenteric ischemia	100	13	No	No	10	8
6	47	M	Mesenteric ischemia	15	21	No	No	20	5
7	77	M	Mesenteric ischemia	210		Yes	Yes	–	–
8	65	F	Acute abdomen	130		Yes	Yes	–	–
9	60	F	Mesenteric ischemia	110		Yes	Yes	–	–
10	62	F	Mesenteric ischemia	40		Yes	Yes	–	–
11	45	F	Mesenteric ischemia	110		Yes	Yes	–	–
12	52	M	Mesenteric ischemia	80		Yes	Yes	–	–
13	51	F	Mesenteric ischemia	100		Yes	Yes	–	–

Anthropometric measurements were done using standard techniques. Body impedance analysis was performed using a bioelectrical impedance analyzer (BIA 101-A Detroit, MI, USA) after overnight fasting. Analysis of the composition of the habitual diet was based on the Semi-Quantitative Food Frequency Questionnaire, which assesses the food intake for the previous 6 months. The dietetic data was processed by specific software (NutWin 1.5® Professional Software; Federal University of São Paulo, São Paulo, Brazil).

Serum phosphorus, as well as urinary calcium and phosphorus were analyzed by atomic absorption flame spectrophotometry. Calcium serum levels were determined by colorimetry. Ascorbic acid was measured by colorimetric reaction with 2,4-dinitrophenylhydrazine and spectrophotometric detection. Determination of vitamin D_3, retinol, and α-tocopherol was accomplished by ultraviolet high performance liquid chromatography (HPLC-UV), whereas vitamin K was measured on an HPLC device equipped with an electrochemical detector.

BMD was measured by dual-energy x-ray absorptiometry (DXA) using a Hologic QDR 4500A scanner® (Hologic Inc, Waltham, MA) in the following sites: total femur, femoral neck and lumbar spine. The results were expressed as T-Score and analyzed in accordance to the World Health Organization (WHO).

Data are reported as mean ± SDs, and by the median and range. For correlation between variables, data were analyzed by Spearman's test. Statistical significance was set at $p < 0.05$. Data analyses were performed with the Statistica software (version 8.0, StatSoft Inc, Tulsa, Oklahoma).

RESULTS

Four patients presented BMI below 18.5 kg/m² and reduced fat and lean body mass (Table 3). The subjects had an adequate intake of nutrients related to bone health, except for calcium (Table 3). However, considering the addition of daily mineral and vitamin supplement tablets, all nutrients exceeded the nutritional recommendations. Table 4 shows serum concentrations of vitamins and serum and urinary concentrations of calcium and phosphorus in SBS patients. All patients presented low vitamin D_3 level. Vitamin E deficiency affected eight patients and hypovitaminosis A and C occurred in one subject. Furthermore, low calcium serum levels were documented in five patients, whereas hypercalciuria was observed in three individuals.

The T-score values were: -1.40 (range: -3.70 to -0.20) in the total femur, -1.90 (range: -4.0 to -0.30) in the femoral neck and -2.20 (range: -4.20 to -0.60) in the lumbar spine. Only one patient presented a nor-

Table 3. Body composition and daily oral nutritional intake in 13 patients with short bowel syndrome

	Mean ± SD	Median (range)	Reference range
Body composition			
Weight (kg)	54 ± 12	51 (39-85)	-
Height (cm)	162 ± 8	160 (147-178)	-
BMI (kg/m²)	21 ± 5	20 (14-33)	18.5-25.0
Lean body mass (%)	70 ± 24	80 (18-95)	M:15-19 / F:23-26
Fat body mass (%)	30 ± 23	21 (11-82)	M:81-85 / F:74-77
Nutritional intake			
Energy (kcal)	2216 ± 865	2095 (1052-3890)	-
Energy (kcal/kg)	42 ± 16	39 (19-87)	-
Protein (g)	90 ± 37	87 (56-118)	M:56/F:46 (AI)
Protein (g/kg)	1.6 ± 0.5	1.6 (0.9-2.5)	0.66 (EAR)
Vitamin A (ER)	1421 ± 1155	2006 (199-3789)	M:625/F:500 (EAR)
Vitamin E (mg)	11 ± 6	12 (2.3-20)	12 (EAR)
Vitamin K (µg)	146 ± 65	215 (3.6-327)	M:120/F:90 (AI)
Vitamin C (mg)	173 ± 140	265 (26-359)	M:75/F:60 (EAR)
Phosphorus (mg)	1288 ± 573	1057 (667-2232)	580 (EAR)
Calcium (mg)	855 ± 368	876 (240-2306)	M:800/F:1000 (EAR)
Magnesium (mg)	327 ± 88	370 (205-457)	M:350/F:265 (EAR)

BMI: body mass index; M: male; F: female; EAR: estimated average requirement; AI: adequate Intake.

mal BMD; osteopenia was observed in 6 patients (46%) and osteoporosis in 6 individuals (46%). There was no correlation between the T-score and variables such as patient age, the length of remaining small bowel and the time elapsed after intestinal resection.

There were positive correlations between T-score and BMI in the three study sites (Figure 1), although we could not establish any correlations between T-score and lean/fat body masses. Moreover, there were no correlations between T-score and serum levels of vitamins D and A in the three study sites. Serum vitamins C (Figure 2) and E (Figure 3) associated with the T-scores of neck and total femur. Vitamin K serum levels correlated with neck femoral T-score (Figure 4). There was no correlation between T-scores and serum and urinary calcium and phosphorus.

Table 4. Serum concentration of vitamins and serum and urinary concentration of calcium and phosphorus in 13 patients with short bowel syndrome

	Mean ± SD	Median (range)	Reference value
Serum			
Vitamin C (mg/dL)	1.08 ± 0.46	1.04 (0.3-2.0)	0.6-2.0
Vitamin A (µmol/L)	2.77 ± 1.26	2.61 (0.71-5.39)	1.04-2.43
Vitamin D (µg/µL)	0.01± 0	0.01 (0.01-0.02)	0.03-0.05
Vitamin E (µmol/L)	21.4 ± 8.2	20.4 (9.8-41.9)	23-27
Vitamin K (ng/mL)	0.37 ± 0.19	0.32 (0.2-0.9)	0.09-2.22
Magnesium (mg/dL)	1.26 ± 0.29	1.29 (0.78-1.62)	1.5-2.3
Phosphorus (mg/dL)	3.62 ± 0.98	3.5 (1.5-5.8)	2.5-4.9
Total calcium (mg/dL)	9.25 ± 1.17	9.2 (7.7-11.7)	8.5-10.1
Ionic calcium (mmol/L)	1.23 ± 0.10	1.20 (1.11-1.51)	1.12-1.28
Urinary			
Calcium (mg/24h)	187.2 ± 160.2	106.2 (20-480)	100-300
Phosphorus (mg/24h)	922.7 ± 337.5	946.7 (400-1600)	700-1500

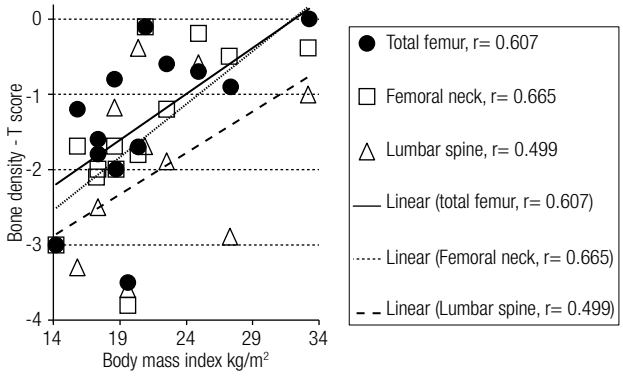

Figure 1. Correlation between BMI and T-scores of total femur, femoral neck and lumbar spine in short bowel syndrome patients.

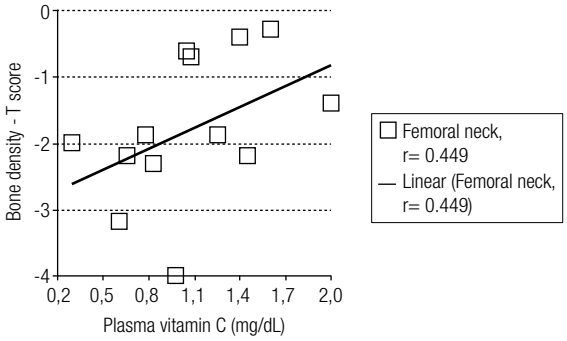

Figure 2. Correlation between plasma vitamin C and T-scores of femoral neck in short bowel syndrome patients.

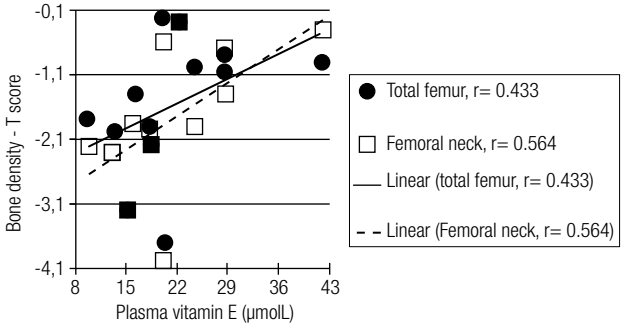

Figure 3. Correlation between plasma vitamin E and T-scores of total femur and femoral neck in short bowel syndrome patients.

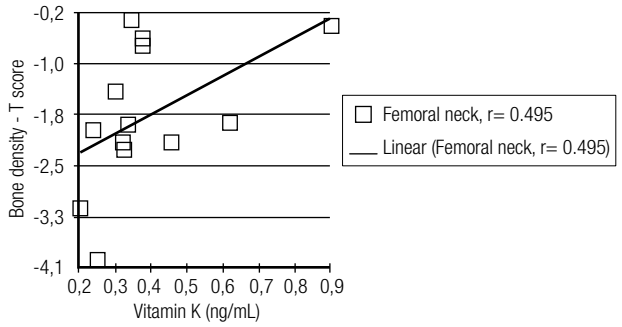

Figure 4. Correlation between serum vitamin K and T-score of femoral neck in short bowel syndrome patients.

DISCUSSION

The frequency of alterations in bone mass observed in the present study was greater than those documented in previous investigations. Osteomalacia occurred in 36% of SBS subjects even with normal calcium and phosphorus serum levels (9) and with daily vitamin D supplementation (10). Bone disease occurred in 67% (11) and 84% (12) of patients who were receiving PN over a long period. Osteoporosis was documented in 46% in the lumbar spine and 12.7% in the femural head; os-

teopenia occurred in 18% in the lumbar spine and 18% in the femoral head among patients receiving PN (13).

Disturbances in nutrient absorption may be partially responsible for the high frequency of osteoporosis/osteopenia. Even after small intestinal resections (jejunoileal bypass) there was a decrease in the serum levels of calcium, phosphorus, 25-OH vitamin D_3, and 1,25-(OH)2 vitamin D_3 (10). Short-term PN was associated with an improvement of bone metabolism, which evolved from a hyperkinetic turnover pattern to a positive balance on bone remodeling, whereas long-term PN was associated with decreased bone formation (12). Except for patients whose intestinal disease started prior to reaching the peak bone mass, long-term PN had no deleterious effect on cortical bone and improved trabecular bone (11). Metabolic acidosis, excessive aminoacid infusion, insufficient vitamin D and magnesium supply are some other factors ascribed to the onset of bone disease (13). Vitamin D is not effective in increasing calcium absorption in patients with intestinal failure, and its excess may be associated with bone disease (14). Hypomagnesemia is common in SBS patients, and may decrease the parathormone secretion and action, resulting in an insufficient production of 1,25-hydroxy-vitamin D, thus contributing to lower calcium bone storage (15).

Although we did not measure the serum levels of PTH, it can be hypothesized that secondary hyperparathyroidism kept circulatory calcium levels within the normal range (16) in our SBS patients. Hypercalciuria (observed in 3 of our SBS patients) cannot be ascribed to excessive supply of calcium, protein (15) or glucose and sodium (17). It is possible that deficiencies of vitamins D and K, observed in our patients may have been responsible for increased bone reabsorption and impaired calcium incorporation into bone, thus resulting in hypercalciuria.

In this study, there was a positive correlation between BMI and T-score obtained from bone densitometry. Meta-analysis by Laet and cols. (18) showed nearly twofold increase in risk ratio for hip fracture in adults with a BMI of 20 kg/m² when compared with others with 25 kg/m². Subjects with low BMI present reduced muscle mass and nutritional deficiencies, which implies in a substantial risk for all fractures, largely independent on age and gender, but dependent on BMD (18). Furthermore, women with lower body weight present reduced peripheral conversion of gonadal hormones, resulting in deficiency of estrogens and its consequent adverse effects on bone (19). Our data are in accordance with those of Raman and cols. (13), who did not find any correlations between T-score and venous supply of aminoacid, calcium, magnesium and fosphate, apart from the duration of PN. In women undergoing PN, multiple regression showed negative correlation between Z-score and age when PN was initiated (20).

No correlation was found between bone density and serum levels of vitamin D_3, a finding which is in contrast with that observed in others studies (10,17). In our study, all patients presented low serum levels of vitamin D, which makes it impossible to perform correlation analysis of T-scores. On the other hand, there was correlation between BMD and serum levels of vitamins C and K. Vitamin C takes part in the process of lysine and proline hydroxylation, necessary to form bone collagen triple helix structure (21). Apart from that, ascorbic acid stimulates alkaline phosphatase, which plays a role in the synthesis of type I collagen matrix, in mineralization and in the expression of several osteoblast proteins (22). It has been documented since the 1980s that patients with vertebral compression fractures present lower vitamin K serum levels (23). In addition to malabsorption, vitamin supplements used by our SBS patients does not contain vitamin K. The association of vitamin K with BMD has been recently take into consideration in patients under home PN (24). Vitamin K is essential for osteocalcin production by osteoblasts (25) and the stimulation of bone collagen surplus (26) by reducing calcium urinary excretion and inhibiting the production of PGE2 and IL-6, compounds which stimulate bone reabsorption (26).

In the present study, we could also observe a positive correlation between BMD and vitamin E serum levels. Tocopherol inhibits epiphyseal cartilage reabsorption and protects the membranes of chondrocytes by reducing free radical generation and lipidic peroxidation (27). In addition, alpha-tocopherol may reduce osteoclast formation and bone loss, by inhibiting the induction of the receptor activator of nuclear factor-κB (RANK) (28), which is an oxidative stress-responsive transcription factor.

The absence of correlation between BMD and serum vitamin A is in accordance with the literature, although researchers have not reached a consensus (29). Melhus and cols. (30) showed that excessive vitamin A supply associates negatively with BMD, even after adjustments in the other risk factors for osteoporosis. Experimental studies showed that vitamin A deficiency

relates to alterations in osteoclast and osteoblast activity (31). On the other hand, excessive vitamin A reduces bone collagen synthesis (32). Inadequate vitamin A supply is associated with compromised bone health, by mechanisms that are still unknown (33).

Limitations of our study include the small sample. However, once SBS does not have a high prevalence, we could consider our sample size as appropriate. Also, we did not perform the measurements of bone health-related hormones. Multiple hormones, including sex steroids, are considered regulators of bone metabolism and have been associated with bone density (34). Considering the scarcity of studies about BMD in SBS patients, this work is relevant for it assesses possible factors associated to metabolic bone disease simultaneously, including vitamin K.

In conclusion, osteopenia and osteoporosis were common in SBS patients and there was a correlation between BMD and the serum levels of vitamins C, E and K, an indicative that such vitamins may influence bone health. Continuous monitoring of T-score and serum vitamin levels and supplementation with vitamins C, K and E in SBS patients are recommended for a timely intervention. Further investigations involving larger samples could assess the effectiveness of oral and venous supply of vitamins C, K and E in reducing the progression of bone disease in SBS.

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REFERENCES

1. Donohoe CL, Reynolds JV. Short bowel syndrome. Surgeon. 2010;8(5):270-9.
2. Van Gossum A, Vahedi K, Abdel-Malik, Staun M, Pertkiewicz M, Shaffer J, et al.; ESPEN-HAN Working Group. Clinical, social and rehabilitation status of long-term home parenteral nutrition patients: results of a European multicentre survey. Clin Nutr. 2001;20(3):205-10.
3. Braga CB, Vannucchi H, Freire CM, Marchini JS, Jordão AA Jr, da Cunha SF. Serum vitamins in adult patients with short bowel syndrome receiving intermittent parenteral nutrition. JPEN J Parenter Enteral Nutr. 2011;35(4):493-8.
4. Ferreira IM, Braga CB, Dewulf Nde L, Marchini JS, da Cunha SF. Vitamin serum variations between cycles of intermittent parenteral nutrition in adult patients with short bowel syndrome. JPEN J Parenter Enteral Nutr. 2013;37(1):75-80.
5. Keller J, Panter H, Layer P. Management of the short bowel syndrome after extensive small bowel resection. Best Pract Res Clin Gastroenterol. 2004;18(5):977-92.
6. Peacock M. Calcium metabolism in health and disease. Clin J Am Soc Nephrol. 2010;5 Suppl 1:S23-30.
7. Prynne CJ, Mishra GD, O'Connell MA, Muniz G, Laskey MA, Yan L, et al. Fruit and vegetable intakes and bone mineral status: a cross sectional study in 5 age and sex cohorts. Am J Clin Nutr. 2006;83(6):1420-8.
8. ASPEN Board of Directors and the Clinical Guidelines Task Force. Guidelines for the use of parenteral and enteral nutrition in adult and pediatric patients. JPEN J Parenter Enteral Nutr. 2002;26(1 Suppl):1SA-138SA.
9. Compston JE, Ayers AB, Horton LW, Tighe JR, Creamer B. Osteomalacia after small-intestinal resection. Lancet. 1978;1(8054):9-12.
10. Mosekilde L, Melsen F, Hessov I, Christensen MS, Lund BJ, Lund BI, et al. Low serum levels of 1.25-dihydroxyvitamin D and histomorphometric evidence of osteomalacia after jejunoileal bypass for obesity. Gut. 1980;21(7):624-31.
11. Cohen-Solal M, Baudoin C, Joly F, Vahedi K, D'Aoust L, De Vernejoul MC, et al. Osteoporosis in patients on long-term home parenteral nutrition: a longitudinal study. J Bone Miner Res. 2003;18(11):1989-94.
12. Pironi L, Labate AM, Pertkiewicz M, Przedlacki J, Tjellesen L, Staun M, et al. Prevalence of bone disease in patients on home parenteral nutrition. Clin Nutr. 2002;21(4):289-96.
13. Raman M, Gramlich L, Whittaker S, Allard JP. Canadian home total parenteral nutrition registry: preliminary data on the patient population. Can J Gastroenterol. 2007;21(10):643-8.
14. Verhage AH, Cheong WK, Allard JP, Jeejeebhoy KN. Vars Research Award. Increase in lumbar spine bone mineral content in patients on long-term parenteral nutrition without vitamin D supplementation. JPEN J Parenter Enteral Nutr. 1995;19(6):431-6.
15. Nightingale JM. Hepatobiliary, renal and bone complications of intestinal failure. Best Pract Res Clin Gastroenterol. 2003;17(6):907-29.
16. Compher C, Pazianas M, Benedict S, Brown JC, Kinosian BP, Hise M. Systemic inflammatory mediators and bone homeostasis in intestinal failure. JPEN J Parenter Enteral Nutr. 2007;31(2):142-7.
17. Shike M, Sturtridge WC, Tam CS, Harrison JE, Jones G, Murray TM, et al. A possible role of vitamin D in the genesis of parenteral-nutrition-induced metabolic bone disease. Ann Intern Med. 1981;95(5):560-8.
18. De Laet C, Kanis JA, Odén A, Johanson H, Johnell O, Delmas P, et al. Body mass index as a predictor of fracture risk: a meta-analysis. Osteoporos Int. 2005;16(11):1330-8.
19. Buttros Dde A, Nahas-Neto J, Nahas EA, Cangussu LM, Barral AB, Kawakami MS. [Risk factors for osteoporosis in postmenopausal women from southeast Brazilian]. Rev Bras Ginecol Obstet. 2011;33(6):295-302.
20. Pironi L, Tjellesen L, De Francesco A, Pertkiewicz M, Morselli Labate AM, Staun M, et al. Bone mineral density in patients on home parenteral nutrition: a follow-up study. Clin Nutr. 2004;23(6):1288-302.
21. Peterkofsky B. Ascorbate requirement for hydroxylation and secretion of procollagen: relationship to inhibition of collagen synthesis in scurvy. Am J Clin Nutr. 1991;54(6 Suppl):1135S-1140S.
22. Chan D, Lamande SR, Cole WG, Bateman JF. Regulation of procollagen synthesis and processing during ascorbate-induced extracellular matrix accumulation in vitro. Biochem J. 1990;269(1):175-81.
23. Hart JP, Shearer MJ, Klenerman L, Catterall A, Reeve J, Sambrook PN, et al. Electrochemical detection of depressed circulating levels of vitamin K1 in osteoporosis. J Clin Endocrinol Metab. 1985;60(6):1268-9.
24. Aljarallah B, Fernandes G, Jeejeebhoy KN, Gramlich LM, Whittaker JS, Armstrong D, et al. The Canadian Home Total Parenteral Nutrition (HTPN) Registry: vitamin K supplementation and bone mineral density. JPEN J Parenter Enteral Nutr. 2012;36(4):415-20.

25. Furie BC, Furie B. Structure and mechanism of action of the vitamin K-dependent gamma-glutamyl carboxylase: recent advances from mutagenesis studies. Thromb. Haemost. 1997;78(1): 595-8.
26. Tamura T, Morgan SL, Takimoto H. Vitamin K and the prevention of fractures. Arch Int Med. 2007;167(1):94-5.
27. Xu H, Watkins BA, Seifert MF. Vitamin E stimulates trabecular bone formation and alters epiphyseal cartilage morphometry. Calcif Tissue Int. 1995;57(4):293-300.
28. Basu S, Michaëlsson K, Olofsson H, Johansson S, Melhus H. Association between oxidative stress and bone mineral density. Biochem Biophys Res Commun. 2001;288(1):275-9.
29. Rejnmark L, Vestergaard P, Charles P, Hermann AP, Brot C, Eiken P, et al. No effect of vitamin A intake on bone mineral density and fracture risk in perimenopausal women. Osteoporos Int. 2004;15(11):872-80.
30. Melhus H, Michaëlsson K, Kindmark A, Bergström R, Holmberg L, Mallmin H, et al. Excessive dietary intake of vitamin A is associated with reduced bone mineral density and increased risk for hip fracture. Ann Intern Med. 1998;129(10):770-8.
31. Mellanby E. Skeletal changes affecting the nervous system produced in young dogs by diets deficient in vitamin A. J Physiol. 1941;99(4):467-86.
32. Dickson I, Walls J. Vitamin A and bone formation. Effect of an excess of retinol on bone collagen synthesis in vitro. Biochem J. 1985;226(3):789-95.
33. Promislow JH, Goodman-Gruen D, Slymen DJ, Barrett-Connor E. Retinol intake and bone mineral density in the elderly: the Rancho Bernardo Study. J Bone Miner Res. 2002;17(8):1349-58.
34. Laurent M, Gielen E, Claessens F, Boonen S, Vanderschueren D. Osteoporosis in older men: recent advances in pathophysiology and treatment. Best Pract Res Clin Endocrinol Metab. 2013;27:527-39.