Serum levels of vitamin B12 are not related to low bone mineral density in postmenopausal Brazilian women

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ABSTRACT

Introduction: Osteoporosis and vitamin B12 deficiency are conditions with an increasing prevalence over time. It has been described an association between low serum vitamin B12, osteoporosis and increased risk of bone fractures, but the studies are heterogeneous and the results are controversial. **Objective:** To investigate the association between plasma levels of vitamin B12 and bone mineral density in a group of asymptomatic women after menopause. **Methods:** Asymptomatic postmenopausal women were consecutively invited to participate in this cross-sectional study. Bone mineral density (lumbar spine and femur) was measured by DXA Lunar Prodigy Vision, and blood levels of vitamin B12, calcium, phosphorus, bone alkaline phosphatase (BAF), and parathyroid hormone were determined. For the diagnostic of osteoporosis the World Health Organization criteria were considered. **Results:** Seventy women were included, mean age 62.5 ± 7 years. Eighteen (25.7%) women had normal bone mineral density, 33 (47.1%) had osteopenia and 19 (27.1%) had osteoporosis. Six (8.6%) patients had wrist fracture; two (2.8%) reported a diagnosis of vertebral fracture and only one (1.4%) patient had suffered a hip fracture. The levels of vitamin B12 (mean ± SD, pg/mL) of women with normal bone mineral density, osteopenia and osteoporosis were 590.2 ± 364.3, 536.6 ± 452.3, and 590.2 ± 497.9, respectively (P = 0.881). Multiple regression analysis showed that body mass index and BAF were the main predictors of lumbar spine bone mineral density. **Conclusion:** The results indicate that vitamin B12 serum levels are not related to bone mineral density in this group of Brazilian postmenopausal women.

Keywords: osteoporosis, postmenopausal, vitamin B 12, bone density.

INTRODUCTION

Osteoporosis is a systemic bone disorder characterized by loss of bone mass and strength, and microarchitectural deterioration of skeletal structure.[1] It is a widespread disease, affecting 75 million people in Europe, the United States and Japan. The number of incident fractures occurring annually in the United States is > 2 million.[2–4] The concept of the disease includes multiple pathogenic mechanisms that, coupled to factors that increase the risk of falls, contribute to an increase in fragility fractures. In some studies, an impaired vitamin B12 status, mainly assessed by plasma levels, has been associated with low bone mineral density (BMD) and increased fracture risk.[5–8] However, the results reported thus far, including animal experiments, are controversial.[9–14] Vitamin B12 is essentially obtained from the diet by consuming animal products and could interfere with bone metabolism, with positive osteoblast stimulation.[15] In contrast, an increase in circulating homocysteine levels due to vitamin B12 deficiency may be implicated in the early onset of osteoporosis, in impaired bone quality and in a higher fracture risk.[8,16–21] The real impact of vitamin B12 deficiency on bone health and on the mechanisms associated with disorders of bone metabolism have not been clearly defined.

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The identification of individuals who could benefit from earlier screening for osteoporosis and therapeutic intervention could reduce the morbidity and mortality associated with osteoporosis. Since both osteoporosis and cobalamin deficiency can go undetected for several years, and because their clinical consequences may possibly be irreversible,[22] efforts should be devoted to the detection of individuals at higher risk for low bone mass and osteoporotic fractures. Although risk factors for osteoporosis and fractures such as age, glucocorticoid use, and family history have been well documented in Brazilian subjects, plasma levels of vitamin B12 have never been studied in relation to BMD in this population.[23–25] Thus, the aim of the present investigation was to study a possible connection between plasma levels of vitamin B12 and BMD in an asymptomatic group of Brazilian native postmenopausal women.

METHODS

Participants

The present cross-sectional study was approved by the Ethics Committee of the Universidade Federal de Minas Gerais (UFMG). Healthy postmenopausal women (at least five years of natural amenorrhea) were consecutively recruited from those seen for the first time at the Gastroenterology Unit of the Hospital Geral between January and December 2007. All subjects gave written informed consent to participate in the study. Exclusion criteria were use of drugs known to influence bone mineralization (glucocorticoids for more than three months, antiepileptic drugs, calcium supplementation, warfarin, hormone replacement therapy, vitamin D, and bisphosphonates), history of neoplasms, diabetes mellitus or use of metformin, liver or renal dysfunction, tobacco use or alcoholic habit (more than three drinks per day), folate or vitamin B12 supplementation, and consumption of an exclusively vegetarian diet. Body mass index (BMI) was calculated as weight in kilograms divided by height in square meters.

Plasma measurements

A blood sample was collected from an antecubital vein of each woman in the morning following an overnight fasting. Serum vitamin B12 was measured in a single assay and its concentration (pg/mL) was determined using a commercial chemiluminescence immunoassay (reference values 200–950 pg/mL, CV 7%). Serum levels of calcium were measured by an ion-selective electrode with automatic correction of pH (reference values 1,17–1,32 mmol/L), bone alkaline phosphatase (BAF) by an immunocapture assay, (reference values 11,6–43,4 U/L), phosphorus by a standard colorimetric method UV (reference values 2,5–4,8 mg/dL), and parathyroid hormone (PTH) were determined using chemiluminescence immunoassay (reference values 8–80 pg/mL).

Bone mineral density

Hip and lumbar BMD was determined with a Lunar Prodigy Vision DXA (Lunar Corp., Madison, WI). The DXA scans were obtained by standard procedures for scanning and analysis according to manufacturer's instructions. Daily quality control was performed by measuring a Lunar phantom. At the time of the study, phantom measurements showed stable results. The coefficient of variation was 1%. The diagnosis of osteoporosis was made according to the criteria of the World Health Organization (WHO), represented by a T-score below −2.5 SD.[26]

Statistical analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences for Windows, version 17 (SPSS Inc, Chicago, IL). Data are reported as mean ± SD. For continuous variables not normally distributed, the groups were compared by the Mann-Whitney U test. A $P < 0.05$ was considered to indicate a significant difference. Stepwise regression analysis was used to evaluate variables independently related to BMD. Single regression analysis was used to express the relation between an independent variable and BMD. Single-factor analysis of variance or covariance was used to perform group comparisons. The Spearman rank correlation coefficient was used to determine the strength of association between pairs of bone parameters.

RESULTS

Seventy postmenopausal women, all of them with physiological menopause, were included in the study. Considering the whole study population, mean age at first visit was 62.5 ± 6.9 years (range: 50–79), mean BMI was 27.1 ± 4.7 kg/m^2 (range: 17.9–43), mean BMD was 0.913 ± 0.153 g/cm^2 (range: 0.533–1.317) for the total femur, 0.864 ± 0.137 g/cm^2 (range: 0.564–1.234) for the femoral neck, and 1.003 ± 0.175 g/cm^2 (range: 0.670– 1.414) for the lumbar spine. The geometric mean of plasma vitamin B12 levels was 565 ± 439 pg/mL (range: 156–2261). Six (8.6%) patients had suffered a wrist fracture and two (2.8%) reported at least one vertebral fracture. One (1.4%) patient had suffered a hip fracture.

Table 1
Demographic and biochemical parameters of the study population of 70 postmenopausal women according to BMD classification

	Normal (n=18)	Osteopenia (n=33)	Osteoporosis (n=19)	P
Age (years)	61.7 ± 6.5	63.2 ± 7.5	62.5 ± 6.9	0.748
BMI[a] (kg/m²)	30.6 ± 4.9	26.3 ± 4.2	25.2 ± 3.8	0.001
Age of menopause (years)	47.3 ± 4.2	49.2 ± 3.7	48.8 ± 4.9	0.308
Duration of menopause (years)	14.4 ± 6.1	14 ± 7.1	13.8 ± 7.9	0.908
Lumbar spine BMD[b] (g/cm²)	1.213 ± 0.109	1.003 ± 0.097	0.804 ± 0.057	< 0.001
Femoral neck BMD[b] (g/cm²)	1.026 ± 0.089	0.833 ± 0.081	0.765 ± 0.119	< 0.001
Total femoral BMD[b] femoral (g/cm²)	1.090 ± 0.106	0.880 ± 0.097	0.795 ± 0.117	< 0.001
Vitamin B12 (pg/mL)	590.2 ± 364.3	536.6 ± 452.3	590.2 ± 497.9	0.881
Serum calcium (mmol/L)	1.22 ± 0.06	1.24 ± 0.03	1.26 ± 0.06	0.026
Serum phosphorus (mg/dL)	3.8 ± 0.59	3.7 ± 0.59	3.6 ± 0.55	0.480
Serum bone phosphatase alkaline (U/L)	25.6 ± 8.8	26.7 ± 5.7	34.2 ± 12.6	0.006
Serum PTH[c] (pg/mL)	42.2 ± 12.0	45.9 ± 14.4	41.1 ± 16.7	0.463

Values expressed in mean ± SD. [a]: body mass index; [b]: bone mineral density.

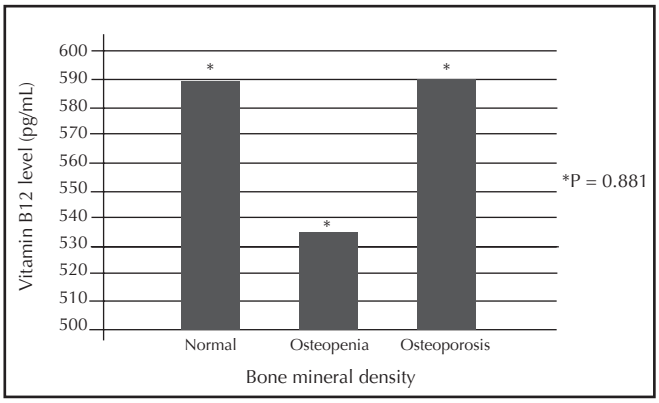

Figure 1
Vitamin B12 levels according to the World Health Organization criteria for bone mineral density.

At DXA evaluation, 19 (27.1%) women were considered to be osteoporotic (T-score below −2.5), 33 (47.1%) had osteopenia (T-score between −2.5 and −1), and 18 (25.7%) had normal BMD values. The demographic and biochemical parameters of the three subgroups according to the densitometric diagnosis are summarized in Table 1. Mean ± SD for vitamin B12 levels (pg/mL) of women with normal BMD, osteopenia and osteoporosis were 590.2 ± 364.3, 536.6 ± 452.3, and 590.2 ± 497.9, respectively (P = 0.881) (Figure 1). Multiple regression analysis showed that BMI and BAF were the main predictors of lumbar spine BMD (Table 2).

Table 2
Correlation between the results of DXA and bone-related variables

Pairs of variables	r[a]	P
BMD[b] and BMI[c]	0.287	0.002
BMD and calcium	−0.16	0.11
BMD and phosphorus	0.03	0.78
BMD and PTH[d]	0.01	0.94
BMD and bone alkaline phosphatase	0.34	0.001
BMD and age	−0.091	0.34
BMD and vitamin B12	0.009	0.93

[a]: Spearman coefficient; [b]: bone mineral density; [c]: body mass index; [d]: parathyroid hormone.

DISCUSSION

Osteoporosis is a skeletal disorder characterized by compromised bone strength, which predisposes the individual to an increased risk for fractures, especially of hip, wrist, and spine. Postmenopausal estrogen deficiency increases the rate of bone remodeling, as well as the amount of bone lost with each remodeling cycle.[27] In addition, many risk factors are associated with osteoporotic fractures, including low peak bone mass, hormonal factors, chronic diseases, drug use, cigarette smoking, low physical activity, low intake of calcium and vitamin D, race, small body size, and a personal or family history of fracture.

Vitamin B12 deficiency has also been related to low bone mass and to an increased risk of fractures, but the results are

Table 3
Overview of studies analyzing serum vitamin B12 levels, fracture risk, and bone mineral density

Author	Subjects	BMD	Fracture risk	Comments
Dhonukshe-Rutten RA et al.[5]	194, men and women	+	NA	
Cagnacci A et al.[12]	161, postmenopausal women	–	NA	
Macdonald HM et al.[40]	1241, women, 45-54 year-old	–	NA	
Stone KL et al.[41]	83, postmenopausal women, >65 years	–	NA	
Tucker KL et al.[6]	2456, men and women	+	NA	
Ravaglia P et al.[42]	702, men and women	NA	–	Fracture risk associated with folate levels
Morris MS et al.[43]	1550, men and women	+	NA	
Dhonukshe-Rutten RA et al.[14]	1267, men and women	+	+	BMD by BUA
Sato Y et al.[44]	433, hemiplegic stroke patients	–	+	
Gjesdal CG et al.[28]	5338, men and women	–	NA	BMD associated with homocysteine levels
Gjesdal CG et al.[45]	4766, men and women	NA	–	
Baines M et al.[46]	328, postmenopausal women	–	NA	BMD associated with folate levels
Gerdhem P et al.[5]	996, women >75 year-old	NA	–	No association with folate levels
McLean RR[7]	1002, men and women	+	+	
Cagnacci A et al.[13]	117, postmenopausal women	–	NA	Five years of follow-up, BMD change associated with folate levels
Ouzzif Z et al.[18]	188, postmenopausal women	+	NA	Association with hip BMD but not lumbar spine
Present study 2012	70, postmenopausal women	–	NA	

+: significant relationship; –: no relationship; BMD: bone mineral density; BUA: broadband ultrasound attenuation; NA: not available.

controversial (Table 3). Tucker et al.[6] showed a relationship between low bone mass in the hip and lumbar spine and low levels of vitamin B12. The same finding was observed in studies by Cagnacci et al.[12,13] and Gjestal et al.[28] Similarly, Rejnmark et al.[11] demonstrated that folate (but not vitamin B12) ingestion was significant associated with bone mass. A study by Dhonukshe-Rutten et al.[5] pointed to the fact that vitamin B12 was associated with low BMD in women, but not in men.

Vitamin B12 deficiency, which is common among the elderly, can lead to neurological complications characterized by paresthesia, loss of proprioception, and reduced vibration sense in the lower extremities, conditions that may increase the propensity for falls.[29,30] Epidemiological studies in the general population have shown a prevalence of vitamin B12 deficiency of about 20% (between 5% and 60%), depending on the definition of cobalamin deficiency.

Cobalamin is an important co-factor in amino acid metabolism, and its deficiency could be responsible for the increase in homocysteine levels, which is also related to osteoporosis and bone fractures.[14,16,17] Homocysteine seems to interfere with cross-links of newly formed collagen and to stimulate osteoblast formation and activity. However, vitamin B12 may influence the bone metabolism through other pathways beyond homocysteine metabolism. Another possible effect could be the direct action of vitamin B12 on osteoblasts, since a functional and proliferative dose-dependent response was observed when two different cell lineages of osteosarcoma were stimulated with cyanocobalamin.[15]

In agreement with our results, Cagnacci et al.,[13] in a study of 117 postmenopausal women, found no significant relation between the change of vertebral BMD and vitamin B12; but the annual rate of vertebral BMD change was independently related to folate levels. No relation was noted with homocysteine. A cross-sectional study by Rejnmark L et al.[11] showed positive correlations between daily intake from the diet and from the diet plus supplements of folate and BMD at the femoral neck, but again, no relation between BMD and vitamin B6 or B12 in perimenopausal women. A sectional study reported the mean values of dietary analysis of food registries with free serum cobalamin and homocysteine in Brazilian adult females and did not demonstrate a deficient intake.[31]

Loss of stomach acidity resulting from aging or atrophic gastritis (which can occur by an autoimmune mechanism or as a late stage of *H. pylori* infection) could be implicated in

subjects with impaired B12 status.[32] Hypochlorhydria has been estimated to affect up to 40% of older adults, and is associated with impaired absorption of protein-bound vitamin B12. Although a retrospective study in women found higher rates of fracture among those with pernicious anemia compared with normal controls,[33] we could not find difference in the BMD between patients with autoimmune gastritis, *H. pylori* gastritis, and normal controls.[34] It is also possible that the increased use of acid blockers may contribute to the development of vitamin B12 deficiency.[35]

Despite of the higher serum calcium in the osteoporosis group, the statistic analysis between calcium and others parameters showed no significant difference, and could be explained by the osteoclastic activity in this group, once no patients had had levels above the normal upper limit neither other hypercalcemic pathological conditions.

Our study has limitations since the sample size does not permit generalization of the results, but, to our knowledge, this is the first investigation of the relationship between vitamin B12 status and BMD in Brazilian postmenopausal women. Although participants were recruited from the community, we observed exclusion criteria such as diseases and situations that could influence bone health. This makes the results less generalizable, but may support the lack of association between vitamin B12 levels and BMD, since studies investigating vitamin B12, BMD and fracture risk are not homogenous.[8] Also, we did not measure methylmalonic acid or homocysteine levels to confirm functional deficiency of vitamin B12 and, because this was an observational study, the participants were not selected on the basis of evidence of vitamin B12 deficiency. We did not perform measurements of vitamin D levels in our study population. Although important, there may be ethnic differences in the effects of low vitamin D status on bone mass or bone metabolism.[36,37] African Americans typically have lower vitamin D levels than Caucasian Americans, yet they have a lower prevalence of osteoporosis.[38] A Brazilian transversal study in postmenopausal women evaluated the correlation of vitamin D deficiency and BMD. Although a high incidence of inadequate serum concentrations of 25-OH vitamin D (68.3%) was found, with the presence of 8% with secondary hyperparathyroidism, no significant differences were found between serum vitamin D concentrations and BMD.[39]

Because the risk of osteoporotic fractures is higher in women than in men, all postmenopausal women over the age of 65 should be screened for osteoporosis. Younger postmenopausal women with fractures or risk factors should be submitted to densitometry and laboratory assessment for osteoporosis. This reinforces the importance of recognizing risk factors in different populations, with direct implications for the public health system. Our results indicate that serum vitamin B12 levels do not seem to be an indicative factor for screening of low bone mineral density in a given population of postmenopausal women. Thus, the results available thus far suggest that a low level of vitamin B12 is not a reliable risk factor for osteoporosis in Brazilian postmenopausal women.

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