Investigating vitamin D deficiency

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This section uses case scenarios to educate doctors on the best approach to the diagnosis and management of patients with different endocrine problems. The appropriate selection of tests and correct interpretation of test results are discussed.

Vitamin D plays an important role in calcium metabolism, bone mineralisation and muscle strength.[1] Over 90% of vitamin D is synthesised in the skin upon exposure to ultraviolet B radiation from sunlight, and the rest is obtained from the diet. Dietary sources of vitamin D include oily fish, liver, egg yolks, fortified margarine and fortified breakfast cereals. However, with the exception of fatty fish such as North Sea salmon, herring or mackerel, the vitamin D content of unfortified food is relatively low.[2] Risk factors for vitamin D deficiency are listed in the Box.

Vitamin D obtained from sun exposure and diet is biologically inert and must undergo endogenous hydroxylation for activation. Vitamin D is first hydroxylated in the liver to 25-hydroxyvitamin D (25-OH vitamin D) and subsequently undergoes a second hydroxylation in the kidney to form physiologically active 1,25-dihydroxyvitamin D (1,25-[OH]2 vitamin D), also known as calcitriol.[3]

1,25(OH)2 vitamin D stimulates the absorption of calcium, and to a lesser degree phosphorus and magnesium, from the small intestine.[4] Disorders in vitamin D action cause a decrease in the net flux of mineral to the extracellular compartment, causing hypocalcaemia and hypophosphataemia. Hypocalcaemia stimulates parathyroid hormone (PTH) synthesis and release from the parathyroid glands, leading to secondary hyperparathyroidism, which in turn lowers serum phosphate levels further by stimulating renal phosphate clearance.

Calcium and phosphorus are essential components for normal bone mineralisation and are incorporated into bone by osteoblasts. In vitamin D deficient states, defective mineralisation of the organic bone matrix occurs as a result of low serum concentrations of calcium and phosphorus plus impaired functional capacity of osteoblasts to incorporate these elements into bone.

Osteomalacia results from a loss of skeletal mass caused by defective bone matrix mineralisation of the normal osteoid tissue after the closure of the growth plates in adults.[5] Rickets results from inadequate mineralisation of bone matrix occurring in children and adolescents before the growth plates have closed. Disorders in vitamin D action also impair the differentiation of osteoblasts and thus their functional capacity to mineralise bone matrix; this also contributes to rickets and osteomalacia.[6,7]

The following three cases illustrate the investigation and management of individuals who present with consequences of defective bone mineralisation as a result of disorders or deficiency of vitamin D.

Case 1

A 23-year-old woman presents with leg pain and difficulty climbing stairs; she is otherwise well. She migrated to Sydney from Sudan four years ago. Since then, she has had three children, and is now three months postpartum and breastfeeding. She has a regular daily intake of dairy food (milk, yoghurt and cheese). For religious reasons, she wears a hijab. Examination shows nonspecific mild tenderness over the quadriceps with mild proximal weakness; other examination results are normal.

Investigations show the following results:

- **corrected calcium, 2.05 mmol/L (reference range, 2.15–2.55 mmol/L)**
- **serum phosphate, 0.87 mmol/L (reference range, 0.8–1.5 mmol/L)**
- **serum alkaline phosphatase (ALP), 124 U/L (reference range, 39–117 U/L)**
- **serum intact PTH (iPTH), 85 ng/L (reference range, 15–65 ng/L).**

What do these results show, and what further investigation should be performed?

This patient's biochemical profile shows mild hypocalcaemia, mildly elevated ALP level with an elevated serum PTH level suggestive of secondary hyperparathyroidism. In view of her biochemical profile and her symptoms of muscle aches and proximal weakness, this patient most likely has osteomalacia as a consequence of severe and prolonged vitamin D deficiency due to lack of supply (reduced sunlight exposure) and increased demand (repeated pregnancies and breastfeeding).

Measurement of the patient's serum 25-OH

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vitamin D level showed it was below 15 nmol/L (reference range, 50 to 140 nmol/L).

What is the diagnosis?

The results are consistent with a diagnosis of osteomalacia due to severe vitamin D deficiency. This would have been caused by lack of sunlight exposure, pregnancy and breastfeeding.

The major source of vitamin D is subcutaneous conversion of cholesterol to cholecalciferol on exposure to ultraviolet (UV) B radiation in sunlight. The opportunity for and efficacy of production may be limited if UV light exposure is reduced by factors such as wearing of clothing that covers most of the skin, darker natural skin pigmentation or migration from lower to higher latitude countries (such as from Sudan to Australia).

Most young adults with serum 25-OH vitamin D levels below 50 nmol/L but above 25 nmol/L do not require any additional investigation other than clinical and biochemical evaluation. Patients with serum 25-OH vitamin D levels below 25 nmol/L are at risk of developing osteomalacia. The diagnosis of osteomalacia is usually based on a combination of clinical features (bone pain, tenderness, fractures, muscle weakness), biochemical parameters and radiological findings. Osteomalacia may also be asymptomatic and present radiologically as osteopenia.

The initial laboratory evaluation should include measurement of serum concentrations of calcium, phosphate, ALP, 25-OH vitamin D, PTH, electrolytes, blood urea nitrogen (BUN) and creatinine.

Radiographs may be helpful when the patient has severe bone pain to assess for fractures and to distinguish osteomalacia from multiple myeloma or Pagetic bone disease. Reduced bone density with thinning of the cortex is the most common finding in those with osteomalacia, but it is nonspecific. More specific are changes in vertebral bodies where inadequate mineralisation of osteoid and loss of secondary trabeculae leads to loss of radiological distinctness of vertebral body trabeculae. Looser zones are another characteristic radiological finding of osteomalacia.[8] These are narrow radiolucent lines, 2 to 5 mm in width, with sclerotic borders that are often bilateral, symmetrical and lie perpendicular to the cortical margins of bones. Common sites include the femoral neck, medial part of the femoral shaft, beneath the lesser trochanter, and the pubic and ischial rami. Bone biopsy and histomorphometry is rarely required and should only be performed when the diagnosis of osteomalacia is in doubt or if its cause is not determined by noninvasive testing.

Several studies have demonstrated markedly reduced spine, hip and forearm bone mineral density (BMD) as measured by dual-energy x-ray absorptiometry (DXA) in patients with osteomalacia related to vitamin D deficiency.[9,10] However, as DXA BMD findings are unable to differentiate osteomalacia and osteoporosis, BMD measurement is not routinely required for the diagnosis of osteomalacia.

How should this patient be managed?

For people living in Australia and New Zealand, the main source of vitamin D is through exposure to sunlight. The minimal erythemal dose (MED) is the amount of low wavelength UVB radiation exposure that just causes faint redness of the skin (erythema). Experimental data indicates exposure of around 15% of the body surface (arms and hands or equivalent) to one-third of the MED near the middle of the day will result in approximately 1000 IU of vitamin D.[11] However, deliberate exposure to sunlight between 10:00 and 14:00 (or 11:00 and 15:00 daylight saving time) in the summer months is not advised in Australia and New Zealand. Given the high incidence of skin cancer in these countries, sunscreens and other UV radiation avoidance measures should be used if exposure is likely to be prolonged when the UV index is 3 or above and/or there is a risk of skin damage.

In people with vitamin D deficiency who are Immunocompromised or have an increased risk of skin cancer, oral vitamin D supplementation may be a more appropriate means of maintaining adequate vitamin D levels.[11] The dietary reference intakes of vitamin D for children aged 1 to 18 years, pregnant women and nonpregnant adults up to and including the age of 70 years is 600 IU, with the dietary reference intakes increasing to 800 IU for those older than 70 years.[12] If adequate sunlight exposure cannot be achieved for personal or cultural reasons, then a higher intake of daily vitamin D up to 600 to 800 IU may be required.[12]

Risk factors for vitamin D deficiency

- Insufficient exposure to sunlight (reduced vitamin D synthesis)
  - ageing
  - dark skin
  - immobility, institutionalisation
  - wearing veils, hijab, hats
- Pregnancy (increased demand for vitamin D)
- Breastfeeding (increased demand for vitamin D)
- Nutritional deficiency (inadequate vitamin D intake)
- Gastrointestinal malabsorption (reduced vitamin D absorption)
  - short bowel syndrome (stomach or bowel resection
  - chronic pancreatic disease
  - cystic fibrosis
  - biliary disorders, primary biliary cirrhosis, biliary fistulae, biliary atresia
  - Crohn's disease
  - coeliac disease
  - liver cirrhosis
- Renal failure (reduced vitamin D synthesis)
- Liver failure (reduced vitamin D synthesis)
- Drugs (enhanced vitamin D degradation)
  - anticonvulsants (phenytoin, carbamazepine, phenobarbitone)
  - rifampicin
  - highly active antiretroviral therapy
  - cholestyramine

The aims of therapy are to replace and then maintain vitamin D levels via supplementation with cholecalciferol (vitamin D3) or ergocalciferol (vitamin D2). Provided parathyroid function and renal function are intact, the vitamin D supplement will undergo physiological renal conversion to physiologically active 1,25-(OH)2 vitamin D as needed. Standard supplementation requires 1000 IU daily; however, in cases of severe deficiency such as in this patient, doses of 3000 to 10,000 IU daily may be required for up to three months, followed by a maintenance dose of 1000 to 2000 IU daily.[13] The 2011 US Endocrine Society's clinical practice guideline on vitamin D deficiency recommends that high risk adults with serum 25-OH vitamin D concentrations below 50 nmol/L

may be treated with 50,000 IU of oral cholecalciferol once weekly for six to eight weeks or its equivalent of 6000 IU daily to replete vitamin D stores and achieve a serum 25-OH vitamin D level higher than 75 nmol/L, followed by maintenance therapy of 1500 to 2000 IU daily.[14] Side effects from over-replacement are extremely rare and usually involve doses well beyond 10,000 IU daily.

If this patient's dietary calcium intake is inadequate, supplementation with calcium carbonate or calcium citrate should also be commenced. All patients should maintain a daily total calcium intake (diet plus supplements) of 1000 mg (for women aged 19 to 50 and men aged 19 to 70 years) to 1200 mg (for women aged 51 to 70 and all adults aged over 70 years).[12]

Upon initiation of treatment for osteomalacia, symptoms of bone pain, muscle aches and proximal myopathy typically disappear within weeks to months.[15,16] During clinical recovery, the ALP level rises temporarily due to enhanced bone formation, and then normalises. The serum 25-OH vitamin D level should be measured three to four months after therapy begins.[13] Some degree of physiological bone resorption may occur during pregnancy and lactation; however, this bone loss is reversible soon after weaning occurs.[17]

Case 2

A 71-year-old woman presents with back pain, which she has had for the past week. She lives in a nursing home and spends most of her time indoors. She does not give any history of fall or trauma. She reached menopause at age 53 years, after which she took oestrogen and progesterone therapy for a year before it was discontinued when she developed deep vein thrombosis. Osteoporosis was diagnosed three years ago when she was found to have a low bone density on DXA scan. Results of her initial DXA scan showed her lumbar spine T-score was -2.5 and her femoral neck T-score, -2.8.

She was commenced on weekly oral alendronate but has not been very compliant because of dyspepsia and abdominal discomfort when taking it. She also takes 1000 mg of calcium supplements daily but eats no dairy products as she suffers from lactose intolerance.

She is 163 cm tall and weighs 53 kg (body mass index [BMI], 19.9 kg/m^2), and has mild kyphosis and some tenderness over her thoracic vertebrae. No notable tenderness is present over other areas of her spine. Her gait is normal, and she uses a cane for stability while walking.

Investigations show:

- **Blood tests:**
  - **haemoglobin level, 124 g/L (normal range for women 120–150 g/L)**
  - **serum urea, 4.8 mmol/L (normal range, 1.7–8.3 mmol/L)**
  - **serum creatinine level, 52 µmol/L (normal range, 50–98 µmol/L)**
  - **corrected serum calcium level, 2.11 mmol/L (normal range, 2.15–2.55 mmol/L).**
- **Anteroposterior and lateral views of thoracic and lumbar spine:**
  - **osteopenic vertebrae**
  - **T10 and T12 compression fractures.**
- **DXA scan:**
  - **lumbar spine T-score, -2.9**
  - **femoral neck T-score, -3.2.**

What should be done next?

In view of the patient's worsening osteoporosis and intolerance to oral bisphosphonates, she was offered annual intravenous (IV) bisphosphonate and received her first infusion. Oral bisphosphonates were discontinued. Ten days later, she presented to the local emergency department with carpopedal spasm and palpitations.

Investigations performed in the emergency department showed the following:

- corrected serum calcium level, 1.62 mmol/L (normal range, 2.15–2.55 mmol/L)
- serum magnesium level, 0.91 mmol/L (normal range, 0.8–1.2 mmol/L)
- serum phosphate level, 0.54 mmol/L (normal range, 0.8–1.5 mmol/L)
- serum ALP level, 486 U/L (normal range, 39–117 U/L)
- QT interval (QTc), 550 ms (normal for women, <460 ms) on ECG.

What diagnosis do these results suggest?

This patient's symptoms are consistent with severe hypocalcaemia as evidenced by her investigation results and prolonged QT interval on ECG. Her magnesium levels are normal.

What further investigations should be performed?

Further investigations that should be performed are measurement of serum PTH and 25-OH vitamin D levels. The results show:

- serum iPTH level, 138 ng/L (normal range, 15–65 ng/L)
- serum 25-OH vitamin D level, 18 nmol/L (normal range, 50–140 nmol/L).

The patient's markedly elevated PTH levels occurring in conjunction with low serum calcium and phosphate levels and an elevated ALP level are suggestive of vitamin D deficiency. Her serum 25-OH vitamin D level of 18 nmol/L indicates she has severe vitamin D deficiency.

What is the diagnosis?

The investigation results are consistent with a diagnosis of hypocalcaemic tetany due to intravenous bisphosphonate administration with uncorrected underlying vitamin D deficiency.

IV bisphosphonates are an option for patients with osteoporosis who cannot tolerate oral bisphosphonates or who have difficulty sitting upright. Hypocalcaemia may occur in patients treated with IV bisphosphonates.[18,19] Zolendronic acid inhibits osteoclast-mediated bone resorption, lowering serum calcium levels; individuals who have received zolendronic acid therefore have difficulty counteracting hypocalcaemic stimuli. Symptomatic hypocalcaemia is more likely to occur in those with vitamin D deficiency receiving IV bisphosphonates. Elderly individuals who are institutionalised or housebound are at high risk of vitamin D deficiency, and in Australia, 80% of women and 70% of men living in hostels or nursing homes in Victoria, New South Wales and Western Australia are vitamin D-deficient.[20,21]

How should this patient be managed?

Patients with hypocalcaemic tetany require IV calcium gluconate with close monitoring until symptoms resolve and ECG changes normalise. Oral cholecalciferol or calcitriol may help resolution of hypocalcaemia. The symptoms may be prolonged and take a few days to resolve.

This patient had a low serum calcium level before commencing IV bisphosphonate therapy

and also risk factors for vitamin D deficiency (elderly, living in a nursing home and lack of sun exposure). Serum 25-OH vitamin D concentration should be assessed and any vitamin D deficiency corrected before starting before a bisphosphonate infusion. Increasing calcium supplementation (doubling of usual dose) for five to seven days, starting on the day of bisphosphonate infusion, may also minimise hypocalcaemia. A maintenance dose of at least 1000 IU vitamin D is required to reduce fracture risk in the elderly.[13] In cases of vitamin D deficiency, oral doses of 10,000 IU daily have been given without adverse effects for at least 90 days in postmenopausal women.[22]

Case 3

A 20-year-old woman, diagnosed with type 1 diabetes at the age of 14 years, presents with complaints of deep pain in her arms and thighs. Her basal–bolus insulin regimen comprises 12 units of insulin aspart three times a day with meals and 26 units of the long-acting insulin glargine at bedtime. She has been having increased bowel movements (up to three or four times a day) for the past eight months, associated with abdominal bloating. She is 165 cm tall and weighs 48 kg (BMI, 17.6 kg/m2). Her diet is well balanced and she gives no history of lactose intolerance. Her menses have always been regular.

Investigations show the following results:

- **corrected serum calcium, 2.01 mmol/L (normal range, 2.15–2.55 mmol/L)**
- **serum phosphate, 0.74 mmol/L (normal range, 0.8–1.5 mmol/L)**
- **serum ALP, 412 U/L (normal range, 39–117 U/L)**
- **serum iPTH level, 125 ng/L (normal range, 15–65 ng/L)**
- **serum 25-OH vitamin D level, 11 nmol/L (normal range, 50–140 nmol/L).**

What do these results show, and what further investigations should be performed?

This patient has hypocalcaemia, hypophosphataemia, elevated ALP along with significant hyperparathyroidism suggestive of vitamin D deficiency. Her symptoms of deep pain in her arms and thighs associated with this biochemical profile suggest she has osteomalacia. Her low serum 25-OH vitamin D level confirms this.

Further investigations that should be performed include qualitative faecal fat analysis, anti-transglutaminase antibody measurement and duodenal biopsy. The results show:

- positive qualitative faecal fat analysis
- anti-transglutaminase antibodies, 28 U/mL (normal range, 4 U/mL or below)
- partial villous atrophy, hyperplastic crypts of Lieberkühn and increased intraepithelial lymphocytes on duodenal biopsy.

What is the diagnosis?

The investigation results are consistent with a diagnosis of vitamin D deficiency secondary to malabsorption due to coeliac disease.

Coeliac disease (gluten-sensitive enteropathy) occurs more frequently in patients with type 1 diabetes. Patients with type 1 diabetes and gastrointestinal symptoms such as chronic or recurrent diarrhoea, malabsorption, weight loss, and abdominal distension or bloating should be evaluated for coeliac disease. Villous atrophy occurs in the proximal bowel, causing calcium malabsorption and increased levels of unbound intraluminal fatty acids; these fatty acids bind to intraluminal calcium, further inhibiting absorption. Hypocalcaemia causes secondary hyperparathyroidism, which in turn results in enhanced metabolic breakdown of vitamin D metabolites.[23-25] The amount of vitamin D-dependent calcium-binding proteins in the intestinal wall has been shown to be reduced in active disease.[26]

How should this patient be managed?

A gluten-free diet is recommended for patients with coeliac disease. The principal sources of dietary gluten are wheat, rye and barley, and foods containing these grains should be avoided. Patients require written information and dietary counselling. Adherence to a gluten-free diet causes improvement and resolution of symptoms of malabsorption. Approximately 70% of patients with coeliac disease have noticeable clinical improvement within two weeks of commencing a gluten-free diet.[27]

Treatment of osteomalacia in this patient would include vitamin D supplementation and adequate calcium supplementation. The desired threshold level of 25-OH vitamin D for optimum skeletal benefit is still an area of controversy and debate. The Australian and New Zealand Bone and Mineral Society advocates an optimal range of serum 25-OH vitamin D of 50 to 70 nmol/L.[11,13] This range is also similar to the US Institute of Medicine recommendation.[12] The 2011 US Endocrine Society, however, recommends maintaining a higher serum level, between 75 and 100 nmol/L, for optimum skeletal health.[14,28] It has been found that 25-OH vitamin D levels of greater than 75 nmol/L are needed to ensure suppression of the PTH level into the normal range.[29] However, as this is an area of controversy, more research may be required to identify the benefits of higher serum 25-OH vitamin D levels in maintenance of skeletal health and fracture reduction.

In patients with malabsorption, at least 6000 to 10,000 IU of oral cholecalciferol per day may be needed to treat vitamin D deficiency, followed by maintenance therapy of at least 3000 IU, and up to 6000 IU, per day, along with adequate daily calcium intake.[14]

As mentioned earlier, the symptoms of osteomalacia resolve within weeks to months of commencing treatment. The serum 25-OH vitamin D level should be monitored after three to four months of therapy and the dose of vitamin D supplement adjusted to prevent hypercalciuria or hypercalcaemia.

Summary

Vitamin D deficiency may occur even in populations living in areas with adequate sunlight. Maintenance of adequate vitamin D levels through vitamin D supplementation improves muscle strength and balance and reduces the risk of falls in community-dwelling and institutionalised individuals. Vitamin D supplements are available in oral form as 1000 IU oral cholecalciferol (vitamin D_3) in Australia and New Zealand.

It is important that clinicians recognise the signs and symptoms of vitamin D myopathy and identify specific populations at risk for vitamin D deficiency as this condition is reversible and easily treated. **ET**

References

A list of references is included in the website version (www.medicinetoday.com.au) of this article.

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References

1. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. Am J Clin Nutr 2008; 87: S1080-S1086.
2. Pearce SH, Cheetham TD. Diagnosis and management of vitamin D deficiency. BMJ 2010; 340: b5664.
3. Institute of Medicine, Food and Nutrition Board. Dietary reference intakes for calcium and vitamin D. Washington DC: National Academy Press; 2010.
4. Mayer E, Kadowabi S, Williams G, Norman AW. Mode of action of 1,25-dihydroxyvitamin D. In: Kumar R, ed. Vitamin D: basic and clinical aspects. Martinus Nijhoff, Boston; 1984. p. 259.
5. Kline MJ. Imaging in osteomalacia and renal osteodystrophy. Medscape 2011 (updated July 2013). Available online at: http://emedicine.medscape.com/article/392997-overview (accessed April 2014).
6. Reichel H, Koeffler P, Norman AW. (1989). The role of the vitamin D endocrine system in health and disease. N Engl J Med 1989; 320: 980-991.
7. Owen TA, Aronow MS, Barone LM, Bettencourt B, Stein GS, Lian JB. Pleiotropic effects of vitamin D on osteoblast gene expression are related to the proliferative and differentiated state of bone cell phenotype: dependency upon basal levels of gene expression, duration of exposure and bone matrix competency in normal rat osteoblast culture. Endocrinology 1991; 129: 3139-3146.
8. Frame B, Parfitt AM. Osteomalacia: current concepts. Ann Intern Med 1978; 89: 966-982.
9. Basha B, Rao DS, Han ZH, Parfitt AM. Osteomalacia due to vitamin D depletion: a neglected consequence of intestinal malabsorption. Am J Med 2000; 108: 296-300.
10. Bhambri R, Naik V, Malhotra N, et al. Changes in bone mineral density following treatment of osteomalacia. J Clin Densitom 2006; 9: 120-127.
11. Nowson CA, McGrath JJ, Ebeling PR, et al. Vitamin D and health in adults in Australia and New Zealand: a position statement. Med J Aust 2012; 196: 686-687.
12. Institute of Medicine (US). Dietary reference intakes for calcium and vitamin D. Report at a glance, report brief, released 30/11/2010. Washington DC: National Academy of Sciences; 2014.
13. Working group of the Australian and New Zealand Bone and Mineral Society, Endocrine Society of Australia and Osteoporosis Australia. Vitamin D and adult bone health in Australia and New Zealand: a position statement. Med J Aust 2005; 182: 281-285.
14. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011; 96: 1911-1930.
15. Garcia-Porrua C, Gonzalez-Gay MA, Vila-Alvarenga S, Rivas MJ, Soilan J, Penedo M. Coeliac disease and osteomalacia: an association still present in Western countries. Rheumatology (Oxford) 2000; 39: 1435.
16. Kozanoglu E, Basaran S, Goncu MK. Proximal myopathy as an unusual presenting feature of celiac disease. Clin Rheumatol 2005; 24: 76-78.
17. Karlsson C, Obrant KJ, Karlsson M. Pregnancy and lactation confer reversible bone loss in humans. Osteoporos Int 2001; 12: 828-834.
18. Champallou C, Basuyau JP, Veyret C, et al. Hypocalcemia following pamidronate administration for bone metastases of solid tumor: three clinical case reports. J Pain Symptom Manage 2003; 25: 185-190.
19. Rosen CJ, Brown S. Severe hypocalcemia after intravenous bisphosphonate therapy in occult vitamin D deficiency. N Engl J Med 2003; 348: 1503-1504.
20. Flicker L, Mead K, MacInnis RJ, et al. Serum vitamin D and falls in older women in residential care in Australia. J Am Geriatr Soc 2003; 51: 1533-1538.
21. Sambrook PN, Cameron ID, Cumming RG, et al. Vitamin D deficiency is common in frail institutionalised older people in northern Sydney [letter]. Med J Aust 2002; 176: 560-561.
22. Mastaglia S, Oliveri B, Parisi MS, et al. 10,000 IU of oral vitamin D per day are required to rapidly (3 months) reach adequate 25OHD in osteoporotic women. J Bone Miner Res 2003; 18 (Suppl 2): S55.
23. Clements MR, Davies M, Fraser DR, Lumb GA, Mawer EB, Adams PH. Metabolic inactivation of vitamin D is enhanced in primary hyperparathyroidism. Clin Sci Lond 1987; 73: 659-664.
24. Corazza GR, Di SA, Cecchetti L, et al. Bone mass and metabolism in patients with celiac disease. Gastroenterology 1995; 109: 122-128.
25. Molteni N, Bardella MT, Vezzoli G, Pozzoli E, Bianchi P. Intestinal calcium absorption as shown by stable strontium test in celiac disease before and after gluten-free diet. Am J Gastroenterol 1995; 90: 2025-2028.
26. Staun M, Jarnum S. Measurement of the 10,000-molecular weight calcium-binding protein in small-intestinal biopsy specimens from patients with malabsorption syndromes. Scand J Gastroenterol 1988; 23: 827-832.
27. Pink IJ, Creamer B. Response to a gluten-free diet of patients with the coeliac syndrome. Lancet 1967; 1(7485): 300-304.
28. Holick MF. Vitamin D deficiency. N Engl J Med 2007; 357: 266-281.
29. Holick MF, Siris ES, Binkley N, et al. Prevalence of vitamin D inadequacy among postmenopausal North American women receiving osteoporosis therapy. J Clin Endocrinol Metab 2005; 90: 3215-3224.