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Vitamin D and Bone Physiology: Demonstration of Vitamin D Deficiency in an Implant Osseointegration Rat Model

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Abstract

Purpose: The patient population varies in nutritional deficiencies, which may confound the host response to biomaterials. The objective of this study was to evaluate the effect of a common deficiency of vitamin D on implant osseointegration in the rat model.
Materials and Methods: Male Sprague-Dawley rats were maintained under the cessation of vitamin D intake and UV exposure. The serum levels of $1,25(OH)_2D_3$, 25 OHD_3, Ca, and P were determined. Miniature cylindrical Ti6Al4V implants (2-mm long, 1-mm diameter) were fabricated with double acid-etched (DAE) surface or modified DAE with discrete crystalline deposition (DCD) of hydroxyapatite nanoparticles. DAE and DCD implants were placed in the femurs of vitamin D-insufficient and control rats. After 14 days of healing, the femur-implant samples were subjected to implant push-in test and nondecalcified histology. The surfaces of recovered implant specimens after the push-in test were further evaluated by scanning electron microscopy (SEM).
Results: The decreased serum level of 25 OHD_3 demonstrated the establishment of vitamin D insufficiency in this model. The implant push-in test revealed that DAE and DCD implants in the vitamin D-insufficient group (15.94 ± 8.20 N, $n = 7$; 15.63 ± 3.96 N, $n = 7$, respectively) were significantly lower than those of the control group (24.99 ± 7.92 N, $n = 7$, $p < 0.05$; 37.48 ± 17.58 N, $n = 7$, $p < 0.01$, respectively). The transcortical bone-to-implant contact ratio (BIC) was also significantly decreased in the vitamin D-insufficient group. SEM analyses further suggested that the calcified tissues remaining next to the implant surface after push-in test appeared unusually fragmented.
Conclusions: The effect of vitamin D insufficiency significantly impairing the establishment of Ti6Al4V implant osseointegration in vivo was unexpectedly profound. The outcome of Ti-based endosseous implants may be confounded by the increasing prevalence of vitamin D insufficiency in our patient population.

Once placed in the host environment, biomaterials are subjected to a complex process of cellular and extracellular reactions involving intrinsic and extrinsic factors. Thus, behaviors of a given biomaterial may vary in different hosts, who carry predisposing pathophysiological conditions. For example, when titanium-based endosseous implants are placed in chemically induced diabetic rodents, the degree of bone-to-implant integration or osseointegration was significantly decreased for the long term;[1,2] however, other studies found that bone remodeling around the implant during early healing periods was not affected by the diabetic condition.[3,4] Besides diagnosed or undiagnosed chronic disorders, our patient populations may be suffering from various degrees of nutritional deficiencies. A report by the Institute of Medicine of the National Academy of Sciences has indicated that approximately 50% of women in the United States are potentially vitamin D deficient.[5]

Vitamin D is a fat-soluble hormone transformed into an active form through the liver and kidney; it plays an essential role in maintaining normal blood levels of calcium and phosphorus, and thus affects sound bone remodeling.[6,7] While severe vitamin D deficiency causes rickets in children and osteomalacia in adults, there is evidence that lesser degrees of vitamin D insufficiency can cause deleterious effects on bone tissues. Increased unmineralized osteoid has been reported in biopsy specimens collected in winter months,[8] and hip fracture patients have been associated with vitamin D insufficiency.[9,10]

We have hypothesized that vitamin D insufficiency affects the establishment of osseointegration, the biological bone tissue response to titanium-based endosseous implants. To test this hypothesis, an experimental implant osseointegration was examined in a vitamin D-insufficient rat model.

Materials and methods

Development of vitamin D insufficiency in rats

At 4 weeks of age, male Sprague-Dawley rats were transferred to a vitamin D-deficient environment for 4 weeks. Rats were depleted of vitamin D through lack of light and a modified diet. A custom-made housing preventing any light transmission was used for light inhibition. Rats were monitored on a regular basis using night-vision goggles to ensure rats were healthy and maintaining their diet. A vitamin D-deficient diet [0.47% calcium (Ca), 0.3% Phosphorus (P)] (Harlan Teklad Custom Research Diets, Madison, WI) was used throughout the experimental period. Control rats were housed in the regular vivarium and fed a nutritionally balanced diet.

Serum chemistry measurements

Approximately 2 to 6 mL of periphery blood was extracted at the time of sacrifice, and serum sample was prepared. Ca and P levels were determined by the ACE Calcium-Arsenazo assay and reacting phosphate ions with molybdic acid polymers in an acidic solution, respectively. The serum levels of 1,25-dihydroxycholecalciferol (1,25D) and 25-hydroxycholecalciferol (25D) were determined using corresponding radioimmunoassays (Anilytics Incorporated, Gaithersburg, MD).

Experimental implants

Miniature cylindrical Ti6Al4V implants (2-mm long, 1-mm diameter) were fabricated with a surface treatment with double acid-etching (DAE) (Osseotite, Biomet3i, Palm Beach Gardens, FL). An additional set of DAE implants further underwent the discrete crystalline deposition (DCD) of hydroxyapatite nanoparticles (Nanotite, Biomet3i). Each implant was gas-sterilized and packaged separately.

Implant placement

The rats were anesthetized with 2% isoflurane inhalation. After their legs were shaved and scrubbed with 10% providone-iodine solution, the distal aspect of the femur was carefully exposed via skin incision and muscle dissection. The flat surface of the distal femur was selected for implant placement. The implant site was prepared 7 mm from the distal edge of the femur by drilling with a 0.8-mm round bur followed by reamers ISO 090 and 100. The osteotomy procedure was performed under irrigation of sterile saline solution to avoid overheating the bone bed. Implant stability was confirmed with a mechanical fit. Surgical sites were then closed in layers. The animals recovered without complications and were given water and vitamin D-deficient rat food or control food ad libitum during the healing process.

Implant push-in test

The femur-implant specimens were harvested after 14 days of healing, and embedded in a custom-made resin block. The implant-bone specimens were then subjected to push-in testing as previously reported.[11,12] The angle of the implant relative to the horizontal plane was measured under an incident light microscope. The testing machine (Instron 5544 electro-mechanical testing system, Instron, Norwood, MA) was equipped with a 2000 N load cell and a custom-made pushing rod (diameter = 0.8 mm). The implant was pushed in at a crosshead speed of 1 mm/min. The applied load and the displacement of the implant were monitored. The raw push-in value was determined by measuring the peak of the load-displacement curve (Fig 1). The raw push-in values were normalized against the deviation angle of the implant as follows: $Y = X/(1 + 0.017q)$, where Y = the normalized push-in value, X = the raw push-in value, and q = the implant deviation angle.

Scanning electron microscopy (SEM)

After the push-in test, the implant-bone specimens were fixed in 10% buffered formalin, and the cortical bone was carefully split using a diamond disc within 1 mm to expose the implant. Samples were desiccated and sputter coated with carbon or gold. A scanning electron microscope (Cambridge Stereoscan 250, Cambridge, UK) was used to validate the sheared surface created by the implant push-in test and to evaluate implant surface and remnant tissue adhered to its surface. Accelerating voltage of 20 kV was used for imaging.

Nondecalcified histology and bone-to-implant contact (BIC) measurement

The implant-bone specimens were fixed in buffered formalin for 1 week and dehydrated in an ascending series of alcohol rinses before being embedded in light-curing epoxy resin (Technovit 7200 VLC, Heraeus Kulzer, Wehrheim, Germany) without decalcification. The specimens were sectioned longitudinally and ground to a thickness of 30 μm with a grinding system (Exakt Apparatebau GmbH, Norderstedt, Germany). The histologic section was stained with Goldner's trichrome stain for light microscopy (BX40, Olympus, Melville, NY), and digital photographing (DP10, Olympus). BIC measurement was performed on the top half of the implant in the longitudinal section, which represented the transcortical bone area.

Statistical analysis

For evaluating the implant push-in test and BIC, the control and vitamin D-insufficient groups were compared using Student's *t*-test at the 5% level.

Results

Establishment of vitamin D insufficiency in rats

The control rat group (n = 5) reported a serum level of 1,25D at 52.4 ± 10.4 pg/mL and 25D at 19.1 ± 9.7 ng/ml. The vitamin D-deprived rat group reported serum levels of 1,25D at 56.2 ±

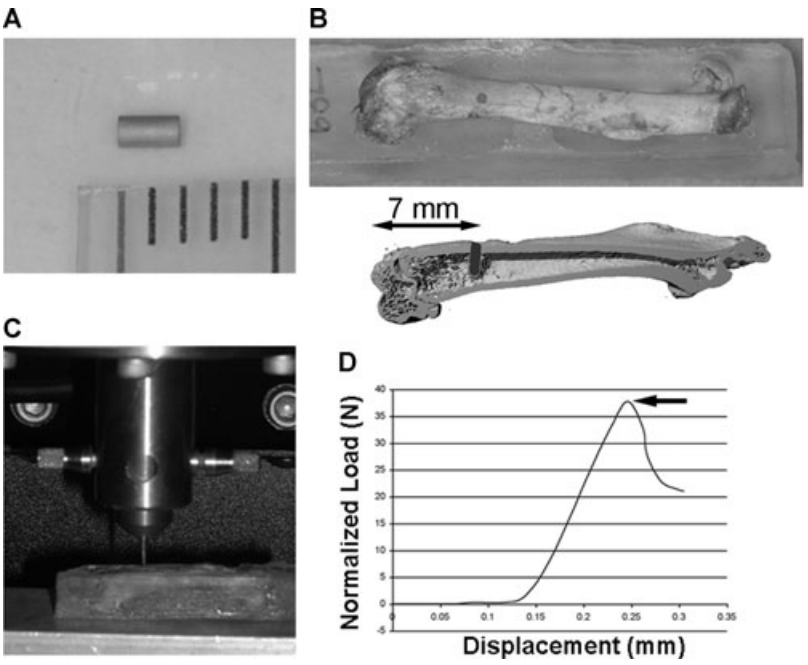

Figure 1 Implant push-in test. (A) Ti6Al4V miniature cylindrical implants (1-mm diameter, 2-mm length) were fabricated with DAE or DCD surfaces. (B) The experimental implant was placed 7 mm from the distal end of the rat femur. The femur specimen was embedded in autopolymerizing resin using a custom-made mold. (C) The implant was pushed in by Instron. (D) The push-in value was obtained as the normalized load at the abrupt break point (arrow).

23.9 pg/ml and 25D at 1.9 ± 0.6 ng/ml (Table 1). While the serum level of 1,25D was not altered, the vitamin D-deprived condition resulted in significant reduction in the serum level of 25D ($p < 0.01$). Blood serum chemistry levels further suggested that the calcium and phosphorous levels were not affected (Table 1). From these data, the vitamin D insufficiency was developed in our rat model.

Biomechanical assessment of osseointegration

The implant push-in values of the DAE implant (n = 7) and DCD implant (n = 7) in the control group were 24.99 ± 7.92 N and 37.48 ± 17.58 N, respectively (Table 2). DCD implants showed significantly higher implant push-in values than those of DAE implants ($p < 0.05$). The implant push-in values of the DAE implant (n = 7) and DCD implant (n = 7) in the vitamin D-insufficient group were 15.94 ± 8.20 N and 15.63 ± 3.96 N, respectively (Table 2). In the vitamin D-insufficient group, both DAE ($p < 0.05$) and DCD ($p < 0.01$) implants showed significant decrease as compared to the corresponding implants in the control group (Fig 2). It was also noted that the difference between the push-in values of DAE implants and DCD implants became indistinguishable in the vitamin D-insufficient group (Table 2, Fig 2).

Histomorphometric assessment of osseointegration

Histological comparison between bone tissues surrounding the implant in the vitamin D-insufficient and the control groups showed no observable differences in the bone marrow and trabecular bone; however, the coronal portion of the implant located in the transcortical bone of vitamin D-insufficient rats suggested unusual soft tissue interface to the implant surface, which was not readily observed in the corresponding site of the control rats (Fig 3). The BIC ratio within the transcortical bone region of DAE implants (n = 4) and DCD implants (n = 4) in the control group were 69.06 ± 11.79% and 70.31 ± 6.95%, respectively. In the vitamin D-insufficient group, the BIC ratio significantly decreased to 45.89 ± 13.49% (n = 4; $p < 0.05$) and 38.13 ± 5.25% (n = 4; $p < 0.01$), respectively (Fig 3).

Table 1 Serum chemistries of vitamin D-deprived rats housed in total darkness and in untreated controls

	25D (ng/ml)	1,25D (pg/ml)	Ca (mg/dl)	P (mg/dl)
Untreated controls (n = 5)	19.1 ± 9.7	52.4 ± 10.4	11.7 ± 1.1	7.1 ± 1.1
Vitamin D deprived (n = 10)	1.9 ± 0.6*	56.2 ± 23.9	11.2 ± 0.7	6.7 ± 1.7

*$p < 0.01$.
25D = 25-hydroxycholecalciferol; 1,25D = 1,25-dihydroxycholecalciferol; Ca = Calcium; P = Phosphorus.

Table 2 Implant push-in values in the control group and the vitamin D-insufficient group

	Control group	Vitamin D-insufficient group
DAE Ti6Al4V implants	24.99 ± 7.92 N (n = 7)	15.94 ± 8.20 N* (n = 7)
DCD Ti6Al4V implants	37.48 ± 17.58 N (n = 7)	15.63 ± 3.96 N** (n = 7)

*$p < 0.05$ against controls; **$p < 0.01$ against controls.

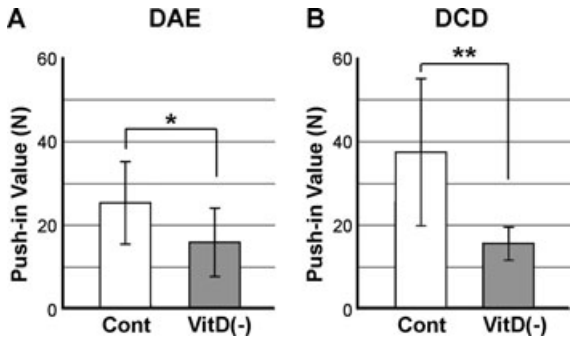

Figure 2 (A) Implant push-in values of the control (Cont) and vitamin D-insufficient (Vit D(–)) groups for DAE implants (n = 7 in each group) $^{*}p < 0.05$; (B) Implant push-in values of DCD implants (n = 7 in each group) $^{**}p < 0.01$.

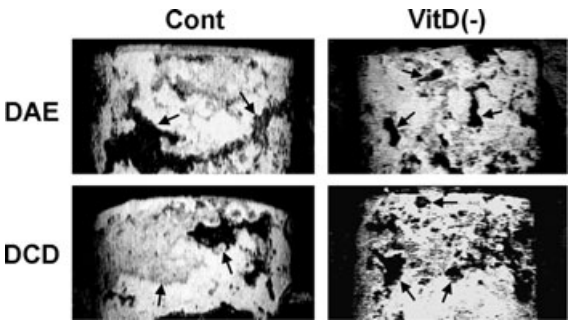

Figure 4 Back-scattered SEM images of DAE and DCD implants recovered after push-in test. The mineralized tissue (arrows) appeared to remain in large areas on the implant surface in the control group (Cont), whereas the remnant mineralized tissue in the vitamin D-insufficient group (Vit D(–)) appeared to be fragmented.

SEM analysis of the implant surface after push-in test

SEM analysis of the implant surface was performed after push-in testing. In the control group, the DAE and DCD implant surfaces show a fracture line between the interface of the new bone and old bone formation. It appeared that calcified tissue tended to remain attached to the DCD implant surface, although quantitative evaluation was not made (data not shown). In the vitamin D-insufficient group, the DAE and DCD implant surface tended to show fracture between the implant and calcified tissue resulting in exposed implant surface. SEM evaluation further showed that the implant was denuded between the implant body and the cortical bone at the transcortical bone area. The backscatter image of this region revealed a thin layer of remnant tissues that appeared to contain mineral components. The backscatter images of the remnant calcified tissues in the control group were spread over large areas on the implant, whereas those of the vitamin D-insufficient group appeared to be interrupted, resulting in a cluster of small tissue remnants (Fig 4).

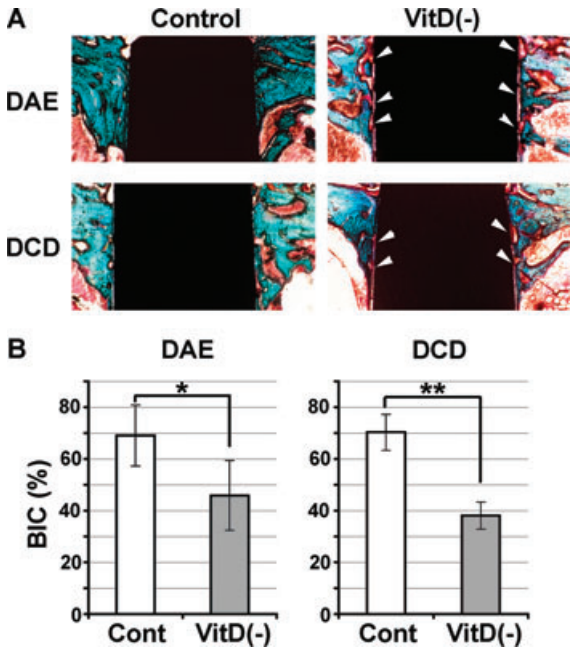

Figure 3 (A) Nondecalcified histology of DAE and DCD implants at the transcortical bone region. The control group showed close bone-to-implant adhesion, whereas the vitamin D-insufficient group (VitD(–)) showed soft tissue interface (arrowheads). (B) Bone-to-implant contact ratio (BIC) of DAE and DCD implants in control and Vit D(–) groups (n = 4 in each group). $^{*}p < 0.05$; $^{**}p < 0.01$.

Discussion

This study reports the unexpectedly profound negative effect of vitamin D insufficiency on bone and implant integration. The major source for vitamin D in humans is sunlight exposure to the skin. As the modern lifestyle has migrated away from agricultural pursuits to more urban and sunlight-deprived environments, ultraviolet B radiation (UVB)-driven cutaneous vitamin D synthesis has plummeted. In so doing, prevalence rates for vitamin D insufficiency in European,[13] North American,[14] and Australian[15] populations, especially in those countries where dietary vitamin D supplementation is not routinely practiced, now approach alarmingly high levels during the winter months. Therefore, our patient population may possibly experience a certain level of vitamin D insufficiency.

Vitamin D from skin and diet is metabolized in the liver to 25D, whose serum level is currently used to assess a patient's vitamin D status. It is generally accepted that a patient with less than 20 ng/ml of 25D is considered vitamin D insufficient.[16] The control rat group in this study showed the serum 25D at 19.1 ± 9.7 ng/ml, which was decreased in the experimental group to 1.9 ± 0.6 ng/ml (Table 1). Therefore, our experimental group may represent severely insufficient vitamin D.

In the kidney, 25D is further activated by the enzyme, 25D-1α-hydroxylase (CYP27B1) and becomes the active form, 1,25D. Without 1,25D, dietary calcium absorption is decreased to the 10% to 15% level at the intestine.[17] Serum calcium homeostasis is achieved by the oversecretion of parathyroid hormone or secondary hyperparathyroidism. Our vitamin D-insufficient

rat group did not develop hypocalcaemia (Table 1), which may be due to the induced secondary hyperparathyroidism.

Deficiency of calcium and vitamin D results in malformation of skeletal tissues and bone remodeling,[18–20] causing rickets in children and osteomalacia in adults. The increased parathyroid hormone also induces osteoclastogenesis and increases bone resorption.[21] A number of clinical reports further indicate the high prevalence of vitamin D insufficiency among elderly patients with bone fracture.[22,23] Vitamin D supplementation has been investigated for prevention of bone fracture. A meta-analysis of such clinical studies indicated that 700 to 800 IU of vitamin D supplementation per day reduced the relative risk of nonvertebral fracture by 23%.[16] We have postulated, therefore, that vitamin D insufficiency could similarly decrease bone remodeling activities around dental implants and potentially prolong the healing time for osseointegration to take place. In our study, vitamin D insufficiency appeared to cause a negative effect on the host response to the titanium implants as depicted by biomechanical (Fig 2) and histomorphometric (Fig 3) assessments.

It has been well established that roughened surface topography at submicron levels, such as a DAE-treated implant, positively induces osseointegration, which was replicated in the rat model.[11] In this rat model, we previously investigated the systemic effect of ovariectomy on implant osseointegration.[12] The ovariectomy group has shown decreased osseointegration measured by the implant push-in test, bone histomorphometry, and expression of bone-related genes; however, the negative effect of ovariectomy was only limited to the early osseointegration establishment during the initial 2-week period of healing, and the functional bone-implant integration appeared to be established by week 4.[12] In this study, the negative effect of vitamin D insufficiency was demonstrated at week 2; however, the long-term effect of vitamin D remains unknown.

Recently, we developed a nano-scale surface modification on titanium implants through discrete crystalline deposition (DCD) of hydroxyapatite nanoparticles,[24] which has been shown to increase bone-to-implant bonding.[24,25] The outcome of the implant push-in test in the control group (Fig 2) was consistent with previous data. In the vitamin D-insufficient group, the significant loss of osseointegration was experienced in both DAE and DCD implants. The decreased push-in values were equivalent to those of smooth surface (turned) implants.[11] Therefore, the advantage of roughened surface topography at submicron as well as nano-scale levels may be regulated by biological mechanisms involving vitamin D.

The increased bone bonding to DCD implant may be facilitated, in part, by the increased nano-scale integration of cement line, which juxtaposes the implant surface.[24,25] Cement line or reversal line is a layer of collagen-poor but highly mineralized tissue[26] deposited directly on the bone surfaces resorbed by osteoclasts during bone remodeling. In histological specimens, the cement line appears as narrow seams less than 5 μm wide following the outline of osteoclastic lacunae. Paget's disease is often associated with prolific cement lines with abnormal appearances,[27] whereas rickets decreases or even eliminates cement lines.[28] Boyce et al reported that bone biopsy specimens from vitamin D deficiency-related osteomalacia patients were associated with thicker osteoid seams adjacent to cement line than the corresponding lesion of aluminum-induced osteomalacia patients.[29] The implant-associated cement line has been shown to contain osteopontin, osteonectin, osteocalcin, and proteoglycans/glycosaminoglycan.[30,31] Because vitamin D increases the osteoblastic expression of osteopontin and osteocalcin[32] as well as chondroitin sulfate glycosaminoglycans,[33] the decreased osseointegration in the vitamin D-insufficient rats may in part be due to the decreased synthesis of these cement line molecular components. Thus, the establishment of osseointegration during early dates may require vitamin D-dependent regulatory mechanisms.

Conclusion

To our knowledge, this is the first report on the effect of vitamin D insufficiency on implant osseointegration. Our data indicate vitamin D insufficiency significantly impaired the establishment of Ti6Al4V implant osseointegration in vivo, an unexpectedly profound effect. The outcome of Ti-based endosseous implants may be confounded by the increasing prevalence of vitamin D insufficiency in our patient population.

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References

1. Siqueira JT, Cavalher-Machado SC, Arana-Chavez VE, et al: Bone formation around titanium implants in the rat tibia: role of insulin. Implant Dent 2003;12:242-251
2. Margonar R, Sakakura CE, Holzhausen M, et al: The influence of diabetes mellitus and insulin therapy on biomechanical retention around dental implants: a study in rabbits. Implant Dent 2003;12:333-339
3. Gerritsen M, Lutterman JA, Jansen JA: Wound healing around bone-anchored percutaneous devices in experimental diabetes mellitus. J Biomed Mater Res 2000;53:702-709
4. McCracken MS, Aponte-Wesson R, Chavali R, et al: Bone associated with implants in diabetic and insulin-treated rats. Clin Oral Implants Res 2006;17:495-500
5. Calvo MS, Whiting SJ: Prevalence of vitamin D insufficiency in Canada and the United States: importance to health status and efficacy of current food fortification and dietary supplement use. Nutr Rev 2003;61:107-113
6. Adams JS, Liu PT, Chun R, et al: Vitamin D in defense of the human immune response. Ann NY Acad Sci 2007;1117:94-105
7. Holick MF: Vitamin D deficiency. N Engl J Med 2007;357:266-281
8. Aaron JE, Gallagher JC, Nordin BE: Seasonal variation of histological osteomalacia in femoral-neck fractures. Lancet 1974;2:84-85
9. Chalmers J, Conacher WD, Gardner DL, et al: Osteomalacia–a common disease in elderly women. J Bone Joint Surg Br 1967;49:403-423

10. Morris HA, Morrison GW, Burr M, et al: Vitamin D and femoral neck fractures in elderly South Australian women. Med J Aust 1984;140:519-521
11. Ogawa T, Ozawa S, Shih JH, et al: Biomechanical evaluation of osseous implants having different surface topographies in rats. J Dent Res 2000;79:1857-1863
12. Ozawa S, Ogawa T, Iida K, et al: Ovariectomy hinders the early stage of bone-implant integration: histomorphometric, biomechanical, and molecular analyses. Bone 2002;30:137-143
13. Erkal MZ, Wilde J, Bilgin Y, et al: High prevalence of vitamin D deficiency, secondary hyperparathyroidism and generalized bone pain in Turkish immigrants in Germany: identification of risk factors. Osteoporos Int 2006;17:1133-1140
14. Schwalfenberg G: Not enough vitamin D: health consequences for Canadians. Can Fam Physician 2007;53:841-854
15. Van Der Mei IA, Ponsonby AL, Engelsen O, et al: The high prevalence of vitamin D insufficiency across Australian populations is only partly explained by season and latitude. Environ Health Perspect 2007;115:1132-1139
16. Bischoff-Ferrari HA, Giovannucci E, Willett WC, et al: Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr 2006;84:18-28
17. DeLuca HF: Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr 2004;80:1689S-1696S
18. Clemens TL, Tang H, Maeda S, et al: Analysis of osteocalcin expression in transgenic mice reveals a species difference in vitamin D regulation of mouse and human osteocalcin genes. J Bone Miner Res 1997;12:1570-1576
19. Yamamoto H, Shevde NK, Warrier A, et al: 2-Methylene-19-nor-(20S)-1,25-dihydroxyvitamin D3 potently stimulates gene-specific DNA binding of the vitamin D receptor in osteoblasts. J Biol Chem 2003;278:31756-31765
20. Atkins GJ, Anderson PH, Findlay DM, et al: Metabolism of vitamin D3 in human osteoblasts: evidence for autocrine and paracrine activities of 1 alpha,25-dihydroxyvitamin D3. Bone 2007;40:1517-1528
21. Holick MF: Resurrection of vitamin D deficiency and rickets. J Clin Invest 2006;116:2062-2072
22. Dixon T, Mitchell P, Beringer T, et al: An overview of the prevalence of 25-hydroxy-vitamin D inadequacy amongst elderly patients with or without fragility fracture in the United Kingdom. Curr Med Res Opin 2006;22:405-415
23. Sakuma M, Endo N, Oinuma T, et al: Vitamin D and intact PTH status in patients with hip fracture. Osteoporos Int 2006;17:1608-1614
24. Nishimura I, Huang Y, Butz F, et al: Discrete deposition of hydroxyapatite nanoparticles on a titanium implant with predisposing substrate microtopography accelerated osseointegration. Nanotechnology 2007;18:245101, 9
25. Mendes VC, Moineddin R, Davies JE: The effect of discrete calcium phosphate nanocrystals on bone-bonding to titanium surfaces. Biomaterials 2007;28:4748-4755
26. Skedros JG, Holmes JL, Vajda EG, et al: Cement lines of secondary osteons in human bone are not mineral-deficient: new data in a historical perspective. Anat Rec A Discov Mol Cell Evol Biol 2005;286:781-803
27. Rosenberg AE: Skeletal System and Soft Tissue Tumors. Philadelphia, PA, Saunders, 1994.
28. Boyde A, Maconnachie E, Reid SA, et al: Scanning electron microscopy in bone pathology: review of methods, potential and applications. Scan Electron Microsc 1986;4:1537-1554
29. Boyce BF, Byars J, McWilliams S, et al: Histological and electron microprobe studies of mineralisation in aluminium-related osteomalacia. J Clin Pathol 1992;45:502-508
30. Nakamura H, Shim J, Butz F, et al: Glycosaminoglycan degradation reduces mineralized tissue-titanium interfacial strength. J Biomed Mater Res A 2006;77:478-486
31. Rammelt S, Corbeil D, Manthey S, et al: Immunohistochemical in situ characterization of orthopedic implants on polymethyl metacrylate embedded cutting and grinding sections. J Biomed Mater Res A 2007;83:313-322
32. Peleg S, Uskokovic M, Ahene A, et al: Cellular and molecular events associated with the bone-protecting activity of the noncalcemic vitamin D analog Ro-26–9228 in osteopenic rats. Endocrinology 2002;143:1625-1636
33. Slater M, Patava J, Mason RS: Role of chondroitin sulfate glycosaminoglycans in mineralizing osteoblast-like cells: effects of hormonal manipulation. J Bone Miner Res 1994;9:161-169