CLINICAL STUDY

Plasma 1,25(OH)₂D levels decrease in postmenopausal women with hypovitaminosis D

Lars Rejnmark¹, Peter Vestergaard¹, Lene Heickendorff² and Leif Mosekilde¹Departments of ¹Endocrinology and Metabolism C and ²Clinical Biochemistry, Aarhus University Hospital, Aarhus Sygehus, Tage-Hansens Gade 2, DK-8000 Aarhus, Denmark

(Correspondence should be addressed to L Rejnmark; Email: rejnmark@post6.tele.dk)

Abstract

Objective: Although calcitriol (1,25(OH)₂D) is considered the biologically active vitamin D metabolite, several studies have shown that calcidiol (25OHD) is the vitamin D metabolite that is most closely linked to parathyroid function and indices of calcium homeostasis. Moreover, low levels of 25OHD have been associated with increased risk of different diseases including cancer, diabetes, and myopathy.

Design: Cross-sectional study.

Methods: We studied relations between plasma concentrations of 25OHD, 1,25(OH)₂D, and parathyroid hormone (PTH) in fasting plasma samples from 315 healthy postmenopausal women randomly selected from the local background population.

Results: P-1,25(OH)₂D levels varied in a concentration-dependent manner with P-25OHD levels ($P < 0.001$). Thus, P-1,25(OH)₂D levels were the lowest in women with vitamin D insufficiency, i.e., P-1,25(OH)₂D levels were reduced by approximately one-third in subjects with P-25OHD levels below 25 nmol/l compared with levels above 80 nmol/l ($P < 0.01$). The association was most pronounced at P-25OHD concentrations below 80 nmol/l, whereas no major increase in P-1,25(OH)₂D was observed at P-25OHD concentrations above 80 nmol/l. In multiple regression analysis, PTH was a minor although significant predictor of P-1,25(OH)₂D levels.

Conclusions: In normal postmenopausal women, the conversion of 25OHD to active vitamin D depends on the substrate concentration. Our data support that vitamin D insufficiency should be considered at P-25OHD levels below 80 nmol/l.

European Journal of Endocrinology 158 571–576

Introduction

Vitamin D is either absorbed from the diet or synthesized in the skin in response to ultra-violet type B rays (UVB) exposure. Subsequently, vitamin D is hydroxylated to 25-hydroxyvitamin D (25OHD) in the liver. Often, 25OHD is considered biologically inert, as it needs to be activated to calcitriol (1,25(OH)₂D) by a specific 1 α -hydroxylase before it activates the vitamin D receptor (1). Parathyroid hormone (PTH) and 1,25(OH)₂D are the principal regulators of calcium homeostasis and both hormones affect the synthesis of each other. Thus, PTH stimulates the renal 1 α -hydroxylase and 1,25(OH)₂D downregulates the synthesis of PTH (2). Circulating 1,25(OH)₂D is produced in the proximal tubuli of the kidney by a renal 1 α -hydroxylase. In addition to PTH, the 1 α -hydroxylase is activated by hypophosphatemia and hypocalcemia, whereas it is inhibited by its own product (1,25(OH)₂D). Moreover, kidney diseases is a well-recognized cause of decreased 1,25(OH)₂D levels.

Plasma 25OHD (P-25OHD) is considered to mirror the individual 'vitamin D status' since it reflects the sum of vitamin D absorbed from the intestine and produced

in the skin. Although 25OHD is considered biologically inactive, a large amount of studies have shown that P-25OHD is an important determinant of several biological functions including bone mineralization and muscle function (2, 3). In addition, circulating levels of PTH are more closely inversely related to P-25OHD than P-1,25(OH)₂D (4). Finally, an impaired vitamin D status has been associated with an increased risk of cancer and several chronic disorders, including diabetes, multiple sclerosis, periodontal disease, and cardiovascular diseases (2, 5). Recently, it has been shown that the biological effects associated with circulating 25OHD may be explained by the conversion of 25OHD to 1,25(OH)₂D in many different tissues, including parathyroid glands, malignant cells, immune competent cells, smooth muscle cells, and pancreatic β -cells (3).

Despite the fact that 25OHD is a precursor for 1,25(OH)₂D, and the finding that impaired vitamin D status is very common in the general population, there is a paucity of data on relations between plasma levels of 25OHD and 1,25(OH)₂D in normal individuals. It has often been stated that low 25OHD levels cause a compensatory secondary hyperparathyroidism (SHPT)

that increases the renal conversion of 25OHD to 1,25(OH)₂D and thereby maintains normal or slightly increased plasma levels of 1,25(OH)₂D until the vitamin D deficiency is severe enough (frank osteomalacia) to limit the availability of 25OHD and thereby reduce 1,25(OH)₂D production (6). On the other hand, other investigators have reported a positive association between plasma levels of 25OHD and 1,25(OH)₂D (7–11). In order to investigate further whether vitamin D status affects P-1,25(OH)₂D levels we studied the associations between these two vitamin D metabolites in a group of healthy postmenopausal women.

Subjects and methods

Through direct mailings, we invited women, randomly selected from the local background population to participate in clinical studies concerning the effects of diuretics on calcium homeostasis and bone metabolism (12–14). The data presented are based on the data from baseline measurements.

To be included, participants were required to be more than 12 months post menopause, and to be healthy as assessed by a standard questionnaire and a biochemical screening program. Subjects with illnesses or taking medications known to affect bone mineral metabolism (including hormone replacement therapy, diuretics, and pharmacological doses of calcium and vitamin D supplements) were excluded. The studies were carried out in accordance with the Declaration of Helsinki II and were approved by the regional Ethical Committee and the Danish National Board of Health. Each individual gave verbal and written informed consent prior to the study.

Blood samples were drawn between 0700 and 0930 h after an overnight fast. Plasma levels of calcium, phosphate, and creatinine were determined by standard laboratory methods. Total plasma calcium was corrected for individual variations in albumin according to the formula: adjusted plasma calcium (mmol/l) = plasma calcium_{total} (mmol/l) – 0.00086 × (650 – plasma albumin (μmol/l)). Samples for analysis of calcitropic hormones were divided in aliquots and stored immediately at –80 °C until analysis.

Plasma intact PTH was measured by an IMMULITE automated analyser (Diagnostic Products Corporation, Los Angeles, CA, USA). The total coefficient of variation (CV) was <7%. Plasma 25-hydroxyvitamin D (25-OHD) was measured by an equilibrium RIA procedure (DiaSorin Inc., Stillwater, MN, USA). The inter- and intra-assay CV were 13 and 10% respectively. Plasma 1,25(OH)₂D was measured by a competitive radio-receptor assay (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The inter- and intra-assay CV were 10 and 8% respectively.

Statistical analysis

Differences between groups were compared by one-way ANOVA or the non-parametric Kruskal–Wallis test, as appropriate, after testing for normal distributions. When indicated, we performed logarithmic transformation of data. Bivariate as well as linear and non-linear regression analysis were used to assess the correlations between variables. Differences between groups were adjusted for using a general linear regression model. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS 14.0, Chicago, Illinois, USA) for Windows.

Results

Table 1 shows characteristics of the 315 included subjects. The women had a median age of 64 (range: 50–82) years. In the study group, 39% had vitamin D insufficiency as defined as P-25OHD <50 nmol/l and 9% had vitamin D deficiency, i.e., P-25OHD <25 nmol/l, whereas 22% had P-25OHD levels above 80 nmol/l (Table 1).

As shown in Table 2, P-1,25(OH)₂D levels varied with plasma concentrations of its precursor, 25OHD (P<0.001). P-1,25(OH)₂D levels were the lowest in women with vitamin D deficiency and the highest in women with P-25OHD levels above 80 nmol/l. This was not changed by the adjustment for differences between categories of vitamin D status; i.e., after an adjustment for age, body weight, and plasma creatinine levels, those with P-25OHD levels below 25 nmol/l had lower P-1,25(OH)₂D levels than subjects with P-25(OH)₂D levels above 25 nmol/l, and the between-group difference remained significant after further adjustment for plasma levels of PTH, calcium, and phosphate (Table 2).

In order to disclose a possible threshold level for further increase in P-1,25(OH)₂D with increasing P-25OHD, we plotted 1,25(OH)₂D concentrations across 10 nmol/l steps of P-25OHD concentrations (Fig. 1).

Table 1 Characteristics of included women.

	All subjects (n=315)
Age (years)	64 (60–70)
Body weight (kg)	66.7 (60.0–73.5)
BMI (kg/m ²)	25.3 (23.1–27.9)
Plasma	
Calcium (mmol/l)	2.43 (2.39–2.48)
Phosphate (mmol/l)	1.0 (1.0–1.2)
Creatinine (μmol/l)	78 (71–84)
PTH (pmol/l)	4.8 (3.7–6.2)
25OHD (nmol/l)	57 (40–78)
No. with p-25OHD <25 nmol/l (%)	27 (9%)
No. with p-25OHD <50 nmol/l (%)	124 (39%)
No. with p-25OHD <80 nmol/l (%)	246 (78%)
1,25(OH) ₂ D (pmol/l)	118 (97–147)

Median with interquartile (25–75%) range.

Table 2 Unadjusted and adjusted plasma 1,25(OH)₂D levels (pmol/l) according to vitamin D status (mean with 95% confidence interval).

	Unadjusted 1,25(OH) ₂ D (<i>P</i> <0.01 by ANOVA)	Adj. 1,25(OH) ₂ D ^a (<i>P</i> <0.01 by ANOVA)	Adj. 1,25(OH) ₂ D ^b (<i>P</i> <0.01 by ANOVA)
Plasma 25OHD levels			
Group 1. p-25OHD: <25 nmol/l	92 (83–102)*	96 (84–109)*	91 (78–103)*
Group 2. p-25OHD: 25–50 nmol/l	111 (104–118)*	112 (105–118)*	111 (105–117)*
Group 3. p-25OHD: 50–80 nmol/l	130 (123–136) [†]	128 (122–134) [†]	129 (124–135)*
Group 4. p-25OHD >80 nmol/l	137 (118–126) [†]	137 (130–145) [†]	139 (131–146)*

Post hoc tests: *significantly different (*P*<0.05) from the other three groups, [†]significantly different (*P*<0.05) from groups 1 and 2.

^aAdjusted for differences in age, body mass index, and plasma creatinine levels.

^bAdjusted for differences in age, body mass index, and plasma levels of PTH, calcium, phosphate, and creatinine.

P-1,25(OH)₂D levels increased gradually until P-25OHD reached ~70–80 nmol/l with no further increases at concentrations above 80 nmol/l.

A bivariate analysis showed a significant correlation (*r*=0.40, *P*<0.001) between P-1,25(OH)₂D and P-25OHD levels. As expected, PTH and 25OHD levels also correlated significantly (*r*=0.35; *P*<0.001) with increasing PTH levels at P-25OHD <50 nmol/l. No correlation was found between P-PTH and P-1,25(OH)₂D (*r*=0.08, *P*=0.14). However, in a multiple regression analysis including indices known to affect P-1,25(OH)₂D levels, PTH levels did influence P-1,25(OH)₂D (*r*_p=0.145, *P*=0.01), although the major determinant was P-25OHD levels (*r*_p=0.379, *P*<0.01). The results of the multiple regression analysis are shown in Table 3 (model summary: *r*=0.542; *P*<0.001). Finally, we studied the relations between P-25OHD and P-1,25(OH)₂D stratified by PTH levels. In our studied subjects, the median P-PTH concentration was 4.8 pmol/l. As shown in Fig. 2, P-1,25(OH)₂D decreased significantly with decreasing P-25OHD levels and in accordance with the results from

the multiple regression analysis, P-PTH levels only affected the relationship to a minor degree.

Discussion

In a group of healthy postmenopausal women, we found vitamin D status, as assessed by the measurements of plasma 25OHD levels, to be a major determinant of plasma levels of 1,25(OH)₂D. Apparently, at P-25OHD levels above 80 nmol/l, P-1,25(OH)₂D levels do not increase further, whereas at levels below 80 nmol/l, P-1,25(OH)₂D concentrations decrease with decreasing 25OHD levels.

In previous studies, conflicting results have been reported on the effect of low P-25OHD levels on P-1,25(OH)₂D levels. Some studies found no relationship between plasma levels of 25OHD and 1,25(OH)₂D (15–17), while other studies have shown either a positive (7–11, 18) or an inverse association (19–21). Similar, discrepant results have been reported on the

Figure 1 Boxplot summarizing plasma 1,25(OH)₂D levels at different categories of plasma 25OHD levels. Each box shows the median, quartiles, and extreme values within a category.

Table 3 Multiple regression analysis on determinants of plasma 1,25(OH)₂D levels.

Partial correlation coefficients (<i>r_P</i>)		
Age (years)	0.094	(<i>P</i> =0.10)
BMI (kg/m ²)	−0.182	(<i>P</i> <0.01)
Plasma		
Calcium (mmol/l)	−0.130	(<i>P</i> =0.02)
Phosphate (mmol/l)	−0.186	(<i>P</i> <0.01)
Creatinine (μmol/l)	−0.212	(<i>P</i> <0.01)
PTH (pmol/l)	0.145	(<i>P</i> =0.01)
25OHD (nmol/l)	0.379	(<i>P</i> <0.01)

Overall, model summary: *r*=0.542; *P*<0.001.

effect of treatment with ergo- or cholecalciferol on P-1,25(OH)₂D levels. An increase in 1,25(OH)₂D levels has been reported in patients with vitamin D insufficiency (9, 19, 22), primary hyperparathyroidism (23), and severe osteomalacia (24), whereas no effect has been found in other studies including healthy subjects (25–27) or patients with SHPT due to hypovitaminosis D (28, 29). Moreover, a biphasic response has recently been noted in an Australian study with a positive correlation between plasma levels of 25OHD and 1,25(OH)₂D if baseline 25OHD is in the normal range, but a negative correlation if baseline 25OHD is subnormal (30). The size of our study is larger than the most previous studies and supports the notion of a positive relationship between plasma levels of 25OHD and 1,25(OH)₂D. In some prior studies, negative correlations between plasma levels of 25OHD and 1,25(OH)₂D have been justified by the inclusion of a large proportion of patients with severe vitamin D deficiency, who might have been exposed immediately before the evaluation to vitamin D supplements (31). However, an increase in 1,25(OH)₂D levels in response to the supplementation with vitamin D may merely be due to a positive association as demonstrated in our study than an inverse relationship.

p < 0.001 (ANOVA) in both PTH groups.
Figure 2 Plasma 1,25(OH)₂D concentrations by groups of plasma 25OHD levels stratified by PTH groups (mean with 95% confidence intervals).

Previously, it has been suggested that variations within the physiological range of P-25OHD concentrations are of minor importance for the conversion of 25OHD to 1,25(OH)₂D, as plasma levels are more than 100 times higher for 25OHD than 1,25(OH)₂D. Our data do not support this hypothesis. Most likely, the 1 α -hydroxylase enzyme is set to handle the conversion of 25OHD at the concentrations normally present in plasma, and apparently it has a set point at which it becomes dependent on the concentration of the substrate, i.e., at a P-25OHD concentration of ~80 nmol/l. Nevertheless, despite low P-25OHD concentrations, 1,25(OH)₂D is still synthesized, although the plasma concentration is reduced by approximately one-third compared with the subjects with P-25OHD levels above 80 nmol/l.

During recent years, increased attention has been paid to the importance of a sufficient vitamin D status and several investigators have sought to determine the plasma levels of 25OHD that is sufficient to meet physiological needs (5). Traditionally, the hallmark for an insufficient vitamin D status is SHPT, and several studies have shown that P-25OHD levels below 50 nmol/l cause increased PTH levels. Accordingly, it has been suggested that vitamin D insufficiency should be defined as P-25OHD concentration below 50 nmol/l (32). However, recent research using other biomarkers than PTH, including biochemical markers of bone turnover, lower extremity function, BMD, risk of falls and fractures, periodontal disease, intestinal calcium absorption, and insulin sensitivity, has indicated that P-25OHD concentrations above 80 nmol/l are needed to ensure an optimal vitamin D status (3, 33). Interestingly, our results suggest that vitamin D insufficiency defined as P-25OHD levels <80 nmol/l is also associated with impaired plasma levels of 1,25(OH)₂D.

Circulating 1,25(OH)₂D is synthesized in the kidney by the renal 1 α -hydroxylase. Our study, therefore, indicates that the amount of 1,25(OH)₂D produced by the renal 1 α -hydroxylase in postmenopausal women depends on the plasma concentrations of its precursor, 25OHD. However, in addition to the renal synthesis an increasing body of evidence suggests that 1,25(OH)₂D also is synthesized in a variety of non-renal cells by the action of local 1 α -hydroxylase enzymes (34). The non-renal production of 1,25(OH)₂D may be of major importance to cell function, as 1,25(OH)₂D is known to enhance cell differentiation and inhibit cell proliferation. The regulation of the non-renal 1 α -hydroxylase enzyme is largely unknown. Nevertheless, in vascular smooth muscle cells the local 1-hydroxylase system has been shown to be upregulated by PTH, indicating a hormonal regulation of the local 1-hydroxylase that is similar to the regulation of the renal enzyme (27). Apart from certain diseases (like sarcoidosis and other granulomatose diseases), it is usually believed that this extra-renal produced 1,25(OH)₂D is used locally and

does not enter the systemic circulation. Thus, in healthy subjects the extra-renal 1,25(OH)₂D is unlikely to contribute to mineral homeostasis. However, in active sarcoidoses, the amount of 1,25(OH)₂D released to the circulation depends on dietary vitamin D intake and sun exposure, indicating that the production of 1,25(OH)₂D is substrate regulated (35). Further studies should focus on whether this is the case in other tissues. If so, it may help explaining that an impaired vitamin D status is associated with an increased risk of cancer and different chronic disorders, including diabetes and cardiovascular diseases.

Previous studies have shown that the elimination half-time of 25OHD correlates inversely with P-1,25(OH)₂D concentrations (36). The reduced P-1,25(OH)₂D concentrations in subjects with low P-25OHD levels may therefore help preserve P-25OHD levels, and thereby maintain the amount of 25OHD available for conversion in peripheral tissues.

It is well known that PTH has a stimulatory effect on the renal 1 α -hydroxylase and that treatment with 1 α -hydroxylated vitamin D analogs is needed to normalize plasma calcium levels in patients with hypoparathyroidism (37). In our study, P-PTH did not correlate with P-1,25(OH)₂D levels in the bivariate analysis. However, in our multiple regression analysis, PTH did contribute significantly to the model. This may implicate that PTH is necessary for the synthesis of 1,25(OH)₂D, but in the setting of vitamin D insufficiency the effects of low P-25OHD levels are not compensated for by increased PTH levels.

Limitations to study

In our study, we did not measure calcium intake. In the setting of vitamin D insufficiency, daily intake of calcium has been shown to be of importance to P-PTH levels (38) and may as well influence P-1,25(OH)₂D levels (39). Thus, further studies should focus on whether the positive correlation between plasma levels of 25OHD and 1,25(OH)₂D is influenced by calcium intake. Moreover, it should be studied whether age or polymorphisms in the 25-hydroxyvitamin D₃-1 α -hydroxylase gene (CYP27B1) influence the relationship (27, 40). In addition, the cross-sectional design of our study does not allow for causal conclusions, and therefore the relationship between plasma levels of 25OHD and 1,25(OH)₂D should be evaluated further in experimental studies.

Conclusion

Our data suggest that the conversion of 25OHD to 1,25(OH)₂D depends on the concentration of the substrate. It seems that the positive correlation is most pronounced at P-25OHD concentrations below 80 nmol/l. This supports the findings by other

investigators of an impaired vitamin D status at P-25OHD concentrations below 80 nmol/l. As the conversion of 25OHD to active vitamin D occurs in many tissues, a decreased conversion due to low substrate concentrations may have generalized biological consequences.

Acknowledgements

The studies included in this analysis were funded by Institute of Clinical Medicine and Centre for Clinical Pharmacology, University of Aarhus, and The LEO Pharma Research Foundation. None of the authors have any financial or other relationships which might lead to a conflict of interest.

References

- 1 Valdivielso JM & Fernandez E. Vitamin D receptor polymorphisms and diseases. *Clinica Chimica Acta* 2006 **371** 1–12.
- 2 Holick MF. The vitamin D epidemic and its health consequences. *Journal of Nutrition* 2005 **135** 2739S–2748S.
- 3 Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T & Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *American Journal of Clinical Nutrition* 2006 **84** 18–28.
- 4 Need AG, Horowitz M, Morris HA & Nordin BC. Vitamin D status: effects on parathyroid hormone and 1, 25-dihydroxyvitamin D in postmenopausal women. *American Journal of Clinical Nutrition* 2000 **71** 1577–1581.
- 5 Mosekilde L. Vitamin D and the elderly. *Clinical Endocrinology* 2005 **62** 265–281.
- 6 Holick MF. Resurrection of vitamin D deficiency and rickets. *Journal of Clinical Investigation* 2006 **116** 2062–2072.
- 7 Sahota O, Mundy MK, San P, Godber IM, Lawson N & Hosking DJ. The relationship between vitamin D and parathyroid hormone: calcium homeostasis, bone turnover, and bone mineral density in postmenopausal women with established osteoporosis. *Bone* 2004 **35** 312–319.
- 8 Gloth FM, III, Gundberg CM, Hollis BW, Haddad JG, Jr & Tobin JD. Vitamin D deficiency in homebound elderly persons. *Journal of the American Medical Association* 1995 **274** 1683–1686.
- 9 Lips P, Wiersinga A, van Ginkel FC, Jongen MJ, Netelenbos JC, Hackeng WH, Delmas PD & van der Vijgh WJ. The effect of vitamin D supplementation on vitamin D status and parathyroid function in elderly subjects. *Journal of Clinical Endocrinology and Metabolism* 1988 **67** 644–650.
- 10 Vivek A, Rajiv B, Godbole M & Ambrish M. Vitamin D status and its relationship with bone mineral density in healthy Asian Indians. *Osteoporosis International* 2004 **V15** 56–61.
- 11 Bettica P, Bevilacqua M, Vago T & Norbiato G. High prevalence of hypovitaminosis D among free-living postmenopausal women referred to an osteoporosis outpatient clinic in Northern Italy for initial screening. *Osteoporosis International* 1999 **V9** 226–229.
- 12 Rejnmark L, Vestergaard P, Pedersen AR, Heickendorff L, Andreasen F & Mosekilde L. Dose–effect relations of loop- and thiazide-diuretics on calcium homeostasis: a randomized, double-blinded Latin-square multiple cross-over study in postmenopausal osteopenic women. *European Journal of Clinical Investigation* 2003 **33** 41–50.
- 13 Rejnmark L, Vestergaard P, Heickendorff L, Andreasen F & Mosekilde L. Effects of long-term treatment with loop diuretics on bone mineral density, calcitropic hormones and bone turnover. *Journal of Internal Medicine* 2005 **257** 176–184.

- 14 Rejnmark L, Vestergaard P, Heickendorff L, Andreasen F & Mosekilde L. Effects of thiazide- and loop-diuretics, alone or in combination, on calcitropic hormones and biochemical bone markers: a randomized controlled study. *Journal of Internal Medicine* 2001 **250** 144–153.
- 15 Ho SC, Mac DD, Chan C, Fan YK, Chan SS & Swaminathan R. Determinants of serum 1,25-dihydroxyvitamin D concentration in healthy premenopausal subjects. *Clinica Chimica Acta* 1994 **230** 21–33.
- 16 Tjellesen L & Christiansen C. Vitamin D metabolites in normal subjects during one year. A longitudinal study. *Scandinavian Journal of Clinical and Laboratory Investigation* 1983 **43** 85–89.
- 17 Vieth R, Ladak Y & Wallish PG. Age-related changes in the 25-hydroxyvitamin D versus parathyroid hormone relationship suggest a different reason why older adults require more vitamin D. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 185–191.
- 18 Egsmose C, Lund B, McNair P, Lund B, Storm T & Sorensen OH. Low serum levels of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D in institutionalized old people: influence of solar exposure and vitamin D supplementation. *Age and Ageing* 1987 **16** 35–40.
- 19 Bouillon RA, Auwerx JH, Lissens WD & Pelemans WK. Vitamin D status in the elderly: seasonal substrate deficiency causes 1,25-dihydroxycholecalciferol deficiency. *American Journal of Clinical Nutrition* 1987 **45** 755–763.
- 20 Kinyamu HK, Gallagher JC, Balhorn KE, Petranick KM & Rafferty KA. Serum vitamin D metabolites and calcium absorption in normal young and elderly free-living women and in women living in nursing homes. *American Journal of Clinical Nutrition* 1997 **65** 790–797.
- 21 Heaney RP. Ethnicity, bone status, and the calcium requirement. *Nutrition Research* 2002 **22** 153–178.
- 22 Ooms ME, Roos JC, Bezemer PD, van der Vijgh WJ, Bouter LM & Lips P. Prevention of bone loss by vitamin D supplementation in elderly women: a randomized double-blind trial. *Journal of Clinical Endocrinology and Metabolism* 1995 **80** 1052–1058.
- 23 LoCascio V, Adami S, Galvanini G, Ferrari M, Cominacini L & Tartarotti D. Substrate-product relation of 1-hydroxylase activity in primary hyperparathyroidism. *New England Journal of Medicine* 1985 **313** 1123–1125.
- 24 Thacher TD, Fischer PR, Isichei CO & Pettifor JM. Early response to vitamin D2 in children with calcium deficiency rickets. *Journal of Pediatrics* 2006 **149** 840–844.
- 25 Heaney RP, Barger-Lux MJ, Dowell MS, Chen TC & Holick MF. Calcium absorptive effects of vitamin D and its major metabolites. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 4111–4116.
- 26 Barger-Lux MJ, Heaney RP, Dowell S, Chen TC & Holick MF. Vitamin D and its major metabolites: serum levels after graded oral dosing in healthy men. *Osteoporosis International* 1998 **V8** 222–230.
- 27 Miller WL & Portale AA. Vitamin D 1(alpha)-hydroxylase. *Trends in Endocrinology and Metabolism* 2000 **11** 315–319.
- 28 Chapuy MC, Chapuy P & Meunier PJ. Calcium and vitamin D supplements: effects on calcium metabolism in elderly people. *American Journal of Clinical Nutrition* 1987 **46** 324–328.
- 29 Honkanen R, Alhava E, Parviainen M, Talasniemi S & Monkkonen R. The necessity and safety of calcium and vitamin D in the elderly. *Journal of the American Geriatrics Society* 1990 **38** 862–866.
- 30 Need AG, Horowitz M, Morris HA & Nordin BC. Vitamin D status: effects on parathyroid hormone and 1,25-dihydroxyvitamin D in postmenopausal women. *American Journal of Clinical Nutrition* 2000 **71** 1577–1581.
- 31 Papapoulos SE, Fraher LJ, Clemens TL, Gled J & O'Riordan JLH. Metabolites of vitamin D in human vitamin-D deficiency: effect of vitamin D3 or 1,25-dihydroxycholecalciferol. *Lancet* 1980 **316** 612–615.
- 32 Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. *Endocrine Reviews* 2001 **22** 477–501.
- 33 Hathcock JN, Shao A, Vieth R & Heaney R. Risk assessment for vitamin D. *American Journal of Clinical Nutrition* 2007 **85** 6–18.
- 34 Zehnder D, Bland R, Williams MC, McNinch RW, Howie AJ, Stewart PM & Hewison M. Extrarenal expression of 25-hydroxyvitamin d(3)-1 alpha-hydroxylase. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 888–894.
- 35 Conron M, Young C & Beynon HLC. Calcium metabolism in sarcoidosis and its clinical implications. *Rheumatology* 2000 **39** 707–713.
- 36 Davies M, Heys SE, Selby PL, Berry JL & Mawer EB. Increased catabolism of 25-hydroxyvitamin D in patients with partial gastrectomy and elevated 1,25-dihydroxyvitamin D levels. Implications for metabolic bone disease. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 209–212.
- 37 Marx SJ. Hyperparathyroid and hypoparathyroid disorders. *New England Journal of Medicine* 2000 **343** 1863–1875.
- 38 Steingrimsdottir L, Gunnarsson O, Indridason OS, Franzson L & Sigurdsson G. Relationship between serum parathyroid hormone levels, vitamin D sufficiency, and calcium intake. *Journal of the American Medical Association* 2005 **294** 2336–2341.
- 39 Anderson PH, O'Loughlin PD, May BK & Morris HA. Determinants of circulating 1,25-dihydroxyvitamin D3 levels: the role of renal synthesis and catabolism of vitamin D. *Journal of Steroid Biochemistry and Molecular Biology* 2004 **89–90** 111–113.
- 40 Armbricht HJ, Boltz MA & Hodam TL. PTH increases renal 25(OH)D3-1alpha-hydroxylase (CYP1alpha) mRNA but not renal 1,25(OH)2D3 production in adult rats. *American Journal of Physiology. Renal Physiology* 2003 **284** F1032–F1036.

Received 6 January 2008

Accepted 20 January 2008