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ORIGINAL ARTICLE

Effect of vitamin D supplementation on vitamin D status and bone turnover markers in young adults

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Objective: To assess the vitamin D status of healthy young people living in Northern Ireland and the effect of vitamin D supplementation on vitamin D status and bone turnover.
Design: Double-blinded randomised controlled intervention study.
Setting: University of Ulster, Coleraine, Northern Ireland.
Subjects: In total, 30 apparently healthy students (15 male and 15 female subjects), aged 18–27 years, were recruited from the university, with 27 completing the intervention.
Interventions: Subjects were randomly assigned, to receive either 15 μg (600 IU) vitamin D_3 and 1500 mg calcium/day (vitamin D group), or 1500 mg calcium/day (control group) for 8 weeks between January and March. Vitamin D status, bone turnover markers, serum calcium and parathyroid hormone concentrations were measured at baseline and post intervention.
Results: At baseline, vitamin D status was low in both the vitamin D group (47.9 (s.d. 16.0)) and the control group (55.5 (s.d. 18.6) nmol/l 25(OH)D). Post intervention vitamin D status was significantly higher in the vitamin D-treated group (86.5 (s.d. 24.5)) compared to the control group (48.3 (s.d. 16.8) nmol/l) ($P<0.0001$). There was no significant effect of supplementation on bone turnover markers or PTH concentrations.
Conclusions: This study suggests that young adults in Northern Ireland do not consume an adequate daily dietary intake of vitamin D to maintain plasma vitamin D concentrations in the wintertime. A daily supplement of 15 μg vitamin D_3 significantly increased vitamin D status in these individuals to levels of sufficiency. Achievement of an optimum vitamin D status among young adults may have future positive health implications.
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Introduction

Vitamin D plays a central role in bone mineralisation by regulating calcium and phosphate metabolism (Suda *et al*., 2003), such that vitamin D deficiency leads to hyperparathyroidism, increased bone turnover, rickets, osteoporosis and mild osteomalacia, and an increased risk of hip and other fractures (Dawson-Hughes *et al*., 1991; Hutchison and Bell, 1992; Martinez *et al*., 1994; Aguado *et al*., 2000; Cheng *et al*., 2003; Isaia *et al*., 2003). More recently, observational studies have also reported that vitamin D intake is inversely associated with the incidence of a number of conditions, including cancer and autoimmune disorders such as rheumatoid arthritis, multiple sclerosis and type I diabetes mellitus (Bostick *et al*., 1993; Hypponen *et al*., 2001; Merlino *et al*., 2004; Munger *et al*., 2004).

In humans, vitamin D is obtained predominantly from cutaneous synthesis through a process initiated via sunlight exposure, by UV irradiation on the skin (Webb *et al*., 1988). In countries around the equator, UV light and, therefore, the production of vitamin D, is constant throughout the year (Glerup *et al*., 2000). However, seasonal variation in UV intensity, as occurs both far North and South of the equator, results in marked variation in vitamin D status throughout the year, with highest concentrations found in late summer

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and markedly lower concentrations observed in late winter (McLaughlin *et al*., 1974; McKenna, 1992). This seasonal pattern is apparent in all European countries, but is particularly evident in northern European countries, including the UK and Ireland, which are located at 50–60°N (Ovesen *et al*., 2003; Zittermann, 2003). There is currently no dietary reference value (DRV) for vitamin D for adults aged 18–50 years, in the UK and Ireland, as it is assumed that there is sufficient cutaneous synthesis of vitamin D from sunlight exposure (SACN report, 2003). Moreover, it has generally been assumed that enough of the vitamin is synthesised in the body to meet daily requirements. However, vitamin D intakes are generally low (Lehtonen-Veromaa *et al*., 1999; Brot *et al*., 2001; Ginty *et al*., 2004; Moore *et al*., 2004) compared to the DRVs of 7–8.5 μg/day for young children and 10 μg/day for those over 65 years and pregnant and lactating women (SACN report, 2003), groups who are known to be at-risk of deficiency. A recent study has shown that Irish adults have very low vitamin D intakes, averaging around only 3.2 μg/day (128 IU/day) from food alone (Hill *et al*., 2004). This may be because there are only a limited number of food sources containing small amounts of vitamin D and include: oily fish, liver, egg yolks and a few fortified margarines and breakfast cereals. Therefore, during the winter period, when vitamin D synthesis is negligible, most people in northern Europe rely on the stores built up in the body during the previous summer. There is ongoing debate on the plasma 25(OH)D concentrations which are regarded as sufficient. Studies have used various biomarkers to define vitamin D sufficiency, such as changes in PTH concentrations and effects on bone metabolism (Lips *et al*., 1988; Bates *et al*., 2003). Plasma 25(OH)D concentrations of <25 (Parfitt, 1998; SACN report, 2003), 30 (Lips *et al*., 1988) and 50 (Lips, 2004) nmol/l have been suggested as cutoff levels for insufficiency, with the recommendation of <50 nmol/l being widely used in recent studies investigating vitamin D status.

It is perhaps not surprising that epidemiological studies have reported low vitamin D status in countries which have low UV exposure for much of the year (McKenna, 1992; Lips, 2001; Ovesen *et al*., 2003). The majority of these studies have been carried out on postmenopausal women and elderly people owing to the greater risk of osteoporosis in these individuals. More recent studies have also reported low vitamin D status in younger adults, adolescents and children living at higher latitudes (Ala-Houhala *et al*., 1984; Lamberg-Allardt *et al*., 1986; McKenna, 1992; Lehtonen-Veromaa *et al*., 1999; Fuleihan *et al*., 2001; Guillemant *et al*., 2001; Tangpricha *et al*., 2002; Ginty *et al*., 2004; Gordon *et al*., 2004). Given the role of vitamin D in bone mineralisation, vitamin D insufficiency is highly likely to have deleterious effects on bone mineral density (BMD) in this group, and thereby decreasing their ability to achieve their potential peak bone mass (Bonjour *et al*., 1994; Lehtonen-Veromaa *et al*., 2002a, b). In contrast, a high dietary vitamin D intake in adolescence has been reported to positively influence BMD in young adults (Neville *et al*., 2002). Optimisation of BMD at a young age through enhanced vitamin D status may reduce the risk of osteoporosis in such individuals in later life.

The current study aims to assess the vitamin D status of healthy young people living in Northern Ireland at a latitude of 55°N, in wintertime when vitamin D synthesis is negligible. It also aims to assess the effect of vitamin D supplementation on plasma 25-hydroxyvitamin D (25(OH)D) levels (the status marker for the vitamin) and on bone turnover markers, which are reported to be the most sensitive methods for monitoring acute changes in bone metabolism.

Subjects and methods

Design

This double-blinded randomised controlled intervention study was approved by the Research Ethics Committee of the University of Ulster and all participants gave written informed consent before the commencement of the study. By using data collected previously (Vieth *et al*., 2001) and allowing for a 10% drop-out rate, it was calculated that 30 individuals should be recruited to see a significant effect of supplementation (at $P<0.05$) on vitamin D status.

Subjects

In total, 30 apparently healthy individuals, 15 male and 15 female, aged 18–27 years, were recruited from among the student population within the University of Ulster. Subjects were randomly assigned to two groups, to receive either Calcichew® D_3 supplements, containing 15 μg (600 IU) vitamin D_3 and 1500 mg calcium/day (vitamin D group), or Calcichew®, containing 1500 mg calcium/day (control group) (Shire Pharmaceuticals, UK) for 8 weeks between January and March. A commercially available source of vitamin D alone was not obtainable at the time of the study, so supplements with added calcium at high levels were needed in order to achieve the desirable dose of vitamin D. Height and weight were measured at baseline and body mass index (BMI) was calculated. All subjects had a BMI of less than 30 kg/m^2, were nonsmokers, not on any special diets, not taking any dietary supplements, did not normally expose themselves to artificial sources of UV light, had not taken a sun holiday before the study and were not planning a holiday during the intervention period.

Dietary assessment

A 4-day food diary was completed by all subjects at baseline and daily calcium and vitamin D intakes were analysed using the dietary analysis program WISP (WISP for WINDOWS, version 3.0, Tinuviel Software, Warrington, UK).

Blood collection
Venous blood was collected following an overnight (12-h) fast, by a trained phlebotomist between 0830 and 1000 hours at baseline and post intervention. Blood was collected into a 9 ml serum separator tube and a 4 ml EDTA tube for analysis of bone turnover markers, vitamin D status and parathyroid hormone (PTH) concentration. A 4 ml serum tube was collected last, with the tourniquet off for measurement of calcium. All tubes were allowed to stand at room temperature before centrifugation at 2000 r.p.m. for 20 min. Samples were aliquoted and frozen at –20°C for a maximum of two months.

Calcium, PTH concentration and vitamin D status
Total serum calcium was assessed immediately following sample collection, at baseline and post intervention, using the Roche Diagnostics colorimetric assay on a Hitachi 912 analyser (Roche Diagnostics, UK). Intact plasma PTH concentrations were measured by an electrochemiluminescence immunoassay (ECLIA) on the Elecsys Module (Roche Diagnostics, UK).

Vitamin D status was assessed by quantitatively measuring plasma 25(OH)D using OCTEIA 25(OH)D enzyme immunoassay (ELISA) kit (Immunodiagnostic Systems Limited (IDS), UK), following the manufacturer's instructions. The IDS OCTEIA 25(OH)D kit is an ELISA in a microtitre strip format utilising 25(OH)D sheep polyclonal antibody coated on the strip to capture 25(OH)D in the sample. The enzyme activity of the captured 25(OH)D was detected with a tetramethylbenzidine (TMB) substrate at a wavelength of 450 nm (reference 650 nm). All samples were measured in duplicate and mean absorbance for each sample calculated. Percentage binding (B/Bo%) of each calibrator, control and sample was calculated as: mean absorbance divided by mean absorbance for '0' calibrator $\times 100$. A point-to-point smoothed spline calibration curve was plotted using GraphPad Prism 3 (GraphPad Software Inc., USA) on Microsoft Windows XP and the 25(OH)D concentration of each sample was calculated. The sensitivity of the OCTEIA 25(OH)D ELISA kit is 5 nmol/l. The interassay coefficient of variation (CV) was 3.88% and the intra-assay CV was 4.41%.

Bone turnover markers
Bone formation. Bone-specific alkaline phosphatase (BAP) was quantitatively measured by Metra BAP ELISA (Quidel, Oxford Biosystems, UK). Metra BAP is an immunoassay in a microtitre strip format utilising a monoclonal anti-BAP antibody coated on the strip to capture BAP in the sample. The enzyme activity of the BAP was detected with a *p*-nitrophenyl phosphate substrate at a wavelength of 405 nm. The sensitivity of this kit was 0.7 U/l, the interassay CV was 2.57% and the intra-assay CV was 2.71%.

Bone resorption. An ELISA for the quantification of degradation products of C-terminal telopeptides of type I collagen in human serum (Serum CrossLaps, Nordic Bioscience Diagnostics, Denmark) was used as a marker of bone resorption. The serum crosslaps immunoassay is a microtitre strip coated with streptavidin and uses a biotinylated antibody, a peroxidase-conjugated murine monoclonal antibody specific for serum crosslaps and a TMB substrate to measure enzyme activity at a wavelength of 450 nm (reference 650 nm). The sensitivity of the assay was 0.9 ng/ml, the interassay CV was 9.19% and the intra-assay CV was 2.93%

Statistical analysis
All analyses were performed using SPSS (SPSS Inc., version 11.0, Chicago) on Microsoft Windows XP. Data were normally distributed and are presented as means (standard deviations). Baseline characteristics and mean daily nutrient intakes reported by the control and vitamin D-treated groups, were compared by means of a two-tailed independent samples *t*-test. A one-way between groups analysis of covariance (ANCOVA) was conducted to compare the effect of treatment, with a *P*-value <0.05 considered significant in all analyses.

Results

Of the 30 individuals originally recruited to the study, one subject did not complete because of illness and two subjects failed to give a blood sample post intervention. Thus, the results of 27 subjects who provided complete data were included in the final analyses.

There were no statistically significant differences at baseline in anthropometric characteristics or nutritional intakes between the control group and the vitamin D-treated group (Table 1). The mean daily intakes of calcium were above the recommended nutrient intake (RNI) of 700 mg/day (Department of Health, 1991) and the main dietary sources of vitamin D, which contributed more than 10% of the dietary vitamin D were eggs, cheese, milk, margarine and cereals (data not shown).

Table 1 Baseline characteristics of subjects supplemented with either calcium (control group) or calcium and vitamin D (vitamin D group)

	Control group (males n = *8, females* n = *7)*	*Vitamin D group (males* n = *7, females* n = *5)*	P-*value**
Age (years)	20.6 (1.35)	21.6 (2.64)	0.222
Height (m)	1.72 (0.06)	1.73 (0.07)	0.675
Weight (kg)	68.0 (6.27)	74.6 (15.6)	0.190
BMI (kg/m^2)	22.9 (1.83)	24.8 (4.41)	0.154
Calcium intake (mg/day)	882.6 (427.3)	749.3 (179.6)	0.287
Vitamin D intake (μg/day)	2.39 (2.72)	1.62 (1.10)	0.367

All values are mean (s.d.).
**P*-values obtained from two-tailed independent samples *t*-test.
BMI = body mass index.

Table 2 Baseline and post intervention plasma concentrations of 25(OH)D, PTH and serum concentrations of calcium, bone-specific alkaline phosphatase (BAP) and serum crosslaps (CTx) in subjects supplemented with either calcium (control group) or calcium and vitamin D (vitamin D group) for 8 weeks

	Control group (males n = *8, females* n = *7)*		*Vitamin D group (males* n = *7, females* n = *5)*		P-*value**
	Baseline	*Post*	*Baseline*	*Post*	
Calcium (mmol/l)	2.37 (0.13)	2.63 (0.12)	2.34 (0.17)	2.65 (0.10)	0.578
25(OH)D (nmol/l)	55.5 (18.6)	48.3 (16.8)	47.9 (16.0)	86.5 (24.5)	0.000**
PTH (pg/ml)	28.6 (10.8)	25.1 (12.8)	29.2 (16.7)	30.6 (9.56)	0.230
Bone turnover markers					
BAP (U/l)	25.3 (8.04)	25.5 (7.13)	27.2 (8.51)	27.2 (8.97)	0.988
CTx (ng/ml)	0.63 (0.26)	0.53 (0.21)	0.59 (0.21)	0.50 (0.29)	0.959

All values are mean (s.d.).
**P*-values obtained from one-way analysis of covariance (ANCOVA), ***P*-value < 0.001.
PTH = parathyroid hormone. BAP = bone-specific alkaline phosphatase. CTx = serum telopeptide.

Laboratory results for baseline and post supplementation measurements for both groups are presented in Table 2. At baseline, one subject was vitamin D deficient according to the cutoff level of <25 nmol/l (Parfitt *et al*., 1982; Department of Health, 1998), whereas no subjects were deficient post supplementation. A level of 30 nmol/l has also been suggested as the cutoff for vitamin D deficiency (Lips *et al*., 1988). According to this higher level, 14.8% ($n = 4$, all in control group) of our subjects were deficient at baseline and post supplementation. In 2004, Lips (2004) re-evaluated this recommendation and suggested a level of plasma 25(OH)D <50 nmol/l as insufficient. By using this cutoff, 50% of the subjects were classified as insufficient at baseline, with the majority of the remaining subjects verging on insufficiency, mean (s.d.) 55.5 (18.6) and 47.9 (16.0) nmol/l 25(OH)D in the control and vitamin D groups, respectively. At baseline, there was no significant difference in vitamin D status between the control and vitamin D-treated groups. Post supplementation vitamin D status was significantly higher in the vitamin D-treated group compared to the control group, 86.5 (24.5) vs 48.3 (16.8) nmol/l 25(OH)D ($P < 0.001$). There was no significant change in serum calcium levels post supplementation. The functional marker of vitamin D status, PTH was within the normal range (15–65 pg/ml) in both groups at baseline and post supplementation and were not significantly altered by vitamin D supplementation. Regression analysis was conducted to correct for baseline vitamin D concentrations to assess if changes in biochemical measurements, including PTH, between the two groups were different (data not shown). There were no significant differences between the two groups after adjusting for baseline vitamin D concentrations.

Discussion

Although a considerable number of studies have examined vitamin D status and the effect of supplementation on status in older populations (Lips *et al*., 1988; Liu *et al*., 1997; Harris *et al*., 2000), fewer studies have examined young people. Such studies are of particular importance among those living at higher latitudes where maintenance of sufficient vitamin D levels may be a problem given low UV exposure in wintertime (Tangpricha *et al*., 2002).

Research to date has indicated that the usual dietary vitamin D intake in Europe is not sufficient to maintain adequate vitamin D status during wintertime when synthesis is minimal (Holick, 1994; Glerup *et al*., 2000; Ovesen *et al*., 2003; Hill *et al*., 2004). The results of the current study support this hypothesis. The estimated overall mean dietary intake was 2.05 μg/day (82 IU/day), an intake which is even lower than that observed in an earlier study which estimated the mean dietary intake of Irish adults to be 3.2 μg/day (128 IU/day) (Hill *et al*., 2004). This lower intake may be explained by the age group in the current study and suggest that young adults may have particularly low vitamin D intakes. It is perhaps not surprising, therefore, that we also observed a mean baseline plasma 25(OH)D concentration of 52.1 (17.6) nmol/l, a concentration which indicates a vitamin D status bordering on insufficiency (25(OH)D <50 nmol/l) (Lips, 2004). Our results are comparable with an earlier study conducted among young men living in Boston, (at a latitude of 42°N) which reported 25(OH)D levels of 54.4 (16.8) nmol/l during wintertime (Harris and Dawson-Hughes, 2002). It is worth noting, however, that different vitamin D assays give different results (Chen *et al*., 1990) and the IDS ELISA has 75% specificity for vitamin D_3, and thus may underestimate vitamin D status. Other wintertime studies of vitamin D status have also found insufficient serum 25(OH)D concentrations among young people living in France and Finland (Guillemant *et al*., 2001; Lehtonen-Veromaa *et al*., 2002a, b).

In the current study, 8 weeks supplementation with 15 μg (600 IU) vitamin D_3/day significantly increased vitamin D status. Post supplementation samples were taken at the end of March, when vitamin D status is naturally expected to be at its lowest. Other vitamin D supplementation studies have shown similar increases in serum 25(OH)D concentrations

and corrections of wintertime insufficiency. These studies have, however, only been conducted at geographical latitudes with a higher exposure to UVB irradiation. Harris and Dawson-Hughes (2002) gave 20 μg (800 IU) vitamin D_3/day for 8 weeks during wintertime to young men living in Boston (42°N), and reported that serum 25(OH)D concentrations increased by 22.5 nmol/l from a baseline value of 59.9 nmol/l. Although vitamin D status was significantly improved in the current study, the plasma concentration of 25(OH)D remained well below the level reported by some researchers to be optimal for health (100 nmol/l) (Vieth, 2004). Although plasma 25(OH)D levels were raised by supplementation, it cannot be certain that a correction of insufficiency occurred. Whether longer-term supplementation would further increase vitamin D status in this group requires further investigation.

To our knowledge, there are no studies examining the effect of vitamin D supplementation on bone turnover markers in young adults. A seasonal variation of biochemical markers of bone turnover has been shown, with a higher turnover reported in wintertime compared to summertime (Chapuy *et al*., 1996; Woitge *et al*., 1998). Mezquita-Raya *et al*. (2001) observed no significant differences in biochemical markers of bone turnover in postmenopausal women with high and low vitamin D status, although they did observe that individuals with vitamin D insufficiency (25(OH)D <40 nmol/l) had a significantly lower BMD. Evidence has shown that there is a direct relationship between vitamin D status and BMD (Aguado *et al*., 2000). In the current study, there was no effect of vitamin D supplementation on the bone turnover markers. Although bone turnover markers are good indicators of bone remodelling, their variability is a major problem in clinical application (Siebel *et al*., 2002). Some bone turnover markers also show a marked variation over time, which may cause problems when they are measured in short-term studies. This variation, together with the fact that vitamin D may play a more active role in bone mineralisation rather than bone remodelling (Suda *et al*., 2003), may have contributed to the non-significant effect of vitamin D supplementation on the markers measured. Furthermore, studies have shown that a serum/plasma 25(OH)D level between 78 and 95 nmol/l is the threshold for effects on bone metabolism (Krall *et al*., 1989; Chapuy *et al*., 1997), levels higher than observed in this study. There was an approximate 10% reduction in CTx in both groups post supplementation; this decrease was not significant. Whether the improvement in vitamin D status, observed in the current study following supplementation is sufficient to promote bone health is unclear and requires investigation in longer-term studies.

There has been considerable debate surrounding the use of PTH as a functional indicator of vitamin D status (Department of Health, 1998). While Pepe *et al*. (2005) suggested that 25(OH)D concentrations were the main factor determining the circulating levels of PTH, it has also been reported that PTH concentrations only rise when 25(OH)D concentrations are deficient and fall below 40 nmol/l (Mezquita-Raya *et al*., 2001; Jesudason *et al*., 2002). In the current study, we did not observe a direct association between 25(OH)D and PTH levels, and observed that even when 25(OH)D concentrations were very low (<30 nmol/l), PTH remained within normal reference ranges. A study conducted in postmenopausal women corroborates these findings, reporting no correlation between 25(OH)D and PTH at physiological concentrations (Villareal *et al*., 1991). Vitamin D only plays a role in the synthesis of PTH, whereas the secretion of the hormone is regulated primarily by calcium (Pepe *et al*., 2005; Zittermann *et al*., 1998). We speculate that no change in PTH concentrations was observed in this study as subjects had adequate dietary intakes of calcium, before supplementation with another 1500 mg calcium/day and were not severely deficient in vitamin D.

In conclusion, the results from the current study provide preliminary data to suggest that young adults in Northern Ireland do not consume an adequate daily dietary intake of vitamin D to maintain plasma vitamin D concentrations in the wintertime. As a consequence, vitamin D insufficiency is likely to be apparent in young adults in Northern Ireland. Clearly there is a need to confirm these results at a population level. A daily supplement of 15 μg (600 IU) vitamin D for 8 weeks significantly increased vitamin D status in these individuals, such that all individuals had sufficient vitamin D status. Achievement of an optimum vitamin D status among young adults may have positive health implications for maintaining bone health, and potentially reducing the incidence of other vitamin D-associated diseases such as multiple sclerosis and type I diabetes. Undoubtedly, further research is required to define public health policy and guidelines with respect to vitamin D intakes among young adults residing at higher latitudes.

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References

Aguado P, del Campo M, Garces M, Gonzalez-Casau M, Bernad M, Gijon-Banos J *et al*. (2000). Low vitamin D levels in outpatient postmenopausal women from a rheumatology clinic in Madrid, Spain: their relationship with bone mineral density. *Osteoporos Int* **11**, 739–744.

Ala-Houhala M, Parviainen M, Pyykko K, Visakorpi J (1984). Serum 25-hydroxyvitamin D levels in Finnish children aged 2–17 years. *Acta Peadiatr Scand* **73**, 232–236.

Bates C, Carter G, Mishra G, O'Shea D, Jones J, Prentice A (2003). In a population study, can parathyroid hormone aid the definition of adequate vitamin D status? A study of people aged 65 years and over from the British National Diet and Nutrition Survey. *Osteoporos Int* **4**, 152–159.

Bonjour J, Theintz G, Law F, Slosman D, Rizzoli R (1994). Peak bone mass. *Osteoporos Int* **1**, S7–S13.

Bostick R, Potter J, Sellers T, McKenzie D, Kushi L, Folsom A (1993). Relation of calcium, vitamin D and dairy food intake to incidence of colon cancer among older women. The Iowa Women's Health Study. *Am J Epidemiol* **137**, 1302–1317.

Brot C, Vestergaard P, Kolthoff N, Gram J, Hermann A, Sorensen O (2001). Vitamin D status and its adequacy in healthy Danish perimenopausal women: relationships to dietary intake, sun exposure and serum parathyroid hormone. *Br J Nutr* **86**, S97–S103.

Chapuy M, Preziosi P, Maamer M, Arnaud S, Galan P, Hercberg S (1997). Prevalence of vitamin D insufficiency in an adult normal population. *Osteoporos Int* **7**, 439–443.

Chapuy M, Schott A, Garnero P, Hans D, Delmas P, Meunier P (1996). Healthy elderly French women living at home have secondary hyperparathyroidism and high bone turnover in winter. EPIDOS Study Group. *J Clin Endocrinol Metab* **81**, 1129–1133.

Chen T, Turner A, Holick M (1990). Methods for the determination of the circulating concentration of 25-hydroxyvitamin D. *J Nutr Biochem* **1**, 315–319.

Cheng S, Tylavsky F, Kruger H, Kurkkainen M, Lvytikainen A, Koistinen A *et al*. (2003). Association of low 25-hydroxyvitamin D concentrations with elevated parathyroid hormone concentrations and low cortical bone density in early pubertal and prepubertal Finnish girls. *Am J Clin Nutr* **78**, 485–492.

Dawson-Hughes B, Dallal G, Krall E, Harris S, Sokoll L, Falconer G (1991). Effect of vitamin D supplementation on wintertime and overall bone loss in healthy postmenopausal women. *Ann Intern Med* **115**, 505–512.

Department of Health (1991). *Dietary references values for food energy and nutrients for the United Kingdom*. Report of the panel on dietary reference values of the Committee on Medical Aspects of Food Policy, no 41. HMSO: London. pp 123–127.

Department of Health (1998). *COMA. Nutrition and bone health*. Report of the subgroup on bone health, working group on the nutrient status of the population of the Committee on Medical Aspects of Food and Nutrition Policy. no 49. HMSO: London.

Fuleihan G, Nabulsi M, Choucair M, Salamoun M, Shahine C, Kizirian A *et al*. (2001). Hypovitaminosis D in healthy schoolchildren. *Pediatrics* **107**, 53–60.

Ginty F, Cavadini C, Michaud P, Burchkhardt P, Baumgartner M, Mishra G *et al*. (2004). Effects of usual nutrient intake and vitamin D status on markers of bone turnover in Swiss adolescents. *Eur J Clin Nutr* **58**, 1257–1265.

Glerup H, Mikkelsen K, Poulsen L, Hass E, Overbeck S, Thomsen J *et al*. (2000). Commonly recommended daily intake of vitamin D is not sufficient if sunlight exposure is limited. *J Intern Med* **247**, 260–268.

Gordon C, DePeter C, Feldman H, Grace E, Eman S (2004). Prevalence of vitamin D deficiency among healthy adolescents. *Arch Pediatr Adolesc Med* **158**, 531–537.

Guillemant J, Le H, Maria A, Allemandou A, Peres G, Guillemant S (2001). Wintertime vitamin D deficiency in male adolescents: effect on parathyroid function and response to vitamin D_3 supplements. *Osteoporos Int* **12**, 875–879.

Harris S, Dawson-Hughes B (2002). Plasma vitamin D and 25(OH)D responses of young and old men to supplementation with vitamin D_3. *J Am Coll Nutr* **21**, 357–362.

Harris S, Soteriades E, Coolidge J, Mudgal S, Dawson-Hughes B (2000). Vitamin D insufficiency and hyperparathyroidism in a low income, multiracial, elderly population. *J Clin Endocrinol Metab* **85**, 4125–4130.

Hill T, O'Brien M, Cashman K, Flynn A, Kiely M (2004). Vitamin D intakes in 18–64 year old Irish adults. *Eur J Clin Nutr* **5**, 1–9.

Holick M (1994). McCollum award lecture. Vitamin D: new horizons for the 21st century. *Am J Clin Nutr* **60**, 619–630.

Hutchison F, Bell N (1992). Osteomalacia and rickets. *Semin Nephrol* **12**, 127–145.

Hypponen E, Laara E, Reunanen A, Jarvelin M, Virtanen S (2001). Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. *Lancet* **358**, 1500–1503.

Isaia G, Giorgino R, Rini G, Bevilacqua M, Maugeri D, Adami S (2003). Prevalence of hypovitaminosis D in elderly women in Italy: clinical consequences and risk factors. *Osteoporos Int* **14**, 577–582.

Jesudason D, Need A, Horowitz M, O'Loughlin P, Morris H, Nordin B (2002). Relationship between serum 25 hydroxyvitamin D and bone resorption markers in vitamin D insufficiency. *Bone* **31**, 626–630.

Krall E, Sahyoun N, Tannenbaum S, Dallal G, Dawson-Hughes B (1989). Effect of vitamin D intake on seasonal variations in parathyroid hormone secretion in postmenopausal women. *N Engl J Med* **321**, 1777–1783.

Lamberg-Allardt C, Ala-Houhala M, Ahola M, Parviainen M, Rasanen L, Visakorpi J (1986). Vitamin D status of children and adolescents in Finland. *Ann Nutr Metab* **30**, 267–272.

Lehtonen-Veromaa M, Mottonen T, Irjala K, Karkkainen M, Lamberg-Allardt C, Hakola P *et al*. (1999). Vitamin D intake is low and hypovitaminosis D common in healthy 9 to 15 year old Finnish girls. *Eur J Clin Nutr* **53**, 746–751.

Lehtonen-Veromaa M, Mottonen T, Nuotiio I, Irjala K, Viikari J (2002a). The effect of conventional vitamin D_2 supplementation on serum 25(OH)D concentration is weak among peripubertal Finnish girls: a 3 year prospective study. *Eur J Clin Nutr* **56**, 431–437.

Lehtonen-Veromaa M, Mottonen T, Nuotio I, Irjala K, Leino A, Viikari J (2002b). Vitamin D and attainment of peak bone mass among peripubertal Finnish girls: a 3 year prospective study. *Am J Clin Nutr* **76**, 1446–1453.

Lips P (2001). Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. *Endocr Rev* **22**, 477–501.

Lips P (2004). Which circulating level of 25-hydroxyvitamin D is appropriate? *J Ster Biochem Mol Biol* **89**, 611–614.

Lips P, Wiersinga A, van G (1988). The effect of vitamin D supplementation on vitamin D status and parathyroid function in elderly subjects. *J Clin Endocrinol Metab* **67**, 644–650.

Liu B, Gordon M, Labranche J, Murray TM, Vieth R, Shear N (1997). Seasonal prevalence of vitamin D deficiency in institutionalized older adults. *J Am Geriatric Soc* **45**, 598–603.

Martinez M, del Campo M, Sanchez Cabezudo M, Garcia J, Sanchez Calvin M, Torrijos A *et al*. (1994). Relations between calcidiol serum levels and bone mineral density in postmenopausal women with low bone density. *Calcif Tissue Inter* **55**, 253–256.

McKenna M (1992). Differences in vitamin D status between countries in young adults and elderly. *Am J Med* **93**, 69–77.

McLaughlin M, Raggatt P, Fairney A, Brown D, Lester E, Wills M (1974). Seasonal variations in serum 25-hydroxycholecalciferol in healthy people. *Lancet* **303**, 536–538.

Merlino L, Curtis J, Mikuls T, Cerhan J, Criswell L, Saag K (2004). Vitamin D intake is inversely associated with rheumatoid arthritis. *Arthritis Rheum* **50**, 72–77.

Mezquita-Raya P, Munoz-Torres M, Luna J, Luna V, Lopez-Rodriguez F, Torres-Vela E *et al*. (2001). Relation between vitamin D insufficiency, bone density and bone metabolism in healthy postmenopausal women. *J Bone Miner Res* **16**, 1408–1414.

Moore C, Murphy M, Keast D, Holick M (2004). Vitamin D intake in the United States. *J Am Diet Assoc* **104**, 980–983.

Munger K, Zhang S, O'Reilly E, Hernan M, Olek M, Willett W *et al*. (2004). Vitamin D intake and incidence of multiple sclerosis. *Neurology* **62**, 60–65.

Neville C, Robson P, Murray L, Strain J, Twisk J, Gallagher A *et al*. (2002). The effect of nutrient intake on bone mineral status in young adults: The Northern Ireland Young Hearts Project. *Calcif Tissue Int* **70**, 89–98.

Ovesen L, Andersen R, Jakobsen J (2003). Geographical differences in vitamin D status, with particular reference to European countries. *Proc Nutr Soc* **62**, 813–821.

Parfitt A (1998). Osteomalacia and related disorders. In: Avioli L, Krane S (eds.). *Metabolic Bone Disease and clinically related disorders*. Academic Press: San Diego, California. pp 327–386.

Parfitt A, Gallagher J, Heaney R, Johnston C, Neer R, Whedon G (1982). Vitamin D and bone health in the elderly. *Am J Clin Nutr* **36**, 1014–1031.

Pepe J, Romagnoli E, Nofroni I, Pacitti M, De Geronimo S, Letizia C *et al*. (2005). Vitamin D status as the major factor determining the circulating levels of parathyroid hormone: a study in normal subjects. *Osteoporos Int* **16**, 805–812.

SACN (2003). *Scientific Advisory Committee on Nutrition*. Report of the subgroup on maternal and child nutrition (SMCN). Paper: Vitamin D deficiency in children. Agenda item 2. SMCN/03/02.

Siebel M, Roeller M, Vander Velden B, Diel L (2002). Long term variability of bone turnover markers in patients with non-metastatic breast cancer. *Clin Lab* **48**, 11–12.

Suda T, Ueno Y, Fujii K, Shinki T (2003). Vitamin D and bone. *J Cell Biochem* **88**, 259–266.

Tangpricha V, Pearce E, Chen T, Holick M (2002). Vitamin D insufficiency among free-living healthy young adults. *Am J Med* **112**, 659–662.

Vieth R (2004). Why the optimal requirement for vitamin D_3 is probably much higher than what is officially recommended for adults. *J Steroid Biochem Mol Biol* **89–90**, 575–579.

Vieth R, Chan P, MacFarlane G (2001). Efficacy and safety of vitamin D_3 intake exceeding the lowest observed adverse effect level (LOAEL). *Am J Clin Nutr* **73**, 288–294.

Villareal D, Civitelli R, Chines A, Avioli L (1991). Subclinical vitamin D deficiency in postmenopausal women with low vertebral bone mass. *J Clin Endocrinol Metab* **72**, 628–634.

Webb A, Kline L, Holick M (1988). Influence of season and latitude on the cutaneous synthesis of vitamin D_3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D_3 synthesis in human skin. *J Clin Endocrinol Metab* **67**, 373–378.

Woitge H, Scheidt-Nave C, Kissling C, Leidig-Druckner G, Meyer K, Grauer A *et al*. (1998). Seasonal variation of biochemical indexes of bone turnover: results of a population based study. *J Clin Endocrinol Metab* **83**, 68–75.

Zittermann A (2003). Vitamin D in preventive medicine: are we ignoring the evidence? *Br J Nutr* **89**, 552–572.

Zittermann A, Scheld K, Stehle P (1998). Seasonal variations in vitamin D status and calcium absorption do not influence bone turnover in young women. *Eur J Clin Nutr* **52**, 501–506.