Vitamin D status and its relationship with parathyroid hormone and bone mineral status in older adolescents

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Osteoporosis is an important contributor to the global burden of disease, and in the UK alone results in one in three women and one in twelve men aged >50 years experiencing a fragility fracture. Optimising peak bone mass in early adulthood is thought to reduce osteoporosis risk by offsetting bone losses in later life. Ensuring sufficient vitamin D status (measured as 25-hydroxyvitamin D (25OHD) in plasma), among other factors, is believed to facilitate the achievement of optimum peak bone mass. Lower 25OHD is associated with a higher plasma concentration of parathyroid hormone (PTH). As PTH is associated with increased bone turnover and bone loss, maintenance of sufficient 25OHD is thought to have a protective effect on bone health. However, there is a lack of consensus internationally on what constitutes an optimum 25OHD concentration, and values between 30 and 80 nmol/l have been suggested. These values have been based on findings from various studies in adults in which PTH has been observed to plateau at a 25OHD concentration of >30 nmol/l; however, not all studies have found such a plateau. Although studies in younger adolescents (14–16 years) have shown an inverse relationship between PTH and 25OHD, the concentration of 25OHD required for achievement of optimum peak bone mass is unknown. The present review examines the evidence defining vitamin D insufficiency thresholds, and the relevance of such thresholds to adolescent bone health.

Vitamin D: 25OHD: Adolescence: Peak bone mass: Bone mineral status: Bone turnover

Osteoporosis is a multifactorial progressive skeletal disease characterised by low bone mass and micro-architectural deterioration of bone tissue with a consequent increase in bone fragility and risk of fracture (Consensus Development Conference, 1993). It results in a reduced quality of life and a marked increase in mortality risk (Gilsanz, 1998). Osteoporosis is an important contributor to the global burden of disease and results in $> 1.6 \times 10^6$ hip fractures each year (Cooper *et al*. 1992). In the UK alone one in three women and one in twelve men aged >50 years currently experience a fragility fracture as a result of the condition (National Osteoporosis Society Online, 2004), and the numbers are rising. The estimated annual cost of the treatment of osteoporotic fractures in the UK is £1·7 × 10^9 (Russell *et al*. 2003). Although osteoporosis is primarily a disease of the elderly, it is believed that the foundations of the condition are laid in childhood and adolescence (Root, 2002). Adolescence is a critical time for bone mineral accrual (Kun *et al*. 2001), resulting in approximately 90% of peak bone mass being attained by the age of 18 years (Slemenda *et al*. 1994). Optimising peak bone mass in early adulthood is thought to reduce the risk of osteoporosis by offsetting bone losses later in life.

A multiplicity of interacting endogenous and exogenous factors influence the achievement of peak bone mass (Heaney *et al*. 2000; Saggese *et al*. 2001), including genotype (Jouanny *et al*. 1995; Rubin *et al*. 1999), physical activity (Fehily *et al*. 1992; Rubin *et al*. 1999) and diet (Wang *et al*. 2003). Furthermore, ensuring a sufficient vitamin D status is also believed to be an important factor for achieving optimal peak bone mass. In the transition from adolescence to adulthood and the cessation of bone growth and mineral accrual it is not known whether variations in vitamin D status result in variations in bone mineral status, and whether there are site-specific effects. The present paper reviews the current definitions of

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vitamin D insufficiency thresholds and explores their relevance to adolescent bone health.

Bone changes during adolescence

Adolescence is characterised by changes in height, weight and body composition, and is also a crucial time for bone development and mineralisation (Kun *et al.* 2001). The attainment of maximum peak bone mass is thought to be an important determinant of osteoporosis, as the mass of bony tissue present at any time during adult life is simply the difference between the amount achieved at maturity and that lost with aging (Bonjour *et al.* 1994). Adolescents are thus an important demographic group in terms of osteoporosis prevention, and it is clear that efforts should be made to ascertain that adolescents attain 100 % of their peak bone mass potential (Cromer & Harel, 2000).

Bone is a metabolically-active tissue that constantly undergoes phases of turnover, involving systematic bone breakdown (resorption) and new formation. If the balance of bone formation to bone resorption is disturbed, over time bone architecture, resistance and elasticity can be adversely affected (Ballabriga, 2000). Two processes comprise bone growth: cortical bone develops by peri-osteal apposition that widens the bone shaft; the trabecular bone assembles by endochondral ossification as bones grow in length (Parfitt, 1994). Bone modelling (where a greater rate of bone formation occurs compared with bone resorption) ceases shortly after the growth spurt and is largely replaced by bone remodelling (an equal rate of bone formation and bone resorption). The function of remodelling is to both maintain the structural integrity of the skeleton and subserve its metabolic functions as a store of Ca and P (Raisz, 1999). Various skeletal sites grow at differential rates. For example, growth of the appendicular skeleton (i.e. the limbs) is greater, relative to that of the axial skeleton (such as the spine and hip), before puberty but then decelerates at puberty when axial growth accelerates (Bass *et al.* 1999; Szulc *et al.* 2000). The timing of peak bone mass is also variable between skeletal sites (e.g. hip and forearm; because at a given point there may still be bone formation in one skeletal site, relative equilibrium in another and bone loss in a further site; Szulc *et al.* 2000), between individuals and especially between the genders. Boys generally start puberty at about the age of 14 years, approximately 2 years later than girls, and their pubertal growth spurt lasts for about 4 years compared with 3 years in girls (Riggs *et al.* 1999). It is thought that these differences between the genders (i.e. prolonged puberty and greater body size) account for the greater peak bone mass in boys (Riggs *et al.* 1999).

Little is known about environmental determinants of mineral accretion and bone development in the older adolescent age-group. Parsons *et al.* (1996) have shown that bone mineral is still being accumulated in young adults aged 18–21 years, but lifestyle or anthropometric factors that influenced these bone mineral changes could not be identified. Ginty *et al.* (2005*b*) have found a positive association between bone mineral status and daily participation in high-impact physical activity in 16–18-year-old adolescent boys. In another study (Gustavsson *et al.* 2003) 16–19-year-old adolescent male badminton and ice hockey players have also been found to have a higher bone mineral status than age-matched controls over 3 years.

Assessment of bone health

Dual-energy X-ray absorptiometry has become the standard clinical method for the assessment of areal bone mineral density (BMD) at prominent fracture sites, such as the forearm, lumbar spine and femur (Reid, 2003). BMD provides a measurement of the bone mineral content, which is then partially corrected for size by correcting for the area of the bone assessed (Reid, 2003). Bone size, however, can markedly influence BMD, thus adjustment of bone mineral content in multiple regression analysis for bone area, body weight and height, for example, is a useful method of correcting for this confounder (Cole & Prentice, 1992; Prentice, 2004). The issue of size correction is of particular importance when studying subjects who are still growing, e.g. adolescents. There is now widespread agreement that BMD should not be used as a measure of bone mineral status during growth because of changes in bone size, and that bone mineral content should be assessed instead, preferably, if appropriate, with adjustment for body and bone size (Fewtrell, 2003; Heaney, 2003; Prentice, 2004).

Bone turnover can be assessed by the measurement of turnover markers in blood and urine, in which the end products of bone formation or resorption are excreted. Examples of markers of bone formation include osteocalcin and bone-specific alkaline phosphatase, both of which are specific products of osteoblasts (bone-forming cells) and can be measured in blood (Szulc *et al.* 2000; Woitge & Seibel, 2001). Bone resorption may be measured, for example, by urinary markers such as deoxypyridinoline and N-telopeptides of type I collagen, which form cross-links to stabilise collagen and during bone matrix resorption are released into the circulation and excreted in urine (Szulc *et al.* 2000).

During adolescence bone turnover is elevated compared with that in adults, consistent with skeletal growth and consolidation. During the pubertal growth spurt bone marker levels are up to four to five times higher than those in adults (Saggese *et al.* 2002). In older adolescents the biochemical markers of turnover decrease, but they decrease later and more slowly in boys than in girls, because of their later and longer growth spurt (Szulc *et al.* 2000).

Vitamin D

Vitamin D synthesis

Vitamin D is the generic descriptor for ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3), and is acquired in man from the diet and by cutaneous synthesis following exposure to sunlight. It is estimated that about 80–90 % of the body's vitamin D requirement is acquired via the skin and the remainder from food sources (Holick, 1999).

During sun exposure UVB photons of light stimulate the conversion of 7-dehydrocholesterol in the skin to pre-cholecalciferol by photolysis (Webb & Holick, 1988; Holick, 1995). The precholecalciferol is then transformed in the epidermis through thermal isomerisation to chole-calciferol. Cholecalciferol selectively (in preference to precholecalciferol) exits the plasma membrane of the skin into the extracellular space by binding to the vitamin D-binding protein in the capillary bed of the epidermis (Holick, 1981). The amount of photoconversion occurring is dependent not only on the appropriate wavelengths of UV light (295–315 nm; Chen et al. 2000) but also on the quantity of UV radiation reaching each layer of the skin (Webb & Holick, 1988).

While the cutaneous pathway for vitamin D synthesis provides the main source of the vitamin, the main dietary sources of ergocalciferol and cholecalciferol in the UK are oily fish, full-fat dairy foods, such as milk, butter and cheese, egg yolk, liver and fortified products such as margarine and cereals (Department of Health, 1998) and are intestinally absorbed. Ergocalciferol or cholecalciferol, as such, are not believed to have important biological activity and are stored in fat deposits in the body (Garrow et al. 2000).

Ergocalciferol and cholecalciferol are transported to the liver with the same relative affinity by vitamin D-binding protein (Norman, 1992; Holick, 1996), which is a multi-functional plasma protein synthesised in the liver that provides a high-affinity high-capacity binding reservoir for, and regulates cellular access of, vitamin D and its metabolites (Haddad, 1999). Within the liver ergocalciferol and cholecalciferol are converted to their corresponding 25-hydroxy derivative by the enzyme 25-hydroxylase. 25-Hydroxyvitamin D (25OHD) is the circulating metab-olite of vitamin D and is typically measured as an index of vitamin D status. The half-life of 25OHD has been shown to be approximately 3 weeks (Lund et al. 1980; Holick, 2004).

Vitamin D requirements

The level of vitamin D required (whether by cutaneous synthesis or via dietary intake) in order to maintain sufficient plasma 25OHD levels for the achievement of optimal peak bone mass is not known. Holick (1999, 2001) has shown that exposure of 6–10% of the body surface to one minimal erythemal dose of sunlight, defined as enough sun to cause pinkness to the skin (Holick, 2004), is equivalent to ingesting approximately 15–25 µg vitamin D, although the effect on plasma levels of 25OHD was not explored. Hence, exposure of the hands, arms and face two to three times per week to about 0·5 minimal erythemal dose (about 5 min for a skin-type-2 adult in Boston (latitude 42·2° N) at noon in July) in the spring, summer and autumn would, according to Holick (2004), provide adequate exposure.

In terms of dietary intakes of vitamin D the UK reference nutrient intake for at-risk adults, such as pregnant and lactating women, adults >65 years old and individuals with limited sun exposure, is currently 10 µg/d (Department of Health, 1998). However, there is currently no recommended daily intake of vitamin D for adolescents in the UK, as it has been considered that they will generally achieve an adequate amount from normal exposure to sunlight (Department of Health, 1998). In France and the USA vitamin D intake recommendations have been made, and the recommended intake is 10 µg/d for 15–18 year olds (Department of Health, 1998).

Determinants of vitamin D status

Season and sunlight exposure

25OHD has been consistently demonstrated to be markedly lower during the winter months than during the summer months in studies of adolescents from various temperate countries, including China (Du et al. 2001), France (Guillemant et al. 1999), Finland (Lehtónen-Veromaa et al. 1999; Outila et al. 2001), Lebanon (El-Hajj Fuleihan et al. 2001) and the UK (Ellis et al. 1977). Generally, this seasonal variation in 25OHD is apparent in countries at a distance from the equator because less time is spent outdoors in winter and because 25OHD is not synthesised from UVB exposure during the winter months. In the UK National Diet and Nutrition Survey (NDNS; Gregory & Lowe, 2000) 25OHD levels for both boys and girls (11–18 years old) were found to be markedly higher when measured during the summer study wave (July–September) than at other times of the year. Similarly, in 116 boys and 109 girls (aged 16–18 years) from the Cambridge Bone Studies (CBS; Stear et al. 2003) 25OHD levels during the winter (January–March) were observed to be significantly lower than those during the autumn (September to January) in both boys (33·3 (SD 12·8) mmol/l v. 49·6 (SD 16·6) nmol/l respectively, $P < 0·0001$; Willett et al. 2005a) and girls (38·8 (SD 14·0) v. 53·4 (SD 14·7) nmol/l respectively, $P < 0·0001$; Willett et al. 2004).

Diet: vitamin D

At northern latitudes during the winter months cutaneous synthesis of vitamin D is limited, and dietary and supplementary sources of vitamin D may be important to maintain a sufficient 25OHD status and avoid prolonged elevated PTH concentrations. It has also been suggested that while winter sunlight cannot produce vitamin D in the skin, it may actually promote the photo-degradation of nutritionally-obtained vitamin D (Webb et al. 1989). It is thought that UV light exposure during the winter can photoisomerise cholecalciferol, thus causing its destruction (Webb et al. 1989). The UVB wavelengths responsible for this photodestruction are longer, with lower energy radiation (316–330 nm), than those that synthesise cuta-neous vitamin D, but unlike the latter wavelengths they are present all year round. It is thought that in summer months these longer wavelengths help to prevent vitamin D toxicity via their catabolic mechanism; however, during the winter it is proposed that this mechanism may lead to decreasing vitamin D and, subsequently, 25OHD levels (Webb et al. 1989).

Data from 298 11–18-year-old boys in the NDNS has shown that during the winter study wave (January–March) 25OHD is significantly positively associated ($P < 0.01$) with dietary vitamin D intake (Willett, 2004). A similar association has also been found in 16–18-year-old boys from the CBS (Willett *et al.* 2005*b*). Furthermore, a positive relationship between 25OHD and vitamin D intake has been reported for UK preschool children during the winter (Davies *et al.* 1999), and for elderly subjects in the UK NDNS in the autumn, winter and spring but not the summer (Bates *et al.* 2003). It is possible that dietary intakes of vitamin D have the most marked impact when cutaneous synthesis of the vitamin is limited and 25OHD stores are low, i.e. during the winter. The intakes of vitamin D in the boys (3·1 (SD 1·7) µg/d) in the CBS are close to the average intake by adults in the UK (Prynne *et al.* 2004). It is surprising that these relatively low intakes of vitamin D should have a marked impact on 25OHD, especially considering the frequent lack of effect seen with vitamin D supplementation at much higher doses (discussed later; see this page).

Vitamin D supplements

Vitamin D supplementation studies have been carried out in an attempt to quantify the effects of known levels of vitamin D intake on 25OHD. Heaney *et al.* (2003) have found that 1 µg cholecalciferol leads to a 0·7 nmol/l increase in winter circulating levels of 25OHD in men (mean age 38·7 (SD 11·2) years), and have estimated that to sustain autumn concentrations of 25OHD a total of approximately 96 µg is needed, with about 12·5 µg of this amount being supplied from oral sources. Thus, typical vitamin D intakes in healthy men are too low to maintain desirable 25OHD levels (i.e. their post-summer levels). However, most vitamin D supplementation studies have been carried out in elderly populations and the results have been variable; both positive effects (Chapuy *et al.* 2002) and no effects (Patel *et al.* 2001; Meyer *et al.* 2002) have been reported. Such variation could be attributable to methodological differences in the type of assay and kit used to quantify 25OHD, and their affinities for ergocalciferol and cholecalciferol (discussed further on p. 197).

Few vitamin D supplementation studies have been conducted in adolescents. Lehtónen-Veromaa *et al.* (1999) have carried out one study in 9–15-year-old Finnish girls (*n* 191) monitored over the course of 12 months. After 6 months all subjects were supplemented for 3 months with a multivitamin containing 10 µg vitamin D. Despite the 93·4% compliance with the supplementation, the final 25OHD concentrations were found to be as low as the baseline measurements. It was concluded that the dose of vitamin D supplementation given to the subjects was too low to maintain a sufficient 25OHD for optimal bone mineral accrual in Finland. However, vitamin D supplementation was discontinued 1 month before the final sample collection, which, given the 3-week half-life of 25OHD, may account for the relative lack of change from the baseline 25OHD concentration.

A study carried out by Guillemant *et al.* (2001) has demonstrated that a 2·5 mg oral dose of cholecalciferol, administered every 2 months for 6 months is sufficient to maintain the concentrations of both 25OHD and PTH at their baseline post-summer levels. While a marked decrease in serum 25OHD values was observed from summer to winter in the control group, the mean 25OHD concentrations in the supplemented subjects were found to show little change. The Guillemant *et al.* (2001) study provided approximately four times the dose of the Lehtónen-Veromaa *et al.* (1999) study, suggesting that this level is more likely to be effective in maintaining 25OHD levels. However, the physiological effects of the dose used (2·5 mg cholecalciferol administered orally every 2 months) are unclear.

Of the boys in the CBS current supplement users were found to have higher 25OHD levels during winter (Willett, 2004). However, despite the correlation with supplement use, no relationship was found between the supplement type (e.g. multivitamin, cod liver oil) and contents (i.e. constituent vitamins and minerals) and 25OHD, although it was noted that 62·2% of the supplement users took supplements (cod liver oil and multivitamins) that contained vitamin D. This positive association between 25OHD and the use of supplements may thus merely reflect a greater personal interest in health by the subjects who took the supplements, rather than a biological effect of the supplements.

Ethnic group

Research has shown that 25OHD concentration can be related to the ethnic group of the individual. A negative relationship has been demonstrated between 25OHD concentration and the natural pigmentation of the skin, and it is well recognised that certain ethnic groups are at greater risk of developing 25OHD deficiency and, subsequently, osteomalacia and rickets (Harris & Dawson-Hughes, 1998; Lawson *et al.* 1999). In ethnic groups with increased melanin a lower 25OHD status has been found, e.g. in populations of Asian origin (O'Hare *et al.* 1984; Awumey *et al.* 1998; Lawson *et al.* 1999) and in those of African-American origin (Harris & Dawson-Hughes, 1998). As melanin functions as a UV light filter, it determines the amount of UVB photons that are able to penetrate the skin (Norman, 1998). The underlying mechanism has yet to be fully elucidated, although it has been shown that melanin absorbs UVB photons in competition with 7-dehydrocholesterol, thus reducing the capacity for vitamin D production (Holick, 1995). Cultural differences, such as diet and clothing choices, may also influence 25OHD status. Asian populations are known to consume chapattis, for example, which have a high phytate content (thought to interfere with the entero-hepatic circulation of vitamin D metabolites; Clemens, 1989), and often have vegetarian diets, thus limiting their vitamin D intake from animal sources. In some ethnic groups the clothing worn almost completely covers the body, so reducing exposure to UVB light. It is often found, therefore, that non-Caucasian populations, such as Asian Indians (Awumey *et al.* 1998; Hampson *et al.* 2003), young American black women

(Harris & Dawson-Hughes, 1998) and Mexican-American girls (Abrams *et al.* 1999) have lower 25OHD levels than their Caucasian counterparts (Awumey *et al.* 1998; Harris & Dawson-Hughes, 1998; Abrams *et al.* 1999; Hampson *et al.* 2003). Also, the 25OHD levels for the 11–18-year-old subjects from the NDNS are significantly higher for both the Caucasian boys ($P < 0.0001$) and girls ($P < 0.0001$; Willett, 2004) compared with the other ethnic groups (categorised as: black Caribbean; black African; black neither Caribbean or African; Indian; Pakistani; Bangladeshi; Chinese; none of these, including mixed race) in all seasonal study waves. Similarly, in the CBS, the Caucasian boys have a significantly higher 25OHD status at all time points of the year than subjects self-categorised as Asian, Black, Indian or Oriental ($P < 0.0001$; Willett, 2004).

Age

25OHD status is thought to change with age, but the evidence thus far is contradictory. Studies that have been undertaken have generally been in adult populations, and less information is available for younger subjects. In several studies of adult populations no significant relationship between 25OHD levels and age has been demonstrated (Sherman *et al.* 1990; Burnand *et al.* 1992; Nakamura *et al.* 2000; Margiloff *et al.* 2001). However, while some research has found a negative correlation between 25OHD levels and age in elderly subjects >65 years old (Dawson-Hughes *et al.* 1997; Jacques *et al.* 1997), a study of relatively younger Finnish adults (30–42 years old) has found a positive relationship between 25OHD levels and age (Lamberg-Allardt *et al.* 2001).

Currently, little is known about the effects of puberty on 25OHD. In a study of Finnish children aged 2–17 years (Ala-Houhala *et al.* 1984) the 11–17 year olds were found to have a lower 25OHD status than the younger children, but in a study of Lebanese children aged 10–16 years (El-Hajj Fuleihan *et al.* 2001) no significant association between 25OHD levels and age was found. In the NDNS (Gregory & Lowe, 2000) it was shown that older teenage boys and girls (15–18 years old) have a significantly lower 25OHD status than younger children (4–6 years old). The 25OHD levels for the 11–18-year-old boys from NDNS were found to show a downward trend (non-significant) with age. However, in a further analysis 25OHD values for boys aged 11–12 years were found to be significantly higher than those for boys aged 16–18 years ($P = 0.02$; Willett, 2004). No such association between 25OHD and age was found in 11–18-year-old girls. A comparable decline in 25OHD levels in boys has been reported for Swiss adolescents, with 25OHD levels being lowest in the most-pubertally-advanced boys (Tanner stage 4–5; see Tanner, 1962) compared with those who were less mature (Ginty *et al.* 2005*a*). In the NDNS it was found that the amount of physical activity (h) decreases with age in both boys and girls (Gregory & Lowe, 2000), and that 16–18-year-old boys undertake significantly less physical activity (h/d) than the younger age-groups ($P = 0.0002$; Willett, 2004), which may correspond to less time outdoors and, subsequently, less sun exposure.

Vitamin D insufficiency

Vitamin D status is conventionally measured as 25OHD concentration in the plasma. The optimum 25OHD concentration for bone health in different age-groups has not been established, and the issue of defining insufficiency thresholds is internationally controversial. The problem of defining vitamin D insufficiency is further compounded by the range of methods used to measure 25OHD, including RIA, HPLC, competitive protein binding assays and ELISA. The results obtained with these methods do not necessarily closely agree. Lips *et al.* (1999) have undertaken a cross-comparison of the methods used for measuring 25OHD levels and have found that the competitive protein binding assay provides a 25OHD value that is 80% higher than that obtained by HPLC, with the RIA giving intermediate results. Binkley *et al.* (2004) have found substantial variation in assay performance in a test of 25OHD measurements across laboratories and have called for method standardisation. The Vitamin D External Quality Assessment Scheme has monitored the performance of nine methods for 25OHD measurement from approximately 125 laboratories across many countries, and marked variation has been found across groups and assays (Vieth & Carter, 2001). For example, a difference in measurements of ≤22% has been found across groups using the Immunodiagnostic Systems Ltd (Boldon, Tyne & Wear, UK) RIA and a difference of ≤25% for the DiaSorin Inc. (Stillwater, MN, USA) RIA. It is therefore apparent that comparisons across studies may be confounded by differing methods of 25OHD measurement.

Approaches for defining 25-hydroxyvitamin D insufficiency thresholds

25OHD insufficiency thresholds have often been suggested that are based on the inverse association between PTH and 25OHD. The hypothesis underlying the application of this threshold is that since PTH is associated with increased bone turnover (Department of Health, 1998) and greater bone loss, maintenance of a sufficient concentration of 25OHD is believed to have a protective effect on bone health.

PTH secretion increases in response to a reduction in plasma Ca concentration (Holick, 1996), either as a result of low Ca intake or possibly increased urinary Ca losses or other pathological states. When the body is in a state of hypocalcaemia (Fig. 1) the parathyroid glands are stimulated to increase the synthesis and secretion of PTH (Holick, 1996), which is a major regulator of Ca metabolism and bone turnover (Gonzalez & Martin, 1998). The production of PTH is regulated by the extracellular Ca concentration acting, via the plasma membrane Ca-sensing receptor, on the secretory cells (Vander *et al.* 2001). In addition to increasing the reabsorption of Ca in the kidney (Holick, 1996), PTH promotes the synthesis of 1α-hydroxylase in the kidney which hydroxylates C-1 of 25OHD to form 1,25-dihydroxyvitamin D (Fraser, 1994). 1,25-Dihydroxyvitamin D is 500–1000 times more biologically active than its precursor, 25OHD (Norman, 1992).
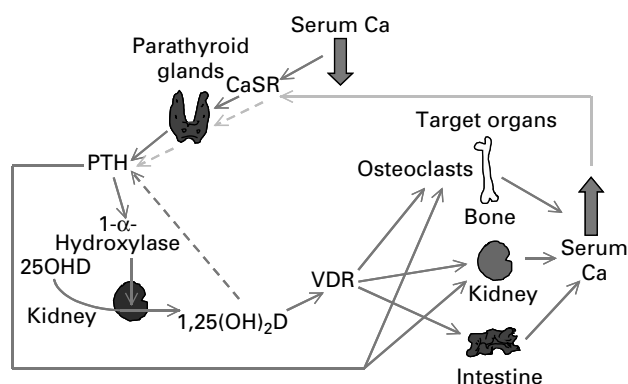
Fig. 1. Calcium metabolism in the body. PTH, parathyroid hormone; 25OHD, 25-hydroxyvitamin D; 1,25(OH)$_2$D; 1,25-dihydroxy-vitamin D; CaSR, calcium-sensing receptor; VDR, vitamin D receptor; ⟶, stimulation; ⇢, suppression.

Both PTH and 1,25-dihydroxyvitamin D mobilise monocytic stem cells to become osteoclasts and increase bone resorption, thus releasing Ca into the circulation (Holick, 1996). 1,25-Dihydroxyvitamin D also increases the efficiency of intestinal Ca absorption, by up regulating active transport, and aids the reabsorption of Ca in the kidney. The net effect is to raise the serum Ca concentration and reduce PTH synthesis and secretion from the parathyroid glands (Holick, 1996). In a negative feedback reaction 1,25-dihydroxyvitamin D also suppresses PTH production (Holick, 1996). The effects of 25OHD on PTH secretion are believed to be indirect, whereby low 25OHD may limit intestinal Ca absorption, resulting in decreased plasma Ca.

It has been hypothesised that persistently low 25OHD may result in prolonged elevation of PTH and a subsequent increase in bone resorption. Previously, a 25OHD threshold of <25 nmol/l was used to define insufficiency, based on 25OHD levels above those found in rickets and osteomalacia (Department of Health, 1998). More recently,

many thresholds for 25OHD insufficiency have been proposed on the basis of the inverse relationship between PTH and 25OHD. However, there is a lack of consensus on what constitutes an optimum 25OHD concentration, and values between 30 and 80 nmol/l have been suggested (Fig. 2). These values have been based on findings from a number of studies of elderly men and women in which PTH has been observed to plateau at a 25OHD concentration of >30 nmol/l (Salamone et al. 1993; Chapuy et al. 1997; Malabanan et al. 1998); however, not all studies have found such a plateau (Bates et al. 2003). In a study of Canadian men and women from different age-groups (Vieth et al. 2003) the relationship between PTH and 25OHD was not found to show a plateau. Although studies in younger adolescents aged 14–16 years have shown an inverse relationship between PTH and 25OHD status (Outila et al. 2001), the concentration of 25OHD required for achievement of optimum peak bone mass has not been established. In the NDNS low 25OHD levels were found to be common in 11–18 year olds, and the prevalence of low values increased with age, particularly in boys. When analysing 25OHD levels from all the seasonal study waves in these subjects it was found that 11% of the 11–14-year-old boys and 16% of the 15–18-year-old boys have 25OHD levels <25 nmol/l (Gregory & Lowe, 2000). In the CBS 19·4% of the girls and 32·2% of the boys aged 16–18 years (n 225) were found to have 25OHD values <25 nmol/l in winter (Willett, 2004). When using higher previously-defined 25OHD thresholds of, for example, <37·5 and <50 nmol/l (Malabanan et al. 1998; Thomas et al. 1998) the percentages increase to 49·1 and 78·7 of the girls and 67·8 and 92·2 of the boys respectively below these thresholds in winter. It is clear that the prevalence of low 25OHD is marked in adolescents, particularly older adolescents, but that the assessment of insufficiency varies greatly depending on the threshold applied.

Many studies have been published to date that report the prevalence of 25OHD insufficiency in their adolescent populations using adult cut-offs (Ala-Houhala et al. 1984;
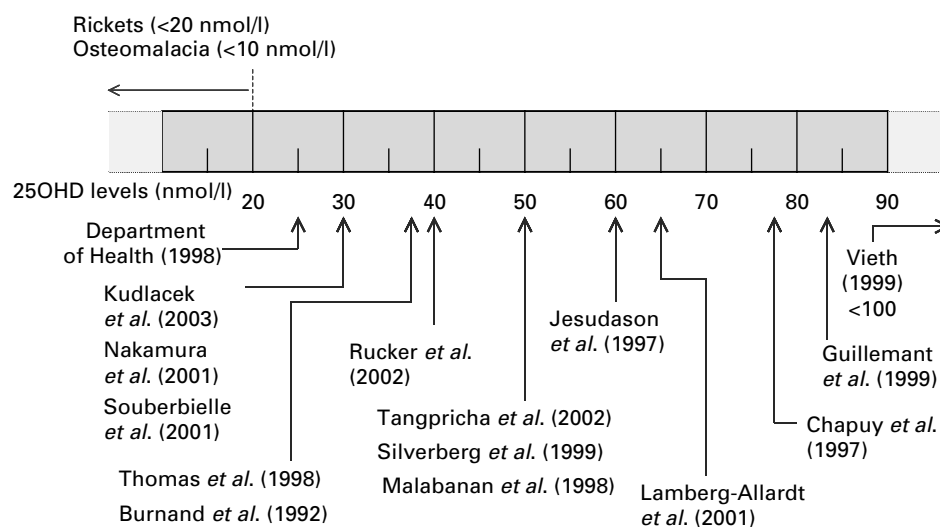
Fig. 2. Proposed 25-hydroxyvitamin D (25OHD) insufficiency thresholds.

O'Hare *et al.* 1984; Lehtónen-Veromaa *et al.* 1999; Du *et al.* 2001; El-Hajj Fuleihan *et al.* 2001; Looker *et al.* 2002). However, it is not known whether it is appropriate to use such thresholds, given the obvious physiological differences relative to the populations from which the thresholds have been derived. It has been suggested that different mechanisms regulate the secretion of PTH during adolescence as compared with adults (Guillemant *et al.* 1995). Elevated PTH concentrations may not be driven by the same mechanism in adolescents as in adults, and may not necessarily be detrimental to bone health; concentrations are normally raised during this time (Krabbe *et al.* 1982; Cadogan *et al.* 1998) as the rate of bone remodelling and consolidation is at a peak. Furthermore, it is not known whether suppressing PTH, and subsequently bone turnover, should be an aim within this population group, as it would be in adult studies, because raised bone turnover is an essential maturation process during this life stage. Inverse relationships between PTH and 25OHD levels have been found in both the 16–18-year-old boys and girls in the CBS, but the association occurs only during winter (Willett, 2004). The average Ca intakes of the adolescents in the CBS suggest that their serum Ca levels will not be compromised, thus avoiding further elevation of PTH. Thus, a sufficient 25OHD status may be more important in subjects with a low Ca intake. Furthermore, as a plateau was not evident, an insufficiency threshold cannot be easily identified in adolescents.

The ongoing difficulties and controversy associated with the relationship between PTH and 25OHD imply that this approach is not the best way to identify vitamin D sufficiency in populations. New approaches to this problem, potentially beyond the PTH–25OHD axis, should be sought.

Effects of vitamin D status on bone health

Ultimately, the importance of vitamin D status lies in its potential impact on bone health and, within adolescents, its possible association with optimum peak bone mass.

Bone mineral status has been found to be positively associated with 25OHD in adults. For example, a positive association between femoral neck BMD (Scharla *et al.* 1995) and bone strength (Nakamura *et al.* 2001) and 25OHD has been shown. Furthermore, vitamin D supplementation treatment for 25OHD insufficiency in 229 older men and women, which led to a marked rise in 25OHD, resulted in marked increases in spine and hip BMD (Kantorovich *et al.* 2000). However, not all studies in adults have found an association between bone mineral status and 25OHD (Sigurdsson *et al.* 2000; Patel *et al.* 2001). Fracture rates have also been used as an outcome in assessing the effect of vitamin D on bone health. Positive associations between reduced fracture rates and both dietary and supplemental vitamin D have been found (Chapuy *et al.* 1992; Feskanich *et al.* 2003; Trivedi *et al.* 2003). Limited studies in adults have also demonstrated an inverse correlation between bone turnover markers and 25OHD (Woitge *et al.* 1998; Jesudason *et al.* 2002).

In adolescents the association between bone health variables and 25OHD is less clear and the evidence is often conflicting. A number of studies in adolescents have observed a positive effect of 25OHD on forearm BMD (Outila *et al.* 2001) and lumbar spine BMD (Lehtónen-Veromaa *et al.* 2002). An association between forearm BMD and 25OHD concentration has been found in a study of 14–16-year-old Finnish girls (Outila *et al.* 2001). However, the association between BMD and 25OHD was only found in subjects with a 25OHD concentration < 40 nmol/l, and a significant correlation between BMD and PTH was not found. A positive association between lumbar spine BMD after 3 years from baseline and baseline 25OHD concentration has been reported in 9–15-year-old Finnish girls (Lehtónen-Veromaa *et al.* 2002). However, the subject group included gymnasts and athletes, whose types of physical activity may have affected their bone mineral status differently from controls; only time spent on activity was adjusted for in the analysis. Furthermore, as mentioned earlier, the use of BMD is not uniformly accepted as an accurate measure of bone mineral accretion in growing subjects.

The lack of an association between 25OHD concentration and bone turnover markers (El-Hajj Fuleihan *et al.* 2001) or bone mineral status (Kristinsson *et al.* 1998; Oliveri *et al.* 2000) has been noted in other studies of children and young adults. In 10–16-year-old Lebanese schoolchildren no significant association was found between 25OHD and the bone turnover markers osteocalcin and bone-specific alkaline phosphatase, even though 52 % of the subjects were categorised as vitamin D insufficient (defined as a 25OHD concentration between 25 and 50 nmol/l; El-Hajj Fuleihan *et al.* 2001). Furthermore, the absence of a significant association between bone resorption marker serum C-terminal telopeptide of type I collagen and bone formation marker N-terminal propeptide of type 1 procollagen and 25OHD has been noted in 11–16-year-old Swiss boys and girls (Ginty *et al.* 2005*a*). In the boys and girls in the CBS no significant association has been found between plasma markers of bone formation (osteocalcin, bone-specific alkaline phosphatase) and urinary resorption markers (N-terminal telopeptide of type I collagen and deoxypyridinoline/creatinine) and baseline 25OHD (Willett *et al.* 2005*b*), change in 25OHD or average 25OHD across the study, after adjusting for confounders (Willett, 2004). The relationship between bone mineral content size-adjusted bone mineral content or BMD and 25OHD has also been assessed at the whole body, lumber vertebrae 1–4, forearm and hip. Some significant positive associations have been found between bone mineral status and 25OHD in the CBS subjects, particularly at the hip, but these associations are largely diminished after size-adjustment (Willett, 2004).

The limited number of established associations between 25OHD and indices of bone health may illustrate the strong confounding effect of puberty on skeletal modelling and remodelling, and that plasma Ca and phosphate concentrations are regulated by factors that are not dependent on 25OHD (Kristinsson *et al.* 1998). In addition, it has been proposed that it is also possible that summer levels of 25OHD may compensate for wintertime deficiencies and,

therefore, that no overall change in bone health is seen (Oliveri *et al*. 2000).

It has also been suggested that during adolescence markers of bone turnover offer limited information, as they are better correlated with rates of linear growth than actual bone mineral accrual (Szulc *et al*. 2000). In addition, these markers are highly variable between individuals, and their concentrations can depend on the amount of marker secreted during bone formation and released from bone during bone resorption, on the catabolism of the marker, on variation associated with circadian rhythms, on the specificity of the marker for bone and on the specificity and precision of assays (Szulc *et al*. 2000).

Conclusions

Vitamin D insufficiency appears to be an important health issue in adolescents, although the optimum 25OHD level that needs to be maintained remains uncertain. In adolescents the CBS adolescents few significant relationships between 25OHD and indices of bone health persist after adjustment for body size. The effect of 25OHD on bone health in this population group remains unknown and may be confounded by stronger physiological influences of puberty. Further studies are needed in order to fully understand the relationship between PTH and 25OHD in older adolescents. Exploring greater numbers of subjects, longitudinally, with wider ranges of 25OHD levels and Ca intakes may help to clarify the nature of this relationship. Furthermore, ways of identifying insufficiency beyond the PTH–25OHD axis are also required. Adolescents are an important yet complex population group and it is suggested that they are considered in their own right, as mechanisms during this period appear to be different from those in children or adults. The work reported provides a further insight into the determinants of 25OHD in older adolescents and will contribute to the debate on the relative importance of 25OHD for achievement of optimum peak bone mass and osteoporosis prevention.

Acknowledgements

References

Abrams SA, Copeland KC, Gunn SK, Stuff JE, Clarke LL & Ellis KJ (1999) Calcium absorption and kinetics are similar in 7- and 8-year-old Mexican-American and Caucasian girls despite hormonal differences. *Journal of Nutrition* **129**, 666–671.

Ala-Houhala M, Parviainen MT, Pyykö K & Visakorpi JK (1984) Serum 25-hydroxyvitamin D levels in Finnish children aged 2 to 17 years. *Acta Paediatrica Scandinavica* **73**, 232–236.

Awumey EMK, Mitra DA, Hollis BW, Kumar R & Bell NH (1998) Vitamin D metabolism is altered in Asian Indians in the southern United States: A clinical research center study. *Journal of Clinical Endocrinology and Metabolism* **83**, 169–173.

Ballabriga A (2000) Morphological and physiological changes during growth: an update. *European Journal of Clinical Nutrition* **54**, Suppl. 1, S1–S6.

Bass S, Delmas PD, Pearce G, Hendrich E, Tabensky A & Seeman E (1999) The differing tempo of growth in bone size, mass, and density in girls is region-specific. *Journal of Clinical Investigation* **104**, 795–804.

Bates CJ, Carter GD, Mishra GD, 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. *Osteoporosis International* **14**, 152–159.

Binkley N, Krueger D, Cowgill CS, Plum L, Lake E, Hansen KE, DeLuca HF & Drezner MK (2004) Assay variation confounds the diagnosis of hypovitaminosis D: a call for standardization. *Journal of Clinical Endocrinology and Metabolism* **89**, 3152–3157.

Bonjour JP, Theintz G, Law F, Slosman D & Rizzoli R (1994) Peak bone mass. *Osteoporosis International* Suppl. 1, S7–S13.

Burnand B, Sloutskis D, Gianoli F, Cornuz J, Rickenbach M, Paccaud F & Burckhardt P (1992) Serum 25-hydroxyvitamin D: distribution and determinants in the Swiss population. *American Journal of Clinical Nutrition* **56**, 537–542.

Cadogan J, Blumsohn A, Barker ME & Eastell R (1998) A longitudinal study of bone gain in pubertal girls: anthropometric and biochemical correlates. *Journal of Bone and Mineral Research* **13**, 1602–1612.

Chapuy MC, Arlot ME, Duboeuf F, Brun J, Crouzet B, Arnaud S, Delmas PD & Meunier PJ (1992) Vitamin D3 and calcium to prevent hip fractures in the elderly women. *New England Journal of Medicine* **327**, 1637–1642.

Chapuy MC, Pamphile R, Paris E, Kempf C, Schlichting M, Arnaud S, Garneo P & Meunier P (2002) Combined calcium and vitamin D$_3$ supplementation in elderly women: confirmation of reversal of secondary hyperparathyroidism and hip fracture risk: The Decalyos II Study. *Osteoporosis International* **13**, 257–264.

Chapuy MC, Preziosi P, Maamer M, Arnaud S, Galan P, Hercberg S & Meunier PJ (1997) Prevalence of vitamin D insufficiency in an adult normal population. *Osteoporosis International* **7**, 439–443.

Chen TC, Parsons KS, Lu K, Mathieu JS & Holick MF (2000) An evaluation of the biologic activity and vitamin D receptor binding affinity of the photoisomers of vitamin D$_3$ and previtamin D$_3$. *Journal of Nutritional Biochemistry* **11**, 267–272.

Clemens MR (1989) The problem of rickets in UK Asians. *Journal of Human Nutrition and Dietetics* **2**, 105–116.

Cole TJ & Prentice A (1992) Bone mineral measurements. *British Medical Journal* **305**, 1223–1224.

Consensus Development Conference (1993) Diagnosis, prophylaxis and treatment of osteoporosis. *American Journal of Medicine* **94**, 646–650.

Cooper C, Campion G & Melton LJ 3rd (1992) Hip fractures in the elderly: a world-wide projection. *Osteoporosis International* **2**, 285–289.

Cromer B & Harel Z (2000) Adolescents: at increased risk for osteoporosis? *Clinical Pediatrics* **39**, 565–574.

Davies PS, Bates CJ, Cole TJ, Prentice A & Clarke PC (1999) Vitamin D: seasonal and regional differences in preschool children in Great Britain. *European Journal of Clinical Nutrition* **53**, 195–198.

Dawson-Hughes B, Harris SS & Dallal GE (1997) Plasma calcidiol, season, and serum parathyroid hormone concentrations in healthy elderly men and women. *American Journal of Clinical Nutrition* **65**, 67–71.

Department of Health (1998) *Nutrition and Bone Health: With Particular Reference to Calcium and Vitamin D*. London: H. M. Stationery Office.

Du X, Greenfield H, Fraser DR, Ge K, Trube A & Wang Y (2001) Vitamin D deficiency and associated factors in adolescent girls in Beijing. *American Journal of Clinical Nutrition* **74**, 494–500.

El-Hajj Fuleihan G, Nabulsi M, Choucair M, Salamoun M, Hajj Shahine C, Kizirian A & Tannous R (2001) Hypovitaminosis D in healthy schoolchildren. *Pediatrics* **107**, E53.

Ellis G, Woodhead JS & Cooke WT (1977) Serum-25-hydroxyvitamin-D concentrations in adolescent boys. *Lancet* **i**, 825–828.

Fehily AM, Coles RJ, Evans WD & Elwood PC (1992) Factors affecting bone density in young adults. *American Journal of Clinical Nutrition* **56**, 579–586.

Feskanich D, Willett WC & Colditz GA (2003) Calcium, vitamin D, milk consumption, and hip fractures: a prospective study among postmenopausal women. *American Journal of Clinical Nutrition* **77**, 504–511.

Fewtrell MS (2003) Bone densitometry in children assessed by dual x ray absorptiometry: uses and pitfalls. *Archives of Disease in Children* **88**, 795–798.

Fraser DR (1994) Effect of calcium deficiency on vitamin D metabolism. In *Nutrient Regulation during Pregnancy, Lactation and Infant Growth*, pp. 237–241 [L Allen, J King and B Lonnerdal, editors]. New York: Plenum Press.

Garrow JS, James WPT & Ralph A (2000) *Human Nutrition and Dietetics*, 10th ed. Edinburgh: Churchill Livingstone.

Gilsanz V (1998) Phenotype and genotype of osteoporosis. *Trends in Endocrinology and Metabolism* **9**, 184–190.

Ginty F, Cavadini C, Michaud PA, Burckhardt P, Baumgartner M, Mishra GD & Barclay DV (2005*a*) Effects of usual nutrient intake and vitamin D status on markers of bone turnover in Swiss adolescents. *European Journal of Clinical Nutrition* (In the Press).

Ginty F, Rennie KL, Mills L, Stear SJ, Jones SC & Prentice A (2005*b*) Positive, site-specific associations between bone mineral status, fitness and time spent at high impact activities in 16–18 year-old boys. *Bone* **36**, 101–110.

Gonzalez EA & Martin KJ (1998) Calcium, phosphorus and vitamin D. In *Handbook of Nutrition and the Kidney*, pp. 87–106 [WE Mitch and S Klahr, editors]. Philadelphia, PA: Lippincott-Raven.

Gregory J & Lowe S (2000) *National Diet and Nutrition Survey: Young People Aged 4 to 18 Years*. vol. 1, *Report of the Diet and Nutrition Survey*. London: The Stationery Office.

Guillemant J, Cabrol S, Allemandou A, Peres G & Guillemant S (1995) Vitamin D-dependent seasonal variation of PTH in growing male adolescents. *Bone* **17**, 513–516.

Guillemant J, Le HT, Maria A, Allemandou A, Pérès G & Guillemant S (2001) Wintertime vitamin D deficiency in male adolescents: effect on parathyroid function and response to vitamin D$_3$ supplements. *Osteoporosis International* **12**, 875–879.

Guillemant J, Taupin P, Le HT, Allemandou A, Pérès G & Guillemant S (1999) Vitamin D status during puberty in French healthy male adolescents. *Osteoporosis International* **10**, 222–225.

Gustavsson A, Thorsen K & Nordstrom P (2003) A 3-year longitudinal study of the effect of physical activity on the accrual of bone mineral density in healthy adolescent males. *Calcified Tissue International* **73**, 108–114.

Haddad JG (1999) The vitamin D binding protein and its clinical significance. In *Vitamin D: Physiology, Molecular Biology and Clinical Applications*, pp. 101–108 [MF Holick, editor]. Totowa, NJ: Humana Press.

Hampson G, Martin FC, Moffat K, Vaja S, Sankaralingam S, Cheung J, Blake GM & Fogelman I (2003) Effects of dietary improvement on bone metabolism in elderly underweight women with osteoporosis: a randomised controlled trial. *Osteoporosis International* **14**, 750–756.

Harris SS & Dawson-Hughes B (1998) Seasonal changes in plasma 25-hydroxyvitamin D concentrations of young American black and white women. *American Journal of Clinical Nutrition* **67**, 1232–1236.

Heaney RP (2003) Bone mineral content, not bone mineral density, is the correct bone measure for growth studies. *American Journal of Clinical Nutrition* **78**, 350–351.

Heaney RP, Abrams S, Dawson-Hughes B, Looker A, Marcus R, Matkovic V & Weaver C (2000) Peak bone mass. *Osteoporosis International* **11**, 985–1009.

Heaney RP, Davies KM, Chen TC, Holick MF & Barger-Lux MJ (2003) Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. *American Journal of Clinical Nutrition* **77**, 204–210.

Holick MF (1981) The cutaneous photosynthesis of previtamin D3: a unique photoendocrine system. *Journal of Investigative Dermatology* **77**, 51–58.

Holick MF (1995) Environmental factors that influence the cutaneous production of vitamin D. *American Journal of Clinical Nutrition* **61**, Suppl., 638S—645S.

Holick MF (1996) Vitamin D and bone health. *Journal of Nutrition* **126**, 1159S–1164S.

Holick MF (1999) Evolution, biologic functions, and recommended dietary allowances for vitamin D. In *Vitamin D: Physiology, Molecular Biology, and Clinical Applications*, pp. 1–16 [MF Holick, editor]. Totowa, New Jersey: Humana Press.

Holick MF (2001) Sunlight dilemma: risk of skin cancer or bone disease and muscle weakness. *Lancet* **357**, 4–6.

Holick MF (2004) Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *American Journal of Clinical Nutrition* **79**, 362–371.

Jacques PF, Felson DT, Tucker KL, Mahnken B, Wilson PW, Rosenberg IH & Rush D (1997) Plasma 25-hydroxyvitamin D and its determinants in an elderly population sample. *American Journal of Clinical Nutrition* **66**, 929–936.

Jesudason D, Need AG, Horowitz M, O'Loughlin PD, Morris HA & Nordin BE (2002) Relationship between serum 25-hydroxyvitamin D and bone resorption markers in vitamin D insufficiency. *Bone* **31**, 626–630.

Jouanny P, Guillemin F, Kuntz C, Jeandel C & Pourel J (1995) Environmental and genetic factors affecting bone mass. Similarity of bone density among members of healthy families. *Arthritis Rheumatology* **38**, 61–67.

Kantorovich V, Gacad MA, Seeger LL & Adams JS (2000) Bone mineral density increases with vitamin D repletion in patients with coexistent vitamin D insufficiency and primary hyperparathyroidism. *Journal of Clinical Endocrinology and Metabolism* **85**, 3541–3543.

Krabbe S, Transbøl I & Christiansen C (1982) Bone mineral homeostasis, bone growth, and mineralisation during years of pubertal growth: a unifying concept. *Archives of Disease in Childhood* **57**, 359–363.

Kristinsson JÖ, Valdimarsson G, Sigurdsson G, Franzson L, Olafsson I & Steingrimsdottir L (1998) Serum 25-hydroxyvitamin D levels and bone mineral density in 16–20 years-old girls: lack of association. *Journal of Internal Medicine* **243**, 381–388.

Kudlacek S, Schneider B, Peterlik M, Leb G, Klaushofer K, Weber K, Woloszczuk W & Willvonseder R (2003) Assessment of vitamin D and calcium status in healthy adult Austrians. *European Journal of Clinical Investigation* **33**, 323–331.

Kun Z, Greenfield H, Xueqin D & Fraser DR (2001) Improvement of bone health in childhood and adolescence. *Nutrition Research Reviews* **14**, 114–151.

Lamberg-Allardt CJ, Outila TA, Karkkainen MU, Rita HJ & Valsta LM (2001) Vitamin D deficiency and bone health in healthy adults in Finland: could this be a concern in other parts of Europe? *Journal of Bone and Mineral Research* **16**, 2066–2073.

Lawson M, Thomas M & Hardiman A (1999) Dietary and lifestyle factors affecting plasma vitamin D levels in Asian children living in England. *European Journal of Clinical Nutrition* **53**, 268–272.

Lehtónen-Veromaa M, Möttönen T, Irjala K, Kärkkäinen M, Lamberg-Allardt C, Hakola P & Viikari J (1999) Vitamin D intake is low and hypovitaminosis D common in healthy 9- to 15-year-old Finnish girls. *European Journal of Clinical Nutrition* **53**, 746–751.

Lehtónen-Veromaa M, Möttönen T, Nuotio I, Irjala K & Viikari J (2002) The effect of conventional vitamin D_2 supplementation on serum 25(OH)D concentration is weak among peripubertal Finnish girls: a 3-y prospective study. *European Journal of Clinical Nutrition* **56**, 431–437.

Lips P, Chapuy MC, Dawson-Hughes B, Pols HAP & Holick MF (1999) An international comparison of serum hydroxyvitamin D measurements. *Osteoporosis International* **9**, 394–397.

Looker AC, Dawson-Hughes B, Calvo MS, Gunter EW & Sahyoun NR (2002) Serum 25-hydroxyvitamin D status of adolescents and adults in two seasonal subpopulations from NHANES III. *Bone* **30**, 771–777.

Lund B, Sorensen OH, Bishop JE & Norman AW (1980) Vitamin D metabolism in hypoparathyroidism. *Journal of Clinical Endocrinology and Metabolism* **51**, 606–610.

Malabanan A, Veronikis IE & Holick MF (1998) Redefining vitamin D insufficiency. *Lancet* **351**, 805–806.

Margiloff L, Harris SS, Lee S, Lechan R & Dawson-Hughes B (2001) Vitamin D status of an outpatient clinic population. *Calcified Tissue International* **69**, 263–267.

Meyer HE, Smedshaug GB, Kvaavik E, Falch JA, Tverdal A & Pedersen JI (2002) Can vitamin D supplementation reduce the risk of fracture in the elderly? A randomized controlled trial. *Journal of Bone and Mineral Research* **17**, 709–715.

Nakamura K, Nashimoto M, Hori Y & Yamamoto M (2000) Serum 25-hydroxyvitamin D concentrations and related dietary factors in peri- and postmenopausal Japanese women. *American Journal of Clinical Nutrition* **71**, 1161–1165.

Nakamura K, Nashimoto M, Matsuyama S & Yamamoto M (2001) Low serum concentrations of 25-hydroxyvitamin D in young adult Japanese women: a cross sectional study. *Nutrition* **17**, 921–925.

National Osteoporosis Society Online (2004) Facts and figures. http://www.nos.org.uk/osteo.asp

Norman AW (1992) Bone biochemistry and physiology from the perspectives of the vitamin D endocrine system. *Current Opinion in Rheumatology* **4**, 375–382.

Norman AW (1998) Receptors for 1(alpha), $25(OH)_2D_3$: past, present and future. *Journal of Bone and Mineral Research* **13**, 1360–1369.

O'Hare AE, Uttley WS, Belton NR, Westwood A, Levin SD & Anderson F (1984) Persisting vitamin D deficiency in the Asian adolescent. *Archives of Disease in Childhood* **59**, 766–770.

Oliveri MB, Wittich A, Mautalen C, Chaperon A & Kizlansky A (2000) Peripheral bone mass is not affected by winter vitamin D deficiency in children and young adults from Ushuaia. *Calcified Tissue International* **67**, 220–224.

Outila TA, Kärkkäinen MUM & Lamberg-Allardt CJE (2001) Vitamin D status affects serum parathyroid hormone concentrations during winter in female adolescents: associations with forearm bone mineral density. *American Journal of Clinical Nutrition* **74**, 206–210.

Parfitt AM (1994) The two faces of growth: benefits and risks to bone integrity. *Osteoporosis International* **4**, 382–398.

Parsons TJ, Prentice A, Smith EA, Cole TJ & Compston JE (1996) Bone mineral mass consolidation in young British adults. *Journal of Bone and Mineral Research* **11**, 264–274.

Patel R, Collins D, Bullock S, Swaminathan R, Blake GM & Fogelman I (2001) The effect of season and vitamin D supplementation on bone mineral density in healthy women: a double-masked crossover study. *Osteoporosis International* **12**, 319–325.

Prentice A (2004) Diet, nutrition and the prevention of osteoporosis. *Public Health Nutrition* **7**, 227–243.

Prynne CJ, Ginty F, Paul AA, Bolton-Smith C, Stear SJ, Jones SC & Prentice A (2004) Dietary acid-base balance and intake of bone-related nutrients in Cambridge teenagers. *European Journal Clinical Nutrition* **58**, 1462–1471.

Raisz LG (1999) Physiology and pathophysiology of bone remodeling. *Clinical Chemistry* **45**, 1353–1358.

Reid DM (2003) An overview of osteoporosis. In *Nutritional Aspects of Bone Health*, pp. 1–12 [SA New and J-P Bonjour, editors]. Cambridge: Royal Society of Chemistry.

Riggs BL, Khosla S & Melton LJ III (1999) The assembly of the adult skeleton during growth and maturation: implications for senile osteoporosis. *Journal of Clinical Investigation* **104**, 671–672.

Root AW (2002) Bone strength and the adolescent. *Adolescent Medicine* **13**, 53–72.

Rubin LA, Hawker GA, Peltekova VD, Fielding LJ, Ridout R & Cole DE (1999) Determinants of peak bone mass: clinical and genetic analyses in a young female Canadian cohort. *Journal of Bone and Mineral Research* **14**, 633–643.

Rucker D, Allan JA, Fick GH & Hanley DA (2002) Vitamin D insufficiency in a population of healthy western Canadians. *Canadian Medical Association Journal* **166**, 1517–1524.

Russell AS, Dennison E & Cooper C (2003) Epidemiology and public health impact of osteoporosis. In *Nutritional Aspects of Osteoporosis*, pp. 13–24 [SA New and J-P Bonjour, editors]. Cambridge: Royal Society of Chemistry.

Saggese G, Baroncelli GI & Bertelloni S (2001) Osteoporosis in children and adolescents: diagnosis, risk factors, and prevention. *Journal of Pediatric Endocrinology and Metabolism* **14**, 833–859.

Saggese G, Baroncelli GI & Bertelloni S (2002) Puberty and bone development. *Best Practice Research Clinical Endocrinology and Metabolism* **16**, 53–64.

Salamone LM, Dallal GE, Zantos D, Makrauer F & Dawson-Hughes B (1993) Contributions of vitamin D intake and seasonal sunlight exposure to plasma 25-hydroxyvitamin D concentration in elderly women. *American Journal of Clinical Nutrition* **58**, 80–86.

Scharla SH, Scheidt-Nave C, Leidig G, Seibel MJ & Ziegler R (1995) Association between serum 25-hydroxyvitamin D and bone mineral density in a normal population sample in Germany. *Challenges of Modern Medicine* **7**, 325–328.

Sherman SS, Hollis BW & Tobin JD (1990) Vitamin D status related parameters in a healthy population: The effects of age, sex and season. *Journal of Clinical Endocrinology and Metabolism* **71**, 405–413.

Sigurdsson G, Franzson L, Steingrimsdottr L & Sigvaldason H (2000) The association between parathyroid hormone, vitamin D and bone mineral density in 70-year old Icelandic women. *Osteoporosis International* **11**, 1031–1035.

Silverberg SJ, Shane E, Dempster DW & Bilezikian JP (1999) The effects of vitamin D insufficiency in patients with primary

hyperparathyroidism. *American Journal of Medicine* **107**, 561–567.

Slemenda CW, Reister TK, Hui SL, Miller JZ, Christian JC & Johnston CC (1994) Influences on skeletal mineralization in children and adolescents: Evidence for varying effects of sexual maturation and physical activity. *Journal of Pediatrics* **125**, 201–207.

Souberbielle JC, Cormier C, Kindermans C, Gao P, Cantor T, Forette F & Baulieu EE (2001) Vitamin D status and redefining serum parathyroid hormone reference range for the elderly. *Journal of Clinical Endocrinology and Metabolism* **86**, 3086–3090.

Stear SJ, Prentice A, Jones SC & Cole TJ (2003) Effect of a calcium and exercise intervention on the bone mineral status of 16–18 y-old adolescent girls. *American Journal of Clinical Nutrition* **77**, 985–992.

Szulc P, Seeman E & Delmas PD (2000) Biochemical measurements of bone turnover in children and adolescents. *Osteoporosis International* **11**, 281–294.

Tangpricha V, Pearce EN, Chen TC & Holick MF (2002) Vitamin D insufficiency among free-living healthy young adults. *American Journal of Medicine* **112**, 659–662.

Tanner JM (1962) *Growth at Adolescence*, 2nd ed. Oxford: Blackwell Scientific Publications.

Thomas MK, Lloyd-Jones DM, Thadhani RI, Shaw AC, Deraska DJ, Kitch BT, Vamvakas EC, Dick IM, Prince RL & Finkelstein JS (1998) Hypovitaminosis D in medical inpatients. *New England Journal of Medicine* **338**, 777–783.

Trivedi DP, Doll R & Khaw KT (2003) Effect of four monthly oral vitamin D3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial. *British Medical Journal* **326**, 469.

Vander A, Sherman J & Luciano D (2001) *Human Physiology: The Mechanisms of Body Function*, 8th ed. New York: McGraw-Hill.

Vieth R (1999) Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. *American Journal of Clinical Nutrition* **69**, 842–856.

Vieth R & Carter G (2001) Difficulties with vitamin D nutrition research: objective targets of adequacy, and assays for 25-hydroxyvitamin D. *European Journal of Clinical Nutrition* **55**, 221–222.

Vieth R, Ladak Y & Walfish PG (2003) 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* **88**, 185–191.

Wang MC, Crawford PB, Hudes M, Van Loan M, Siemering K & Bachrach LK (2003) Diet in midpuberty and sedentary activity in prepuberty predict peak bone mass. *American Journal of Clinical Nutrition* **77**, 495–503.

Webb AR, DeCosta BR & Holick MF (1989) Sunlight regulates the cutaneous production of vitamin D_3 by causing its photodegradation. *Journal of Clinical Endocrinology and Metabolism* **68**, 882–887.

Webb AR & Holick MF (1988) The role of sunlight in the cutaneous production of vitamin D_3. *Annual Review of Nutrition* **8**, 375–399.

Willett AM (2004) Factors affecting vitamin D status in older adolescents and their relevance to bone health. PhD Thesis, University of Cambridge.

Willett AM, Ginty F & Prentice A (2004) Effect of calcium supplementation on vitamin D status in 16–18 year old girls. *Proceedings of the Nutrition Society* **63**, 39A.

Willett AM, Ginty F & Prentice A (2005a) Calcium supplementation does not modify vitamin D status in 16–18 year old boys. *Journal of Bone and Mineral Research* (In the Press).

Willett AM, Ginty F, Prentice A & Laidlaw A (2005b) 25-Hydroxyvitamin D status and effects on PTH, bone metabolism markers and bone mineral status in 16–18 year old boys and girls. *Journal of Steroid Biochemistry and Molecular Biology* (In the Press).

Woitge HW, Scheidt-Nave C, Kissling C, Leidig-Bruckner G, Meyer K, Grauer A, Scharla SH, Ziegler R & Seibel MJ (1998) Seasonal variation of biochemical indexes of bone turnover: results of a population-based study. *Journal of Clinical Endocrinology and Metabolism* **83**, 68–75.

Woitge HW & Seibel MJ (2001) Biochemical markers to survey bone turnover. *Rheumatic Disease Clinics of North America* **27**, 49–80.