Proceedings of the Nutrition Society (2003), **62**, 823–828 DOI:10.1079/PNS2003298

Vitamin D and bone health in early life

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Prolonged vitamin D deficiency resulting in rickets is seen mainly during rapid growth. A distinct age distribution has been observed in the Copenhagen area where all registered hospital cases of rickets were either infants and toddlers or adolescents from immigrant families. Growth retardation was only present in the infant and toddler group. A state of deficiency occurs months before rickets is obvious on physical examination. Growth failure, lethargy and irritability may be early signs of vitamin D deficiency. Mothers with low vitamin D status give birth to children with low vitamin D status and increased risk of rickets. Reports showing increasing rates of rickets due to insufficient sunlight exposure and inadequate vitamin D intake are cause for serious concern. Many countries (including the USA from 2003) recommend vitamin D supplementation during infancy to avoid rickets resulting from the low vitamin D content of human milk. Without fortification only certain foods such as fatty fish contain more than low amounts of vitamin D, and many children will depend entirely on sun exposure to obtain sufficient vitamin D. The skin has a high capacity to synthesize vitamin D, but if sun exposure is low vitamin D production is insufficient, especially in dark-skinned infants. The use of serum 25-hydroxyvitamin D to evaluate vitamin D status before development of rickets would be helpful; however, there is no agreement on cut-off levels for deficiency and insufficiency. Furthermore, it is not known how marginal vitamin D insufficiency affects children's bones in the long term.

Vitamin D: Bone: Early life

Vitamin D is the generic name for a group of closely-related seco-steroids with a biological effect and activity similar to that of cholecalciferol (also termed vitamin D_3) of animal or human origin (van den Berg, 1997). The closely-related ergocalciferol (also termed vitamin D_2) is formed from plant ergosterol. Severe prolonged vitamin D deficiency during growth will result in rickets.

History of rickets

Rickets has been known for at least 2000 years. In approximately 130 AD Soranus was aware of this disease affecting children in smoky Roman cities (Baxter-Jones *et al*. 2003). However, the disease was not considered a major problem until the industrialization of Northern Europe in the 17th century when the incidence of rickets became very high (Holick, 1999). It was also in the 17th century that Whistler and Glisson presented the classical description of rickets, and the relationship between rickets and socio-economic conditions became evident, for example, in England. In the 18th century Scottish fishermen already knew the value of cod oil in relation to rickets and in 1822 Sniadecki observed that children living in Warsaw had a higher incidence of rickets than children living in rural areas outside Warsaw. Based on this observation he recommended exposure to sunlight as a means of curing this disease (Holick, 1999). Palm, 70 years later, published a survey that reached the same conclusion (Holick, 1999). The understanding of the relationship with vitamin D, Ca and P was not resolved until the 1920s (Goel & Arneil, 1985). Despite these observations rickets was very prevalent in infants and toddlers in the first half of the 20th century in both the USA and Europe. In the years after the Second World War approximately 4 % of all infants in Copenhagen developed rickets (Flensborg & Thandrup, 1953), and at the present time rickets is again a growing problem in many industrialized countries, mainly because an increasing number of immigrants are moving to colder climates with less sunshine (Kreiter *et al*. 2000; Shaw & Pal, 2002; Pedersen *et al*. 2003).

Abbreviations: $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; 25OHD, 25-hydroxyvitamin D.

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Vitamin D metabolism and function

Man obtains vitamin D from two main sources, sun exposure and vitamin D-containing foods. The importance of one source depends on the availability of the other source. The skin contains the cholesterol precursor 7-hydrocholesterol. In response to sun exposure 7-hydroxycholesterol absorbs u.v.B light of wavelengths 290–315 nm. The energy uptake results in a bond cleavage and precholecalciferol is formed. The skin temperature induces a thermal isomerization of precholecalciferol resulting in the formation of cholecalciferol, which is transported immediately to the liver in the blood bound to vitamin D-binding protein (Holick, 1999). Foods may contain animal-derived cholecalciferol or plant-derived ergocalciferol. Plants and yeast synthesize ergocalciferol from ergosterol in response to sunlight by a process similar to the synthesis of cholecalciferol from 7-dehydrocholesterol in animals and man. Supplements may contain either cholecalciferol or ergocalciferol. The absorption of the fat-soluble vitamin D is dependent on bile salts and the lymphatic transport system. After synthesis in the skin or absorption from food, cholecalciferol and ergocalciferol undergo similar metabolism in the liver and subsequently in the kidneys (Fraser, 1995). Vitamin D undergoes 25-hydroxylation in the liver and in the human circulation 25-hydroxyvitamin D (25OHD) has a half-life of 3–4 weeks. It is considered to be the best biomarker for the accumulated effect of dietary vitamin D and vitamin D produced in response to sun exposure, and thereby the best indicator of vitamin D status (van der Wielen *et al*. 1995; Utiger, 1998). The most biologically-active form of vitamin D is 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), which is synthesized from 25OHD by 1α-hydroxylation in the proximal convoluted tubule of the kidneys; extra-renal synthesis may also take place (Fraser, 1995; Prentice, 2003). This synthesis is under strict control of calciotropic hormones such as parathyroid hormone. The most important function of $1,25(OH)_2D$ is stimulation of active Ca absorption in the intestines. If concentrations of serum Ca and serum phosphate are too low there will be reduced mineralization of new bone matrix (osteoid) produced by the osteoblast. Unmineralized osteoid results in low mineral:bone matrix (Department of Health, 1998), a condition characteristic of vitamin D deficiency in both childhood and adulthood. Normally, cholecalciferol and ergocalciferol are considered to have the same biological potency in the human body (van den Berg, 1997; Holick, 2002). However, this concept was questioned in a study in which there was a lower increase in serum 25OHD after supplementation with vitamin ergocalciferol than after supplementation with cholecalciferol (Trang *et al*. 1998).

Sunlight exposure

It is very difficult to establish an adequate level of sun exposure. If sun exposure is sufficient there is no requirement for vitamin D from food, and if the diet has a high vitamin D content there is no need for sun exposure. Defining adequate sun exposure is therefore dependent on the amount of vitamin D consumed in the diet. Decreased exposure to sunlight of the appropriate wavelength occurs during winter in countries in the higher and lower latitudes, because the sun's maximum angle is very oblique and the longer passage through the atmosphere decreases the extent of wavelengths that stimulate vitamin D synthesis. Seasonal variation in 25OHD is well known (Lund & Sørensen, 1979); for example, in Copenhagen (latitude 56°NE) exposure to sunlight during the months from October to March results in negligible production of vitamin D. Vitamin D production may also be reduced in sunny areas because of cloud cover or air pollution, a serious problem in many big cities. Agarwal *et al*. (2002) compared a group of 9–24-month-old children living in an area of Delhi, India that had high levels of atmospheric pollution with a comparable age-matched group of children living in an area of Delhi that had less pollution. Mean serum 25OHD levels were 12·4 and 27·1 ng/ml ($P < 0{\cdot}001$) respectively for the two areas. This situation is similar to the situation in many European cities 50–100 years ago (Flensborg & Thandrup, 1953; Holick, 1999). Agarwal *et al*. (2002) suggest that children living in an area with high air pollution should be offered vitamin D supplementation to prevent development of vitamin D-deficiency rickets. Lifestyles or a cultural practice associated with a reduction in time spent outdoors or an increase in the body surface area covered with clothing may also reduce production of vitamin D in the skin (Pedersen *et al*. 2003). Increased skin pigmentation is known to considerably reduce the capacity of the skin to produce vitamin D (Clemens *et al*. 1982). Another important factor in many countries is the use of sunscreens for protection from skin cancer (Etzel *et al*. 1999). Use of sunscreens with protection factor 15 reduces the production of cholecalciferol by 99·5 %. However, the skin has a very high capacity to produce vitamin D. Exposure to sunlight that causes minimum erythema of the skin (one minimal erythemal dose) of children and adults wearing a bathing suit is estimated to be equal to fifty times the recommendation of 5 µg (Holick, 2002). Exposure of one minimal erythemal dose of 6 % of the body is equal to an intake of 15–25 µg cholecalciferol. Thus, there is no need for prolonged periods of sunbathing since the production of precholecalciferol occurs immediately upon exposure to sunlight (Holick, 2002). In children slight tanning is a good indicator that they are making enough cholecalciferol.

Vitamin D-containing foods

Only a limited number of food products contain major amounts of vitamin D (van den Berg, 1997), which is the reason why both children and adults avoiding sun exposure are at risk of developing vitamin D deficiency. Flesh from oily fish such as salmon and mackerel contains high but variable amounts of vitamin D. Eating oily fish three to four times weekly is usually adequate to meet the vitamin D requirement in children (Holick, 2002). Cod-liver oil and oil from other fish are very good sources of vitamin D. Other food items such as meat may contain some 25OHD. As 25OHD is more water soluble than vitamin D it is at least partly absorbed via the portal venous system and is therefore less dependent on bile salts (van den Berg, 1997). In some countries a few foods are fortified with vitamin D. These fortified foods are most often milk products or margarine,

https://doi.org/10.1079/PNS2003298 Published online by Cambridge University Press

but cereals and some breads are also fortified. Unlike human milk, formula milk normally contains a relatively high amount of vitamin D (10 μg/l). Absorption from vitamin D supplements has been estimated to vary between 55 and 99 % in adults when given in oil or with fat that stimulates release of bile salts. Absorption from dietary sources is probably lower (van den Berg, 1997).

Fetus

The fetus is dependent on maternal status and the supply through the placenta for both Ca and vitamin D. Vitamin D is mainly transported through the placenta as 25OHD. During the third trimester there is an increase in the active form of vitamin D, $1,25(OH)_2D$, in the mother (Department of Health, 1998). The placenta synthesizes $1,25(OH)_2D$ from 25OHD for both the mother and the fetus. In the fetus $1,25(OH)_2D$ is also synthesized in the kidneys. The umbilical cord 25OHD level is positively correlated (r 0·81, $P < 0·0001$) with the plasma 25OHD level in the mother (Zeghoud *et al*. 1997), but the level is lower in the cord than in the mother (Nishimura *et al*. 2003). One study has shown that infants born during summer have a lower bone mineral content, higher serum osteocalcin and higher $1,25(OH)_2D$ than infants born during winter (Namgung *et al*. 1994). The authors suggest that vitamin D status during early pregnancy is of particular importance for the newborn's bone mineral content (Namgung *et al*. 1994). A small study comparing vitamin D status in newborns from mothers who underwent long-term hospitalization during pregnancy and mothers with normal pregnancy found significantly lower serum 25OHD in the hospitalized mothers compared with control mothers ($P < 0·01$), but no significant difference between the cord 25OHD in the two groups (Nishimura *et al*. 2003). However, cord 25OHD relative to maternal 25OHD was higher in the hospitalized group than in the control group (82·1 % *v*. 60·3 %), which may indicate a higher placental transfer in the group with the lower 25OHD. Congenital rickets is related to maternal vitamin D deficiency during pregnancy (Specker, 1994; Anatoliotaki *et al*. 2003). Poor maternal vitamin D status may adversely affect foetal growth, bone ossification, tooth enamel formation and neonatal Ca homeostasis (Specker, 1994). These studies underline the importance for the newborn of the mother's vitamin D status during pregnancy. The fight against rickets has to start with the pregnant women. In a recent review Ann Prentice (2003) concluded that supplementation of pregnant women at risk of vitamin D deficiency would reduce the risk of rickets and other problems of Ca handling in the newborn and might promote infant growth. Mothers at risk of vitamin D deficiency during pregnancy will include dark-skinned women with low vitamin D intake from both sunny (Anatoliotaki *et al*. 2003; Dawodu *et al*. 2003) and less sunny countries (Henriksen *et al*. 1995; Shaw & Pal, 2002) who avoid sun exposure.

Infants and toddlers

Neonates born of mothers with low 25OHD levels will have a poor vitamin D status from the start and will be at increased risk of developing rickets (Markestad, 1983). Most of the vitamin D activity in human milk is in the form of 25OHD. The relative amounts of ergocalciferol and cholecalciferol will depend on the mother's dietary intake and extent of sun exposure. Human breast milk normally contains only small amounts of vitamin D metabolites, including 25OHD (Food and Nutrition Board/Institute of Medicine, 1997), and children who are breast-fed and do not receive vitamin D supplementation or adequate exposure to sunlight are at high risk of developing vitamin D deficiency or manifesting rickets (Kreiter *et al*. 2000; Dawodu *et al*. 2003). The 25OHD content of human breast milk is influenced by maternal vitamin D status and dietary vitamin D intake, but there is no correlation between the level in breast milk and plasma 25OHD status in breast-fed infants except when the mother consumes high doses of vitamin D supplements (Department of Health, 1998). Comparison of vitamin D content of milk from white and black women in the same society has shown higher levels in milk from white women (1·58 μg/l *v*. 0·98 μg/l; Specker *et al*. 1985). This finding was explained by the higher vitamin D intake of white women compared with black women, and probably also a higher skin production in white women. There is also a seasonal variation in human milk vitamin D concentration. In Finland vitamin D activity in milk from unsupplemented women was higher in September (3·1 μg/l) compared with February (0·35 μg/l; Ala Houhala *et al*. 1988*b*). In the same study milk from supplemented women (25 μg vitamin D/d) had a higher vitamin D activity compared with milk from unsupplemented women (0·83 μg/l *v*. 0·35 μg/l; Ala Houhala *et al*. 1988*b*). Many years ago it was shown that intake of large quantities of fish liver oil could increase the vitamin D content of human milk (Polskin *et al*. 1945). However, if the infant were to be protected through human milk alone the mother's intake would have to be so high that there would be a risk of adverse effects in the mother (Ala Houhala *et al*. 1986). Thus, the breast-fed infant needs either sun exposure or supplementation to avoid vitamin D deficiency and rickets (Kreiter *et al*. 2000; Dawodu *et al*. 2003). In most countries there are recommendations for vitamin D supplementation during infancy in order to avoid rickets in breast-fed children (Michaelsen *et al*. 2000); doses of 5–10 μg/d are usually recommended (Food and Nutrition Board/Institute of Medicine, 1997; Department of Health, 1998; Michaelsen *et al*. 2000). As cases of rickets continue to be reported in the USA (Kreiter *et al*. 2000), the US National Academy of Sciences has recently recommended that all infants, including those exclusively breast-fed, have a minimum intake of 5 μg vitamin D (Gartner & Greer, 2003).

Rickets, except the congenital type, typically develops during the early months of life. Growth failure, lethargy and irritability are often early signs, followed by more detectable clinical changes such as craniotabes, costochondral beading, swelling of the distal ends of long bones (wrists and ankles) and bowing of the legs (Pedersen *et al*. 2003). Severe cases may present with spasm and hypocalcaemic seizures (Ahmed *et al*. 1995). If rickets is not treated prolonged vitamin D deficiency may lead to permanent dwarfing, gross bowing of the legs and a distorted pelvis that may give severe obstetric complications later for the girls. Providing all children with vitamin D supplements of 5–10 μg/d during

https://doi.org/10.1079/PNS2003298 Published online by Cambridge University Press

infancy should prevent the development of nutritional rickets during the first year of life (Gartner & Greer, 2003), except in cases of very low Ca intake (Okonofua *et al*. 1991). Regulations in most industrialized countries require that infant formula milk is fortified with vitamin D, usually 1–2·5 μg/420 kJ (100 kcal), equivalent to 10 μg/l in a standard formula with 1·5 μg/420 kJ (100 kcal) (Gartner & Greer, 2003).

Children who have received sufficient vitamin D during the first 4–8 months and thereafter an insufficient supply may develop signs of vitamin D deficiency at the age of 1–3 years, with stunting of growth, gross bowing of the legs, muscle weakness, walking problems and deformation of the pelvis. Despite severe leg deformations, healing of these skeletal deformations may still occur with the correct treatment with vitamin D (Biser-Rohrbaugh & Hadley-Miller, 2001).

Puberty

Rickets is seen mainly in periods of rapid growth and most often in the first years of life. However, late rickets or adolescent rickets occurring during the pubertal growth spurt is also well documented in both sunny (Narchi *et al*. 2001) and less sunny countries (Pedersen *et al*. 2003). In accordance with this finding, a distinct age distribution has been observed in the Copenhagen area (Pedersen *et al*. 2003), where all registered hospital cases of nutritional rickets were either infant and toddler or adolescent patients, mainly girls. Growth retardation was only present in the infant and toddler group. In late rickets a waddling gait, lower limb and back pain, bowing of the legs and muscle weakness are common symptoms (Pedersen *et al*. 2003). Hypocalcaemic tetany is also common (Narchi *et al*. 2001). Skeleton deformation is often permanent after late rickets despite corrective treatment. Adolescent rickets is most often seen in females who wear veils for religious reasons (Pedersen *et al*. 2003). In these cases sun exposure is inadequate, and these girls are totally dependent on food sources of vitamin D.

Prevention

It is easy to prevent rickets in all age-groups, and prevention should be given high priority. If sun exposure and vitamin D intake from the habitual diet are not sufficient, supplementation should be recommended (Gartner & Greer, 2003). Thus, supplementation is recommended for most breast-fed infants worldwide and for many immigrant children (and adults) in Western countries (Shaw & Pal, 2002; Pedersen *et al*. 2003), but also for girls and women in sunny countries in which wearing a veil is obligatory for religious reasons (Narchi *et al*. 2001). Children from families with special food customs, such as macrobiotic diets, may also be at particular risk (Dagnelie *et al*. 1990). If it is not practical to give daily supplements, the provision of a high single dose of 3·75 mg vitamin D to 5–11-year-old children at the beginning of autumn has been shown to maintain appropriate vitamin D status during winter without inducing hypercalcaemia or hypercalcuria (Oliveri *et al*. 1996).

However, well-designed randomized blinded intervention studies are needed in order to establish the vitamin D doses required in order to prevent bone problems in different age-groups.

Evaluation of vitmin D status

In order to prevent the development of severe vitamin D deficiency, biochemical markers of vitamin D status could be relevant for use in screening. The 25OHD levels reflect the body content of vitamin D. In patients with vitamin D deficiency the 25OHD level is decreased and levels of parathyroid hormone and alkaline phosphatase are often elevated (Pedersen *et al*. 2003). However, there is no international consensus on cut-off levels of 25OHD for vitamin D deficiency and insufficiency in children or adults. As in adults, an inverse relationship between parathyroid hormone and 25OHD has been found in infants and adolescents (Zeghoud *et al*. 1997; Guillemant *et al*. 1999). One proposal for the definition of a sufficient 25OHD level is the level of 25OHD in a population in which parathyroid hormone has reached a plateau. In a French study of male adolescents this level was as high as 83 nmol/l (Guillemant *et al*. 1999). However, there is no consensus on how to define optimal vitamin D status. Another approach would be to measure the effect of additional vitamin D on bone accretion and establish the level of 25OHD above which additional vitamin D had no further effect on bone accretion. In a double-blinded intervention study from Finland supplementation with 10 μg ergocalciferol five to seven times weekly for 1 year had no effect on height or mineral accretion in the distal radius when compared with a placebo (Ala Houhala *et al*. 1988*a*). The 25OHD level at baseline in January–February was approximately 19 ng/ml (50 nmol/l; Ala Houhala *et al*. 1988*a*). In a 3-year longitudinal study of Finnish girls aged 9–15 years Lehtonen-Veromaa *et al*. (2002) found that baseline 25OHD was positively correlated with unadjusted 3-year change in bone mineral density at the lumbar spine (r 0·35, $P < 0·001$) and femoral neck (r 0·32, $P < 0·001$) in all participants, and in the group with advanced sexual maturation at baseline there was a significant difference (4 %; $P = 0·01$ for trend) between bone accretion in lumbar spine in girls with severe hypovitaminosis D (25OHD < 20 nmol/l) compared with girls with normal vitamin D status (defined as 25OHD > 37·5 nmol/l). Another study from Finland has shown that in girls aged 9–15 years 13·4 and 67·7 % respectively had severe hypovitaminosis (25OHD < 20 nmol/l) and insufficient vitamin D status (25OHD < 37·5 nmol/l) during wintertime. There is no evidence, in terms of lower peak bone mass, to suggest that these levels of vitamin D are likely to be insufficient in the long term. In order to further investigate whether low 25OHD levels affect bone accretion and peak bone mass, more intervention studies are needed. Until their findings have been published it will not be established whether preventive evaluation of vitamin D status by 25OHD is relevant in infants, children and adolescents.

https://doi.org/10.1079/PNS2003298 Published online by Cambridge University Press

Conclusion

A sufficient vitamin D status in early life is essential for bone health. Supplementation with vitamin D should therefore be recommended for known risk groups for development of rickets, such as breast-fed infants, dark-skinned children from both sunny and non-sunny countries who avoid sun exposure, children in areas with severe air pollution and girls who are veiled for religious reasons. However, it is not known whether low vitamin D status without physical signs of insufficiency has any lasting negative effects on bone development in infants, children and adolescents. Until more data on relevant cut-off levels for 25OHD are available, recommendations for a healthy life that include sun exposure and intake of vitamin D-containing food products should be retained, and in cases of doubt about an individual's vitamin D status supplementations should be recommended to avoid possible negative effects.

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