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SYMPOSIUM ON 'NUTRITION AND FOOD PROBLEMS OF DIFFERENT AGE GROUPS'

Rickets and osteomalacia

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Although the relationship between rickets and vitamin D was recognized over 60 years ago it is only relatively recently that the metabolic pathway leading to the active form of vitamin D was recognized. We now understand that vitamin D in the form of one of its active metabolites is necessary for the maintenance of calcium homeostasis and we appreciate in part the mechanisms by which this is brought about. However, much still remains to be discovered about the aetiology of rickets and the role of vitamin D metabolites in bone development. In this article I would like briefly to review our understanding of some aspects of the aetiology of rickets and the research we have carried out into this still unsolved problem.

From the earliest days of vitamin D research it was appreciated that animals had two potential sources of this substance, namely the diet and the action of ultraviolet light (UVL) on skin 7-dehydrocholesterol. UVL of wavelength <315 nm breaks the C₉–C₁₀ bond with varying efficiency according to the particular wavelength, to form pre-vitamin D (Fig. 1). The latter molecule undergoes a temperature-dependent isomerization to yield vitamin D. It also undergoes other intermolecular rearrangements to yield tachysterol and lumisterol. Factors such as the wavelength of the UVL employed, temperature and solvent affect the nature of the products of this photoisomerization but at 37° about 7% is pre-vitamin D, 15% is lumisterol and tachysterol and about 78% is vitamin D. Pre-vitamin D is always present in any sample of vitamin D. In solution at room temperature about 20% may be pre-vitamin D with the percentage reducing to 5–10 at 37°.

Vitamin D is sparsely distributed in food (Table 1) so that without a national fortification policy dietary vitamin D intake would be less than 2.0 µg/d. Because the diet of most people contains only small amounts of vitamin D, and as rickets is

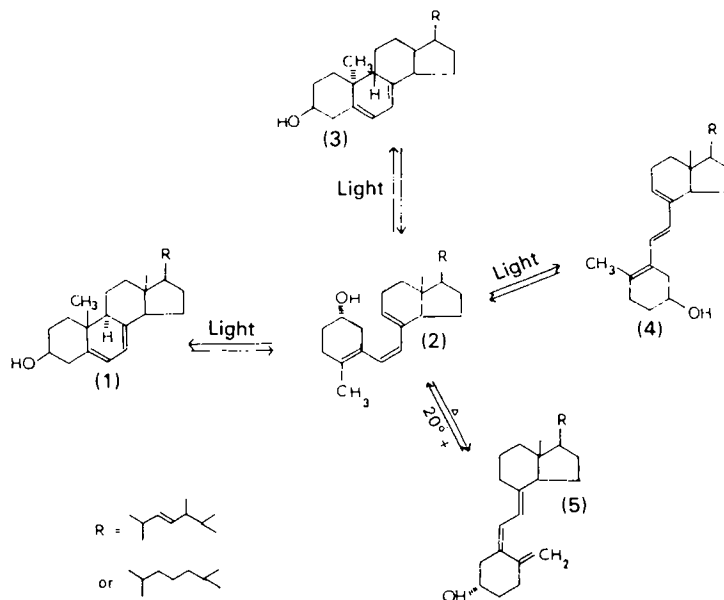


Fig. 1. Photochemical isomerization of 7-dehydrosterols and thermal isomerization of pre-vitamin D: (1), 7-dehydrocholesterol; (2), pre-vitamin D; (3), lumisterol; (4), tachysterol; (5), vitamin D. The two forms of the side chain (R) normally found are given.

Table 1. *Vitamin D content of food*

Food	Vitamin D concentration ($\mu\text{g}/\text{kg}$)
Butter	8
Milk (winter)	0.3
Milk (summer)	1.3
Cheese	0.3
Liver	2
Herring	220
Tuna	60
Sardines	75
Eggs	17

found in some countries with abundant UVL, many countries decided to fortify a specific food with vitamin D. In European countries margarine was frequently the vehicle of choice and in the USA and Canada the vitamin D content of milk was increased. These measures plus the fortification of infant feeding formulae with vitamin D virtually eliminated rickets from the developed nations. This was the position until recently when the reappearance of rickets in Britain and some other European countries has caused a reconsideration of the origin of this disease.

To understand the development of rickets and osteomalacia it is necessary to comprehend the relationship of the various vitamin D metabolites with each other and with the other steroids (Fig 2). Cholecalciferol is formed in the skin by one of the rare reactions in biology involving UVL. Subsequently the liver inserts a

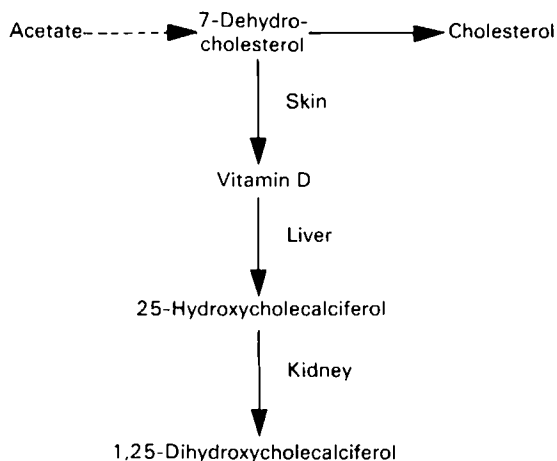


Fig. 2. Metabolic inter-relationship of 1,25-dihydroxycholecalciferol biosynthesis with cholesterol biosynthesis.

hydroxyl group at C-25 and the product, 25-hydroxycholecalciferol (25-OHD), is the main circulating form of substances with anti-rachitic activity. 25-OHD can be hydroxylated at a number of other sites including C-1, C-24 and C-26 but it is now accepted that the only product of physiological importance is 1,25-dihydroxycholecalciferol (1,25-(OH)₂D). The latter substance is the most biologically active of the vitamin-D-derived substances; it is the most rapidly acting metabolite, it is accumulated in its target tissues and it is active when given to anephric animals in contrast to other metabolites without a hydroxyl group at C-1. 1,25-(OH)₂D is synthesized only in the kidney in non-pregnant animals by a mechanism regulated by parathyroid hormone. Consequently 1,25-(OH)₂D is classed as a steroid hormone and the absence of this substance or any interference with its action in target tissues leads quite quickly to the development of all the manifestations of rickets. An interference with 1,25-(OH)₂D formation or function is one of the two reasons for the occurrence of rickets or osteomalacia. Vitamin-D-dependency rickets is an autosomal recessive disease in which the 1-hydroxylation reaction is defective. Such patients respond well to physiological doses of 1,25-(OH)₂D. Vitamin-D-resistant rickets is an X-linked dominant genetic defect. However, it does not respond to 1,25-(OH)₂D and in some cases, at least, is due to an absence of the tissue receptor for 1,25-(OH)₂D. Other clinical conditions are known, e.g. hypoparathyroidism involving an interference of 1,25-(OH)₂D formation or action resulting in defective Ca homeostasis and occasionally rickets. A general review to the background concerning vitamin D formation, metabolism and deficiency states can be found in Fraser (1980).

The second major cause of rickets is an interference with the supply of substrate, 25-OHD, to the renal 1-hydroxylase. The lack of this steroid can be due either to an increased breakdown and excretion or to inadequate supply of vitamin D. Although the turnover of 25-OHD is well known to be increased in almost all cases

of rickets, this increase has never been shown to be a causative factor. A low plasma 25-OHD level is invariably associated with rickets but on theoretical grounds 25-OHD half-lives will be higher at low plasma levels than at higher ones. The only established cause of an inadequate supply of 25-OHD is for the 'nutritional deficiency' rickets. In these cases the disease arises because of one of the following reasons: (1) an inadequate supply of vitamin D, (2) an increased metabolism of vitamin D or 25-OHD, (3) an increased requirement for vitamin D.

A 'nutritional deficiency' of vitamin D has been held as the cause of rickets in the groups listed in Table 2 and it is towards the three reasons listed above that we must look for the causative factor. Of the five groups in Table 2 the osteomalacia in pregnant women is almost certainly due to the demand of the fetus for vitamin D which, in the case of women with a low vitamin D status, could conceivably exhaust their limited tissue pools of this substance. For the other four groups, however, it has been usual to ascribe the disease to an inadequate intake of dietary vitamin D or to a limited exposure to sunshine. It was to try to establish the contribution of these two sources to the total body pool of vitamin D that we carried out a series of studies on the factors affecting vitamin D status. For the reasons given previously, plasma 25-OHD concentration is a good measure of vitamin D status. The plasma levels of this steroid were followed over a 12 month period of a group of children and of elderly people (Lawson *et al.* 1979; Poskitt *et al.* 1979). In both cases the maximum level was reached in early August and the minimum values in February (Fig 3). There was also a significant positive correlation between hours of sunshine and plasma 25-OHD levels. In the elderly there was a correlation between the time spent out of doors and vitamin D status, and between the maximum 25-OHD level and the minimum value in the following winter (Fig. 4).

The dietary vitamin D intake of the elderly was followed throughout the year. There was no relationship of this with the maximum plasma 25-OHD levels but there was a significant correlation with the minimum values seen in winter. Others have observed a correlation between dietary vitamin D levels and status (Hunt *et al.* 1976) but it seems that this relationship can be detected only when the plasma 25-OHD levels are low.

The current position, therefore, is that vitamin D status in summer is primarily governed by the amount of exposure to sunshine with dietary vitamin D in Britain making only a small and almost negligible contribution. During winter months, there is no UVL at northerly latitudes and consequently vitamin D is not formed in

Table 2. *Classification of nutritional deficiency rickets and osteomalacia according to age*

Preterm rickets
 Infant rickets
 Adolescent rickets
 Pregnancy osteomalacia
 Elderly osteomalacia

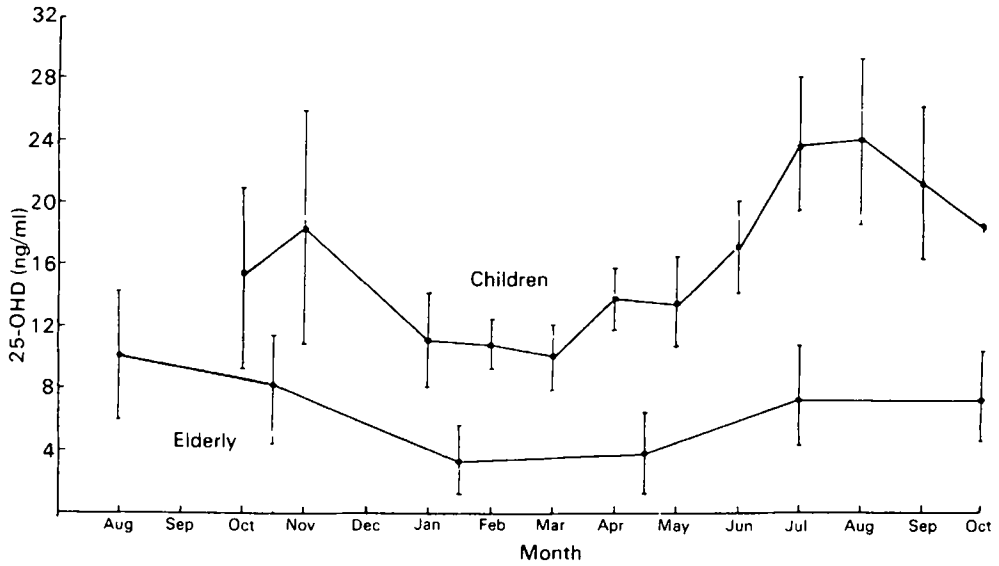


Fig. 3. Plasma 25-hydroxycholecalciferol (25-OHD) concentrations in children (■) and elderly subjects (●) at intervals over a minimum of 1 year. Mean values ± 1 SD represented by vertical bars. For the children a single blood sample was taken from each child from a total of 111 children over the year. The elderly were divided into three groups of eight and a blood sample obtained every 3 months. Only the results from one group are shown here. For the complete results see Lawson *et al.* 1979.

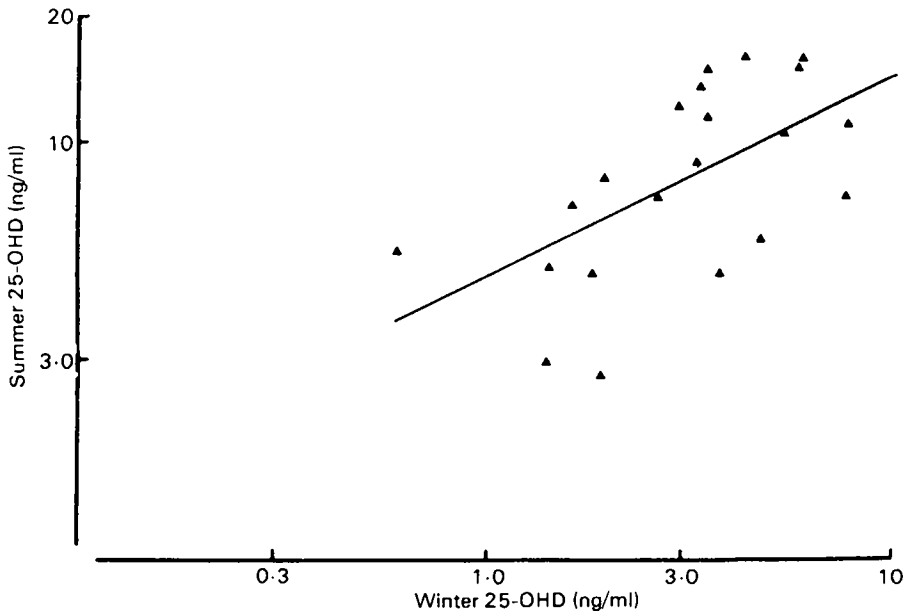


Fig. 4. Plasma 25-hydroxycholecalciferol (25-OHD) levels in elderly subjects in the north of England in late summer plotted against the value (in the same individual) at the end of the following winter. The winter level of 25-OHD appears to be dependent upon the summer level and the graph demonstrates that very few elderly subjects achieve summer levels of plasma 25-OHD adequate to maintain levels above 5 ng/ml in winter. Correlation coefficient (+ 0.58) is significant at the 1% level.

the skin during this period. The body therefore utilizes the vitamin D formed the previous summer and hence UVL is still indirectly the primary factor affecting vitamin D status. The contribution of dietary vitamin D to maintenance of vitamin D status can be observed only when the latter is low.

This conclusion on the relative importance of sunlight in maintaining vitamin D levels caused us to carry out a series of experiments to establish the amount of vitamin D synthesized in the skin of man and rat by a known quantity of UVL. Vitamin D was not detectable in human plasma (<2.0 ng/ml) of normal subjects unless the plasma 25-OHD levels rose above 25.0 ng/ml. Following repeated exposure to UVL, plasma 25-OHD rose until a steady state was reached after 5–6 weeks of irradiation. Skin vitamin D synthesis calculated from the steady-state equation was 0.6 ng/mJ (Davie *et al.* 1982). At northerly latitudes, as in Britain, exposure of the hands and face to sunlight in summer results in the synthesis of about 20 μ g of vitamin D daily. At the concentration of 7-dehydrocholesterol in skin, this level of vitamin D synthesis represents a low yield which could be due to inefficient conversion of the provitamin, or a rapid rate of metabolism and excretion of the vitamin D or the distribution of any newly formed vitamin D to the body tissues. In a series of animal experiments to obtain further information on these points, irradiation of isolated skin with UVL for 1 h showed that only 1% of rat skin 7-dehydrocholesterol was recoverable as vitamin D (Lawson *et al.* 1984). Irradiation of a known area of rat skin for 30 min on 3 d/week for 3 weeks resulted in a rise in the plasma concentration of both vitamin D and 25-OHD. Associated with this rise was an increase in vitamin D levels only in muscle and adipose tissue. 25-OHD was not detectable in adipose tissue irrespective of its level in plasma and only a very small amount was found in muscle. In normal stock rats with a plasma 25-OHD concentration of 9.0 ng/ml, vitamin D was undetectable in plasma but it was present in adipose tissue. In these rats the vitamin D levels of plasma were always lower than those in adipose tissue. Vitamin D concentration in the latter rose rapidly and reached 80 ng/g.

These observations have some interesting implications for our understanding of the processes involved in the maintenance of vitamin D status in the absence of a supply of the vitamin. It is clear that the changes seen in response to UVL are dependent upon the vitamin D status of the animal. In vitamin-D-deficient animals and in those people with a marginal vitamin D status, any exposure to UVL results in a rise in plasma 25-OHD levels. However, the extent of this rise is ultimately limited and eventually the rate of 25-hydroxylation is reduced and the vitamin begins to accumulate in adipose tissue and muscle.

It seems, therefore, that at least in rats there is some constraint on 25-hydroxylation but the conditions which cause it to operate are unknown. A possible factor is plasma 25-OHD concentration. The total amount of vitamin D found in muscle and adipose tissue at the end of the irradiation was very low considering that there are no other realistic sites at which substantial amounts of vitamin D might be found. In the vitamin-D-replete rats, muscle, adipose tissue

and plasma contained about 5% of the vitamin D which could have been produced by irradiating the skin for this period (i.e. 30 min \times 9 = 4.5 h). In the vitamin-D-deficient rats less than 2% of the vitamin D was in muscle and adipose tissue. While further studies are obviously necessary to establish a fuller picture of the fate of vitamin D formed in skin it is clear that substantial amounts are metabolized and probably excreted. Finally, these studies have confirmed previous reports that the formation of vitamin D in skin uses a very small fraction of the 7-dehydrocholesterol available (Yasumura *et al.* 1977).

It is premature to draw any firm conclusions from these investigations, particularly into the causes of the vitamin D deficiency causing rickets and osteomalacia in four of the five groups listed in Table 2. There may well be a different cause in each case. The low vitamin D status of the elderly leading sometimes to osteomalacia is clearly a consequence of the failure of this group to raise their plasma 25-OHD in summer significantly above 15 ng/ml (Fig 4). Although this may be due to inadequate exposure to sunlight at the optimum time of day, it is not obvious that the elderly are exposed to less sunshine than, for example, office workers or miners. Further information is necessary on the UVL received by individuals and the consequent vitamin D response. Adolescent or Asian rickets arises because of the stress of the growth phase at this period precipitating some with an already low vitamin D status into a rachitic state. Again the cause of this low vitamin D status is not clear but it is not always an inadequate exposure to sunlight (Dunnigan *et al.* 1977). The low levels of vitamin D in these cases must therefore be due to increased metabolism of vitamin D or its metabolites.

Although rickets of infancy has been eliminated due to the use of vitamin D supplements and fortification of infant feeding formulae, it is interesting to consider the cause of rickets in this age range in the past. Even today infant rickets occurs in many developing countries. In the absence of vitamin D supplements children born at the beginning of winter have only the vitamin D in their tissues at birth to meet their needs over their first 6 months. Only a better understanding of the size of these pools and their rate of utilization will explain how they can be inadequate. Finally, preterm rickets still awaits an explanation as the controversy is unresolved between whether a lack of vitamin D or inadequate availability of Ca is the causative factor (Tsang, 1983).

These studies emphasize the importance of further information on the following two points. First, there is a need to know the actual amount of UVL received by individuals in different sections of the population in their normal life and the factors limiting the conversion of 7-dehydrocholesterol to vitamin D to less than 5% of the maximum possible. Second, the factors affecting the rate of utilization and metabolism of vitamin D and 25-OHD should be identified. With this information it should be possible to achieve a rational policy on the need for further supplementation of the British diet with vitamin D to improve the vitamin D status of the elderly and immigrants from India and Pakistan.

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