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Old wine in new bottles: vitamin D in the treatment and prevention of tuberculosis

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Tuberculosis (TB) is a major cause of mortality, responsible for 1·68 million deaths worldwide in 2009. The global prevalence of latent *Mycobacterium tuberculosis* infection is estimated to be 32%, and this carries a 5–20% lifetime risk of reactivation disease. The emergence of drug-resistant organisms necessitates the development of new agents to enhance the response to antimicrobial therapy for active TB. Vitamin D was used to treat TB in the pre-antibiotic era, and its active metabolite, 1,25-dihydoxyvitamin D, has long been known to enhance the immune response to mycobacteria *in vitro*. Vitamin D deficiency is common in patients with active TB, and several clinical trials have evaluated the role of adjunctive vitamin D supplementation in its treatment. Results of these studies are conflicting, reflecting variation between studies in baseline vitamin D status of participants, dosing regimens and outcome measures. Vitamin D deficiency is also recognised to be highly prevalent among people with latent *M. tuberculosis* infection in both high- and low-burden settings, and there is a wealth of observational epidemiological evidence linking vitamin D deficiency with increased risk of reactivation disease. Randomised controlled trials of vitamin D supplementation for the prevention of active TB have yet to be performed, however. The conduct of such trials is a research priority, given the safety and low cost of vitamin D supplementation, and the potentially huge public health consequences of positive results.

Tuberculosis: Vitamin D: Immunomodulation: Clinical trials

Tuberculosis (TB) is a major public health problem. The global prevalence of latent *Mycobacterium tuberculosis* (MTB) infection has been estimated at 32%[1], and this carries a 5–20% lifetime risk of reactivation disease in people who are not infected with HIV[2]; reactivation rates higher than 10% per annum have been reported in HIV-infected people[3]. The WHO estimates that in 2009 there were 9·4 million incident cases of active TB, 14 million prevalent cases of TB, 1·3 million deaths from TB in HIV-uninfected people and 0·38 million deaths from TB in HIV-infected people[4]. The development of new agents to prevent acquisition or reactivation of latent MTB infection and to allow shortening of antimicrobial therapy regimens for active TB without loss of efficacy is a research priority. This paper reviews the growing body of evidence from studies conducted both *in vitro* and *in vivo* suggesting that vitamin D might have a role in the prevention and treatment of TB.

Abbreviations: $1,25(OH)_2D$, 1,25-dihydoxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; DBP, vitamin D binding protein; Gc2, group-specific component 2; MTB, *Mycobacterium tuberculosis*; TB, tuberculosis; VDR, vitamin D receptor.

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Immunomodulatory actions of vitamin D in mycobacterial infection

With the exception of a single report[5], vitamin D and its metabolites have not been shown to possess anti-mycobacterial activity in the absence of cells. However, the active metabolite of vitamin D, 1,25-dihydoxyvitamin D ($1,25(OH)_2D$), has long been recognised to induce anti-mycobacterial activity *in vitro* in mononuclear phagocytes, the cells that control growth of MTB[6]. Ligation of macrophage Toll-like receptor 2/1 heterodimers by mycobacterial antigens induces expression of the vitamin D receptor (VDR) and the 1-α hydroxylase enzyme CYP27B1[7,8], which synthesises $1,25(OH)_2D$ from the principal circulating vitamin D metabolite 25-hydroxyvitamin D (25(OH)D). Because extra-renal 1-α hydroxylase follows first-order kinetics, the rate at which it synthesises $1,25(OH)_2D$ depends on the availability of 25(OH)D substrate[9]. Orally ingested vitamin D is freely converted to 25(OH)D[10], and this provides the rationale for administering 'parent' vitamin D to induce anti-mycobacterial responses at the site of disease.

$1,25(OH)_2D$ modulates immune responses by ligating membrane VDR to induce rapid effects (within minutes) or nuclear VDR to induce genomic effects (within hours)[11]. Experiments using selective agonists and antagonists of these two receptors indicate that ligation of nuclear VDR is both necessary and sufficient for induction of anti-mycobacterial responses by $1,25(OH)_2D$ *in vitro*[12]. $1,25(OH)_2D$ modulates the host response to mycobacterial infection by pleiotropic mechanisms including the induction of reactive nitrogen and oxygen intermediates[13,14], down-regulation of the gene encoding tryptophan-aspartate containing coat protein[15], promotion of phagolysosome fusion[16], suppression of matrix metalloproteinase enzymes implicated in the pathogenesis of pulmonary cavitation[17] and induction of antimicrobial peptides including cathelicidin LL-37[7,12] and human β-defensin 2[18]. Cathelicidin LL-37 possesses antimycobacterial activity[7,19] and also induces autophagy[20,21]; $1,25(OH)_2D_3$-induced antimycobacterial activity has been reported to be dependent on expression of the gene encoding cathelicidin LL-37[22].

Vitamin D and tuberculosis: historical aspects

The clinical features of vitamin D deficiency were first described in 1651, when Glisson, Bate and Regemorter published 'A treatise of the rickets: being a disease common to children'[23]. In addition to noting the classical musculoskeletal features of rickets, the authors made the following observation from an autopsy of an infant with the condition: 'One amongst us doth attest, that he saw glandulous knobs and bunches so numerous that they seemed to equalise, if not exceed, the magnitude of the lungs themselves; they were situated between the lungs and the mediastinum … and were extended from the Canel bone to the Diaphragma'. TB is a well-recognised cause of mediastinal lymphadenopathy in children[24] and it is interesting to speculate whether this represents the earliest case report of TB associated with vitamin D deficiency.

Some 200 years later, Chapman reported results of administering cod liver oil to patients with 'consumption', and made the following observation: 'the beneficial effects of the oil were manifested most speedily and most decisively, in the improvement of the appetite, aspect of the countenance, strength and spirits'. He concluded that cod liver oil was 'probably the only remedial agent by which the vital powers may be enabled to struggle successfully against that malady'[25]. This report represents the first circumstantial evidence that administration of a preparation containing vitamin D improved clinical outcome in patients with TB, although it should be noted that no control group was studied, and that any beneficial effects of cod liver oil may have been attributable to its content of vitamin A rather than vitamin D[26]. The first TB sanatorium was opened in Gorbersdorf, Germany (today Sokolowsko, Poland) in 1859, and heliotherapy (exposure of TB patients to sunlight, which induces cutaneous vitamin D synthesis) subsequently became common practice, and was credited with improvements in clinical outcome in many cases[27]. In 1903, Niels Finsen was awarded the Nobel Prize in Physiology or Medicine for his discovery that shortwave UV light was effective in the treatment of cutaneous TB[28]. Vitamin D_2 was purified and crystallised in 1931[29] and Charpy subsequently pioneered the use of pharmacologic doses (≥1·25 mg daily) of vitamin D_2 to treat cutaneous TB[30]. Vitamin D_2 was also used to treat pulmonary TB, both as a single agent and, following the introduction of effective anti-TB chemotherapy, as an adjunct to antibiotic treatment[31].

Association between vitamin D deficiency and susceptibility to tuberculosis

In 1985, Davies observed that people migrating to the United Kingdom from countries with a high incidence of latent MTB infection experienced rates of active TB that exceeded rates in their countries of origin, and that this increased risk coincided with the development of vitamin D deficiency, probably arising as a result of decreased sun exposure[32]. He suggested that vitamin D deficiency may predispose to reactivation of latent MTB infection in this setting, a hypothesis supported by his observation that vitamin D deficiency associated with susceptibility to active TB[33]. Since then, eleven case–control studies investigating the association between vitamin D status and susceptibility to active TB have been published. Of these, seven have reported a statistically significant association between vitamin D deficiency and susceptibility to active TB[34–40], three have reported a non-statistically significant trend towards such an association[41–43] and one[44] has reported that active TB was associated with both 'high' and 'low' serum 25(OH)D concentrations (>140 and <75 nmol/l, respectively). Potential explanations for an association between vitamin D deficiency and active TB include both causality (i.e. vitamin D deficiency impairs host immune response to MTB and causes susceptibility) and reverse causality (i.e. active TB causes vitamin D deficiency, due to anorexia, decreased exposure to sunlight

in debilitated patients, or MTB-induced dysregulation of vitamin D metabolism[38]).

Association between susceptibility to tuberculosis and polymorphisms in the vitamin D receptor and vitamin D binding protein

Human VDR is encoded by the *VDR* gene located on chromosome 12q. This gene is polymorphic, and numerous SNP have been described. The hypothesis that VDR variants might associate with susceptibility to active TB was first investigated by Bellamy *et al*., who reported an association between carriage of the T allele of the *TaqI* VDR polymorphism and susceptibility to active TB in a case–control study conducted in Gambian adults[45]. Wilkinson *et al*. subsequently reported that associations between susceptibility to TB and carriage of the T allele of the *TaqI* VDR polymorphism and the ff genotype of the *FokI* VDR polymorphism in Gujarati Asians living in London were restricted to vitamin D-deficient individuals[36]; this study is the first to report that gene–environment interactions may operate to influence susceptibility to active TB. Numerous case–control studies investigating the association between VDR variants and susceptibility to active TB have since been published; a recent meta-analysis of twenty-three such studies reported that in Asian populations, the *FokI ff* genotype associated with susceptibility to active TB (pooled OR 2·0, 95 % CI 1·3, 3·2), and the BsmI *bb* genotype (defined by the presence of two restriction sites for the *Bsm*1 endonuclease) was associated with protection against active TB (pooled OR 0·5, 95 % CI 0·4, 0·8); no associations were seen in African or Latin American populations, however[46].

Further case–control studies have investigated associations between polymorphisms in the vitamin D binding protein (DBP) and susceptibility to active TB. DBP is a highly expressed multifunctional 58 kDa serum glycoprotein encoded on chromosome 4. Two common polymorphisms at codons 416 and 420 of exon 11 of the *DBP* gene give rise to the three major electrophoretic variants of DBP, termed group-specific component 1 fast, group-specific component 1 slow and group-specific component 2 (Gc2). These variants differ in their functional characteristics: the group-specific component 1 fast and group-specific component 1 slow variants have been reported to have greater affinity for 25(OH)D than the Gc2 variant[47], potentially leading to more efficient delivery of 25(OH)D to the target tissues, while the Gc2 variant is associated with decreased circulating concentrations of 25(OH)D, $1,25(OH)_2D$ and DBP[48,49]. Case–control studies conducted in India, Russia and Kuwait have not reported any association between DBP genotype and susceptibility to TB[50–52], but a more recent study reported an association between the Gc2 allele of DBP and susceptibility to active TB among Gujarati Asians living in London. This association was preserved if serum 25(OH)D concentration was <20 nmol/l, but not if serum 25(OH)D was ≥ 20 nmol/l, suggesting that profound vitamin D deficiency and Gc2 genotype may interact to increase susceptibility to TB[43].

Prospective observational studies

In contrast to the numbers of published cross-sectional studies, relatively few cohort studies investigating associations between vitamin D status or VDR genotype and TB have been conducted. Two studies have examined the influence of VDR genotype on response to antimicrobial therapy: Roth *et al*. reported that the *FF* genotype of the *FokI* VDR polymorphism and the *Tt* genotype of the *TaqI* VDR polymorphism associated with faster sputum culture conversion in a cohort of pulmonary TB patients in Peru[53], while Babb *et al*. reported no difference in time to sputum culture conversion according to *TaqI* or *FokI* VDR genotype among South African TB patients[54]. Recently, a cohort study conducted in Pakistan[55] reported that profound vitamin D deficiency among healthy household TB contacts at baseline associated with increased risk of development of active TB over the subsequent 4 years: seven out of thirty contacts with baseline plasma 25(OH)D $<17{\cdot}5$ nmol/l developed active TB during follow-up, compared with one of thirty-two with plasma 25(OH)D 17·5–33·5 nmol/l and none of thirty with plasma 25(OH)D $>33{\cdot}5$ nmol/l. This association retained significance after adjustment for age and sex, although other potential confounders were not taken into account in the analysis. The observation that increased risk of TB reactivation was almost exclusively confined to individuals with profound vitamin D deficiency is interesting, particularly when taken together with reports from case–control studies that profound vitamin D deficiency is most strongly associated with susceptibility to TB[36]: the implication is that, if vitamin D deficiency does indeed predispose to active TB, then relatively modest elevations of serum 25(OH)D might be effective for the prevention of active disease.

Intervention studies

Despite the compelling results from the laboratory and observational studies reviewed above, no randomised controlled trials of vitamin D supplementation for the prevention of active TB have been published to date. The absence of such studies reflects the very considerable methodological and logistic challenges of conducting them. Because the annual risk of reactivation of latent TB is low in immunocompetent individuals (<1 % even in individuals with a strongly positive and newly converted tuberculin skin test[2]), very large sample sizes and prolonged follow-up will be needed to detect all but the largest effects of vitamin D supplementation on TB incidence in such populations. One study has attempted to circumvent this problem by investigating the effect of vitamin D supplementation on a surrogate outcome measure of antimycobacterial response: the BCG-lux assay, which measures the ability of whole blood to restrict bioluminescence of a reporter mycobacterium[56]. This investigation found that a single dose of 2·5 mg vitamin D enhanced the ability of TB contacts' whole blood to restrict mycobacterial bioluminescence at 24 h post-inoculation[57], providing further evidence that trials of vitamin D supplementation for the prevention of TB are justified.

Table 1. Summary of randomised controlled trials investigating effects of adjunctive vitamin D in patients with tuberculosis (TB)

Reference	Sample size, setting	Vitamin D dose administered	Effect on serum 25(OH)D concentration	Primary outcome
Gwinup *et al.*[58]	Twenty-three adults, USA	125 μg vitamin D_2 daily	Not reported	Serum Ca: no change
Narang *et al.*[60]	Thirty adults, India	10–95 μg daily*	Not reported	Serum Ca: hypercalcaemia in 63 %
Morcos *et al.*[63]	Twenty-four children, Egypt	25 μg daily*	Not reported	Body weight/symptoms: no change
Nursyam *et al.*[64]	Sixty-seven adults, Indonesia	250 μg daily*	Not reported	Smear conversion: increased rate at 6 weeks
Martineau *et al.*[59]	Twenty-five adults, UK	1 × 2·5 mg vitamin D_2 @ 0 months	22 nmol/l increase in active arm	Serum 25(OH)D: small increase at 8 weeks
Wejse *et al.*[65]	365 adults, Guinea Bissau	3 × 2·5 mg vitamin D_3 @ 0/5/8 months	25 nmol/l increase both arms	TB score: no effect
Martineau *et al.*[66]	146 adults, UK	4 × 2·5 mg vitamin D_3 @ 0/2/4/6 weeks	79 nmol/l increase in active arm	Culture conversion: no effect in study population as a whole, but effect seen in subgroup with *tt* genotype of the *TaqI* VDR polymorphism

25(OH)D, 1,25-dihydroxyvitamin D; VDR, vitamin D receptor.
*Type of vitamin D (D_2 *v.* D_3) not reported.

In contrast to prevention studies, randomised controlled trials to determine whether adjunctive vitamin D enhances response to antimicrobial therapy can be powered with more modest numbers of participants, because the majority of TB patients respond rapidly to antimicrobial therapy. Seven such studies have been published to date (summarised in Table 1). Three of these trials had biochemical primary outcomes: two reported no hypercalcaemia in TB patients receiving either 125 μg vitamin D daily[58] or a single oral dose of 2·5 mg vitamin D[59], and one reported hypercalcaemia occurring in nineteen of thirty TB patients receiving daily doses of 10–95 μg vitamin D[60]. However, this third study, by Narang *et al.*, also reported that a daily dose of 60 μg vitamin D elevated mean serum Ca in healthy controls; a finding that contrasts with other studies which demonstrate that identical[61] or considerably higher[62] doses of vitamin D do not induce hypercalcaemia in healthy people. It is possible, therefore, that the actual doses of vitamin D administered in Narang's study were considerably higher than reported. The remaining four clinical trials listed in the table had clinical primary outcomes. Morcos *et al.* investigated the effects of 25 μg vitamin D daily on twenty-four children in Egypt receiving antimicrobial therapy for TB, and showed no effect on body weight or resolution of symptoms[63]. Nursyam *et al.* subsequently conducted a trial of a daily dose of 250 μg vitamin D in sixty-seven pulmonary TB patients in Indonesia[64]. In this study, adjunctive vitamin D enhanced sputum smear conversion at 6 weeks after initiation of antimicrobial therapy (thirty-four out of thirty-four *v.* twenty-five out of thirty-three smear-converted in intervention *v.* control arm at 6 weeks, $P = 0{\cdot}002$); no effect of the intervention was seen at 8 weeks. The vitamin D status of participants was not assessed at either baseline or follow-up in this study, and details of safety monitoring, including monitoring of serum Ca concentrations, were not reported. In the largest treatment trial published to date, Wejse *et al.* randomised 365 adult TB patients in Guinea–Bissau to receive three doses of 2·5 mg vitamin D_3 or placebo at initiation of antimicrobial therapy, and again at 5 and 8 months[65]. The intervention had no effect on the primary outcome measure (a specially designed TB score) or on serum 25(OH)D concentration. Mean serum 25(OH)D concentrations at baseline were 78 nmol/l *v.* 79 nmol/l in intervention *v.* control groups. Most recently, another trial investigated the effect of a 2-weekly dose of 2·5 mg vitamin D on time to sputum culture conversion in 146 patients with smear-positive pulmonary TB in the UK[66]. A 79 nmol/l increase in serum 25(OH)D was seen among participants in the intervention arm of the study, which was associated with a non-statistically significant trend towards faster sputum culture conversion ($P = 0{\cdot}14$). A pre-planned subgroup analysis revealed that adjunctive vitamin D significantly hastened sputum culture conversion by more than 17 d in participants with the *tt* genotype of the *Taq*I VDR polymorphism (hazard ratio 8·09, 95 % CI 1·36, 48·01; $P = 0{\cdot}02$).

Conclusions

Much remains to be done to evaluate whether vitamin D might have a role in the prevention or treatment of TB. A key research priority is to establish randomised controlled trials of vitamin D supplementation for the prevention of TB in individuals with latent MTB infection. Although some question the need for such studies to be conducted on the grounds that data from observational studies are suggestive, and that the methodological challenges of conducting such trials are too great[67], I remain convinced that these trials are necessary, fundable and feasible. Equivalent trials have been conducted to establish the role of chemoprophylaxis for TB prevention[68], and investigation of the role of vitamin D supplementation in this regard should be no less of a research priority, given the safety and low cost of this intervention. Investigations of the potential role of vitamin D as an adjunct to antimicrobial therapy are more advanced, but results from clinical trials published to date have shown little if any benefit in drug-sensitive disease. This is not the end of the road for this line of enquiry, however. First, five other similar trials are ongoing[67]; a meta-analysis of the results

of these studies may reveal a benefit that existing studies have not been powered to demonstrate. Second, the doses of vitamin D administered in trials conducted to date are considerably lower than those reported to be effective historically[31]; the effects of pharmacological dosing regimens are worthy of investigation. Finally, on-going investigations from a recently completed trial[66] reveal that administration of adjunctive vitamin D is associated with favourable immunomodulatory activity; this observation raises the possibility that individuals with multi-drug resistant TB, in whom antimicrobial therapy is less effective, might derive a clinical benefit from enhancement of their antimycobacterial immune response using adjunctive vitamin D therapy.

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References

1. Dye C, Scheele S, Dolin P *et al.* (1999) Consensus statement. Global burden of tuberculosis: Estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. *JAMA* **282**, 677–686.
2. Horsburgh CR Jr (2004) Priorities for the treatment of latent tuberculosis infection in the United States. *N Engl J Med* **350**, 2060–2067.
3. Wood R, Maartens G & Lombard CJ (2000) Risk factors for developing tuberculosis in HIV-1-infected adults from communities with a low or very high incidence of tuberculosis. *J Acquir Immune Defic Syndr* **23**, 75–80.
4. WHO (2010) *Global Tuberculosis Control: WHO Report 2010*. Geneva: WHO Press.
5. Raab W (1946) Vitamin D – its bactericidal action. *Chest* **12**, 409–415.
6. Rook GA, Steele J, Fraher L *et al.* (1986) Vitamin D_3, gamma interferon, and control of proliferation of *Mycobacterium tuberculosis* by human monocytes. *Immunology* **57**, 159–163.
7. Liu PT, Stenger S, Li H *et al.* (2006) Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science* **311**, 1770–1773.
8. Krutzik SR, Hewison M, Liu PT *et al.* (2008) IL-15 links TLR2/1-induced macrophage differentiation to the vitamin D-dependent antimicrobial pathway. *J Immunol* **181**, 7115–7120.
9. Vieth R, McCarten K & Norwich KH (1990) Role of 25-hydroxyvitamin D_3 dose in determining rat 1,25-dihydroxyvitamin D_3 production. *Am J Physiol* **258**, E780–E789.
10. Vieth R (2005) The pharmacology of vitamin D, including fortification strategies. In *Vitamin D* pp. 995–1015 [FH Glorieux, JW Pike and D Feldman, editors]. London: Academic Press.
11. Norman AW, Mizwicki MT & Norman DP (2004) Steroid-hormone rapid actions, membrane receptors and a conformational ensemble model. *Nat Rev Drug Discov* **3**, 27–41.
12. Martineau AR, Wilkinson KA, Newton SM *et al.* (2007) IFN-γ- and TNF-independent vitamin D-inducible human suppression of mycobacteria: The role of cathelicidin LL-37. *J Immunol* **178**, 7190–7198.
13. Rockett KA, Brookes R, Udalova I *et al.* (1998) 1,25-dihydroxyvitamin D_3 induces nitric oxide synthase and suppresses growth of *Mycobacterium tuberculosis* in a human macrophage-like cell line. *Infect Immun* **66**, 5314–5321.
14. Sly LM, Lopez M, Nauseef WM *et al.* (2001) 1alpha,25-dihydroxyvitamin D_3-induced monocyte antimycobacterial activity is regulated by phosphatidylinositol 3-kinase and mediated by the NADPH-dependent phagocyte oxidase. *J Biol Chem* **276**, 35482–35493.
15. Anand K & Kaul D (2003) Vitamin D_3-dependent pathway regulates TACO gene transcription. *Biochem Biophys Res Commun* **310**, 876–877.
16. Hmama Z, Sendide K, Talal A *et al.* (2004) Quantitative analysis of phagolysosome fusion in intact cells: Inhibition by mycobacterial lipoarabinomannan and rescue by an 1alpha,25-dihydroxyvitamin D_3-phosphoinositide 3-kinase pathway. *J Cell Sci* **117**, 2131–2140.
17. Coussens A, Timms PM, Boucher BJ *et al.* (2009) 1alpha,25-dihydroxyvitamin D_3 inhibits matrix metalloproteinases induced by *Mycobacterium tuberculosis* infection. *Immunology* **127**, 539–548.
18. Liu PT, Schenk M, Walker VP *et al.* (2009) Convergence of IL-1beta and VDR activation pathways in human TLR2/1-induced antimicrobial responses. *PLoS ONE* **4**, e5810.
19. Martineau AR, Newton SM, Wilkinson KA *et al.* (2007) Neutrophil-mediated innate immune resistance to mycobacteria. *J Clin Invest* **117**, 1988–1994.
20. Yuk JM, Shin DM, Lee HM *et al.* (2009) Vitamin D_3 induces autophagy in human monocytes/macrophages via cathelicidin. *Cell Host Microbe* **6**, 231–243.
21. Shin DM, Yuk JM, Lee HM *et al.* (2010) Mycobacterial lipoprotein activates autophagy via TLR2/1/CD14 and a functional vitamin D receptor signalling. *Cell Microbiol* **12**, 1648–1665.
22. Liu PT, Stenger S, Tang DH *et al.* (2007) Cutting edge: Vitamin D-mediated human antimicrobial activity against *Mycobacterium tuberculosis* is dependent on the induction of cathelicidin. *J Immunol* **179**, 2060–2063.
23. Glisson F, Bate G & Regemorter A (1651) *A Treatise of the Rickets: being A Disease Common to Children*. London: P. Cole.
24. De Ugarte DA, Shapiro NL & Williams HL (2003) Tuberculous mediastinal mass presenting with stridor in a 3-month-old child. *J Pediatr Surg* **38**, 624–625.
25. Chapman HT (1849) On the use of cod-liver oil in diseases of the bones and joints, in consumption and in other maladies attended by great emaciation. *Pharm Trans*, 1–16.
26. Karyadi E, West CE, Schultink W *et al.* (2002) A double-blind, placebo-controlled study of vitamin A and zinc supplementation in persons with tuberculosis in Indonesia: Effects on clinical response and nutritional status. *Am J Clin Nutr* **75**, 720–727.
27. Mayer E (1938) Heliotherapy of tuberculosis. *Ann Int Med* **11**, 1856–1860.
28. Roelandts R (2005) A new light on Niels Finsen, a century after his Nobel Prize. *Photodermatol Photoimmunol Photomed* **21**, 115–117.
29. Askew FA, Bruce HM, Callow RK *et al.* (1931) Crystalline vitamin D. *Nature* **128**, 758.
30. Charpy J (1950) Quelques traitments vitaminés ou par substances fonctionelles en dermatologie. *Bull Méd* **24**, 505.
31. Martineau AR, Honecker FU, Wilkinson RJ *et al.* (2007) Vitamin D in the treatment of pulmonary tuberculosis. *J Steroid Biochem Mol Biol* **103**, 793–798.
32. Davies PD (1985) A possible link between vitamin D deficiency and impaired host defence to *Mycobacterium tuberculosis*. *Tubercle* **66**, 301–306.

33. Davies PD, Brown RC & Woodhead JS (1985) Serum concentrations of vitamin D metabolites in untreated tuberculosis. *Thorax* **40**, 187–190.
34. Davies PD, Church HA, Brown RC *et al.* (1987) Raised serum calcium in tuberculosis patients in Africa. *Eur J Respir Dis* **71**, 341–344.
35. Davies PD, Church HA, Bovornkitti S *et al.* (1988) Altered vitamin D homeostasis in tuberculosis. *Int Med Thailand* **4**, 45–47.
36. Wilkinson RJ, Llewelyn M, Toossi Z *et al.* (2000) Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in west London: A case-control study. *Lancet* **355**, 618–621.
37. Sasidharan PK, Rajeev E & Vijayakumari V (2002) Tuberculosis and vitamin D deficiency. *J Assoc Physicians India* **50**, 554–558.
38. Sita-Lumsden A, Lapthorn G, Swaminathan R *et al.* (2007) Reactivation of tuberculosis and vitamin D deficiency: The contribution of diet and exposure to sunlight. *Thorax* **62**, 1003–1007.
39. Gibney KB, MacGregor L, Leder K *et al.* (2008) Vitamin D deficiency is associated with tuberculosis and latent tuberculosis infection in immigrants from sub-Saharan Africa. *Clin Infect Dis* **46**, 443–446.
40. Ho-Pham LT, Nguyen ND, Nguyen TT *et al.* (2010) Association between vitamin D insufficiency and tuberculosis in a Vietnamese population. *BMC Infect Dis* **10**, 306.
41. Grange JM, Davies PD, Brown RC *et al.* (1985) A study of vitamin D levels in Indonesian patients with untreated pulmonary tuberculosis. *Tubercle* **66**, 187–191.
42. Chan TY, Poon P, Pang J *et al.* (1994) A study of calcium and vitamin D metabolism in Chinese patients with pulmonary tuberculosis. *J Trop Med Hyg* **97**, 26–30.
43. Martineau AR, Leandro AC, Anderson ST *et al.* (2010) Association between Gc genotype and susceptibility to TB is dependent on vitamin D status. *Eur Respir J* **35**, 1106–1112.
44. Nielsen NO, Skifte T, Andersson M *et al.* (2010) Both high and low serum vitamin D concentrations are associated with tuberculosis: A case-control study in Greenland. *Br J Nutr* **104**, 1487–1489.
45. Bellamy R, Ruwende C, Corrah T *et al.* (1999) Tuberculosis and chronic hepatitis B virus infection in Africans and variation in the vitamin D receptor gene. *J Infect Dis* **179**, 721–724.
46. Gao L, Tao Y, Zhang L *et al.* (2010) Vitamin D receptor genetic polymorphisms and tuberculosis: Updated systematic review and meta-analysis. *Int J Tuberc Lung Dis* **14**, 15–23.
47. Arnaud J & Constans J (1993) Affinity differences for vitamin D metabolites associated with the genetic isoforms of the human serum carrier protein (DBP). *Hum Genet* **92**, 183–188.
48. Lauridsen AL, Vestergaard P, Hermann AP *et al.* (2005) Plasma concentrations of 25-hydroxy-vitamin D and 1,25-dihydroxy-vitamin D are related to the phenotype of Gc (vitamin D-binding protein): A cross-sectional study on 595 early postmenopausal women. *Calcif Tissue Int* **77**, 15–22.
49. Abbas S, Linseisen J, Slanger T *et al.* (2008) The Gc2 allele of the vitamin D binding protein is associated with a decreased postmenopausal breast cancer risk, independent of the vitamin D status. *Cancer Epidemiol Biomarkers Prev* **17**, 1339–1343.
50. Papiha SS, Agarwal SS & White I (1983) Association between phosphoglucomutase (PGM1) and group-specific component (Gc) subtypes and tuberculosis. *J Med Genet* **20**, 220–222.
51. Spitsyn VA & Titenko NV (1990) Subtypes of serum group specific component (Gc) in normal conditions and in pathology. *Genetika* **26**, 749–759.
52. Bahr GM, Eales LJ, Nye KE *et al.* (1989) An association between Gc (vitamin D-binding protein) alleles and susceptibility to rheumatic fever. *Immunology* **67**, 126–128.
53. Roth DE, Soto G, Arenas F *et al.* (2004) Association between vitamin D receptor gene polymorphisms and response to treatment of pulmonary tuberculosis. *J Infect Dis* **190**, 920–927.
54. Babb C, van der Merwe L, Beyers N *et al.* (2007) Vitamin D receptor gene polymorphisms and sputum conversion time in pulmonary tuberculosis patients. *Tuberculosis (Edinb)* **87**, 295–302.
55. Talat N, Perry S, Parsonnet J *et al.* (2010) Vitamin D deficiency and tuberculosis progression. *Emerg Infect Dis* **16**, 853–855.
56. Kampmann B, Gaora PO, Snewin VA *et al.* (2000) Evaluation of human antimycobacterial immunity using recombinant reporter mycobacteria. *J Infect Dis* **182**, 895–901.
57. Martineau AR, Wilkinson RJ, Wilkinson KA *et al.* (2007) A single dose of vitamin D enhances immunity to mycobacteria. *Am J Respir Crit Care Med* **176**, 208–213.
58. Gwinup G, Randazzo G & Elias A (1981) The influence of vitamin D intake on serum calcium in tuberculosis. *Acta Endocrinol (Copenh)* **97**, 114–117.
59. Martineau AR, Nanzer AM, Satkunam KR *et al.* (2009) Influence of a single oral dose of vitamin D_2 on serum 25-hydroxyvitamin D concentrations in tuberculosis patients. *Int J Tuberc Lung Dis* **13**, 119–125.
60. Narang NK, Gupta RC & Jain MK (1984) Role of vitamin D in pulmonary tuberculosis. *J Assoc Physicians India* **32**, 185–188.
61. Tjellesen L, Hummer L, Christiansen C *et al.* (1986) Serum concentration of vitamin D metabolites during treatment with vitamin D_2 and D_3 in normal premenopausal women. *Bone Miner* **1**, 407–413.
62. Stern PH, Taylor AB, Bell NH *et al.* (1981) Demonstration that circulating 1 alpha, 25-dihydroxyvitamin D is loosely regulated in normal children. *J Clin Invest* **68**, 1374–1377.
63. Morcos MM, Gabr AA, Samuel S *et al.* (1998) Vitamin D administration to tuberculous children and its value. *Boll Chim Farm* **137**, 157–164.
64. Nursyam EW, Amin Z & Rumende CM (2006) The effect of vitamin D as supplementary treatment in patients with moderately advanced pulmonary tuberculous lesion. *Acta Med Indones* **38**, 3–5.
65. Wejse C, Gomes VF, Rabna P *et al.* (2009) Vitamin D as supplementary treatment for tuberculosis: A double-blind, randomized, placebo-controlled trial. *Am J Respir Crit Care Med* **179**, 843–850.
66. Martineau AR, Timms PM, Bothamley GH *et al.* (2011) High-dose vitamin D_3 during intensive-phase antimicrobial treatment of pulmonary tuberculosis: A double-blind randomised controlled trial. *Lancet* **377**, 242–250.
67. Vieth R (2011) Vitamin D nutrient to treat TB begs the prevention question. *Lancet* **377**, 189–190.
68. Ferebee SH (1970) Controlled chemoprophylaxis trials in tuberculosis. A general review. *Bibl Tuberc* **26**, 28–106.