Abstract

Introduction: Severe vitamin D deficiency is very common. Evidence-based guidelines for rapid correction with high-dose oral cholecalciferol are not yet available.

Objective: To develop a practical cholecalciferol loading dose regimen.

Materials and methods: A total of 208 vitamin D-deficient subjects (serum 25-hydroxyvitamin D3 level < 50 nmol/l), aged 18–88 years, were treated with solubilized cholecalciferol, 50 000 IU/ml. They received either 25 000 IU every fortnight for 8 weeks (total dose 100 000 IU), 25 000 IU every week for 6 weeks (total dose 150 000 IU), or 25 000 IU every week for 8 weeks (total dose 200 000 IU). Blood samples were collected at baseline and 10 days after the final dose of cholecalciferol.

Results: Most patients were severely vitamin D deficient: 76% had a serum 25-OHD3 level < 30 nmol/l at baseline. Cholecalciferol in a cumulative dose of 100 000, 150 000, and 200 000 IU increased mean serum 25-OHD3 level by 29 nmol/l (95% confidence interval (CI): 23–35 nmol/l), 43 nmol/l (95% CI: 36–50 nmol/l), and 69 nmol/l (95% CI: 64–75 nmol/l) respectively. The change in 25-OHD3 (Δ25-OHD3) was related to the dose per kilogram body weight (R² = 0.38, P < 0.0001), and is described by the equation: Δ25-OHD3 = 0.025 × (dose per kg body weight).

Conclusion: The cholecalciferol loading dose required to reach the serum 25-OHD3 target level of 75 nmol/l can be calculated as follows: dose (IU) = 40 × (75 – serum 25-OHD3) × body weight.

Introduction

Vitamin D status is related to sunlight exposure and therefore depends on latitude (1). As a result, vitamin D deficiency, defined as a serum 25-hydroxyvitamin D3 (25-OHD3) level < 50 nmol/l, is very common in Northern Europe. In Germany, for example, 57% of the 18–79-year-old residents are vitamin D deficient, and this is mainly explained by the lack of sufficient sunlight exposure and low dietary vitamin D (2).

Vitamin D deficiency is well known for its musculoskeletal complications in children and adults (1, 3, 4). However, vitamin D receptors are also present on a large variety of extraskeletal cell types, including cardiomyocytes, vascular endothelial cells, neurons, immune cells, and pancreatic β-cells. A rapidly growing body of evidence indicates that vitamin D deficiency may play a role in the development of common cancers, autoimmune diseases, infectious diseases, and cardiovascular diseases (1, 3, 5). Consequently, improvements in the detection and treatment of vitamin D deficiency may have a major health impact.

There is an emerging consensus that serum 25-OHD3 levels of about 75 nmol/l are optimal for bone health and extraskeletal effects (6, 7). However, there is no consensus on how to achieve this target rapidly. In the USA, ergocalciferol (vitamin D2) is recommended at a dose of 50 000 IU/week for 8 weeks, irrespective of the degree of vitamin D deficiency or body weight (1). In Europe, cholecalciferol (vitamin D3) is mainly used to treat vitamin D deficiency. Usually, this is done with daily supplementation using tablets.

In 2006, the commercially available cholecalciferol tablets (Devaron 400 IU) were suddenly withdrawn from the market in The Netherlands. This forced us to look for alternatives to maintain patient care. We were fortunate to discover a formulation for solubilized cholecalciferol (50 000 IU/ml), which was developed by the Dutch Pharmacists Association (Formularium der Nederlandse Apothekers, FNA) 20 years ago, and which included a guideline for quality control. This formulation is not patented, it is cheap to prepare, and can be used everywhere. Nevertheless, it was hardly prescribed, possibly because of unawareness of its
existence, or due to the lack of evidence-based dosing guidelines. We therefore decided to design a study with the aim to develop a practical cholecalciferol loading dose regimen that would enable rapid correction of vitamin D deficiency in a heterogeneous population such as that encountered in an outpatient clinic of internal medicine. A cumulative dose of 400 000 IU such as that recommended for ergocalciferol was considered to be too high for cholecalciferol. Two studies had provided evidence that cholecalciferol might be 2–3 times more potent than ergocalciferol (8, 9). This indicated that a loading dose for cholecalciferol should be markedly lower than that recommended for ergocalciferol. As the exact proportion of required adjustment was not known, it was decided to perform a dose-escalation study.

Subjects and methods

Participants

A total of 208 patients with established vitamin D deficiency (serum 25-OHD$_3$ level < 50 nmol/l) were included. All patients were visitors of the internal medicine outpatient clinic. They had been tested for vitamin D deficiency because of unexplained fatigue, muscle weakness, myalgia, or unexplained hypophosphatemia. Patients with unstable thyroid disorders, hypoparathyroidism and primary hyperparathyroidism, evidence of malabsorption, and renal insufficiency (glomerular filtration rate < 60 ml/min) and those who used medication interfering with vitamin D metabolism were excluded. All patients gave their informed consent.

Study design

The solubilized cholecalciferol developed by the Dutch Pharmacists Association (Cholecalciferol$_{FNA}$ 50 000 IU/ml) is a watery mixture made of cholecalciferol concentrate in oil, citric acid monohydrate, starch, anise oil, potassium sorbates, polysorbatum 80 (polyoxyethylene sorbitan mono-oleate), sugar syrup, and purified water. The full description of this formulation can be obtained by e-mail, contact: agiesen@alysis.nl. Cholecalciferol is a fat-soluble vitamin that is solubilized by polysorbatum 80, an interface-active substance that causes micelle formation. After oral administration, the solubilised changes into a very fine emulsion with better bioavailability than oily formulations (10, 11).

As dose guidelines were not available, we had to design a pragmatic dose-escalation study that would be safe to conduct as part of regular patient care. To minimize the risk of over supplementation, the first group of patients (group A, n = 30) was treated with a low dose: 25 000 IU every fortnight for 8 weeks, i.e. a total dose of 100 000 IU in 2 months. When the laboratory results had confirmed that this dose did not cause hypercalcemia and that the 25-OHD$_1$ level had increased by 69 nmol/l or less, it was decided to increase the loading dose in the next group of patients. They received 25 000 IU/week for 6 weeks, i.e. a total of 150 000 IU in 2 months (group B, n = 68). Again, hypercalcemia or potentially toxic vitamin D levels were not observed. With this regimen, the four biggest increases in 25-OHD$_3$ were 160, 130, 108, and 100 nmol/l respectively, and the other responses were < 100 nmol/l. Complete suppression of serum parathyroid hormone (PTH) had not occurred with this dose, and therefore, it was considered safe to treat the third group of subjects with 25 000 IU/week for 8 weeks, i.e. a total dose of 200 000 IU in 2 months (Group C, n = 110).

For the analysis of serum 25-OHD$_3$, creatinine, calcium, phosphate, albumin, and PTH, blood samples were collected at baseline and 10 days after the final dose of cholecalciferol. This interval was chosen because it was shown by previous studies that about 10 days are required to convert 90–100% of the absorbed cholecalciferol into 25-OHD$_3$ (9). The blood samples were collected throughout the year, between January 2007 and March 2009.

Serum 25-OHD$_3$ concentrations were measured by RIA (DiaSorin, Stillwater, MN, USA). The detection limit of this assay is 10 nmol/l, and the intra- and inter assay coefficients of variation are 8.1 and 10.2% respectively. Serum intact PTH was measured by chemiluminescent enzyme-labeled immunometric assay (Immulite 2500, Siemens, Los Angeles, CA, USA).

Statistical analysis

Results are expressed as mean values ± S.D. Data that did not follow a normal distribution were log-transformed. Responses to treatment within groups were analyzed by paired, two-sided t-tests. Between-group differences were tested by ANOVA and unpaired t-tests. To enable calculation procedures, 25-OHD$_3$ levels below the assay’s detection limit were given a value of 5 nmol/l. Multivariate regression analysis was used to identify the variables that might predict the cholecalciferol loading dose. Variables included for this analysis were season, sex, age, height, body weight, body mass index (BMI), cholecalciferol dose in IU and in IU/kg body weight, and baseline serum 25-OHD$_3$. To obtain a simple model that would be easy to use in daily practice, only those variables explaining more than 5% of the total variance were considered to be clinically relevant, and variables explaining < 5% of the variance were removed. A P value < 0.05 was considered to be statistically significant.

Results

The baseline characteristics of the patients enrolled in the study are shown in Table 1 and Fig. 1. Sixty-two patients had a diagnosis of osteoporosis, and the remaining subjects had a wide array of diagnoses.
commonly seen in a general internal medicine practice of a teaching hospital. The female: male ratio was 2:1. All variables that might affect the magnitude of the loading dose, such as age, body weight, serum 25-OHD3, and the month of sampling covered a wide range (Fig. 1). A substantial number of patients were obese: 33% had a BMI >30 kg/m², and 5% were morbidly obese (BMI >40 kg/m²).

The laboratory results at baseline and 10 days after the final dose of cholecalciferol are summarized in Table 2. The majority of patients were severely vitamin D deficient. Baseline serum 25-OHD3 level was <10 nmol/l in 12% of the patients, and 76% had levels <30 nmol/l. Cholecalciferol treatment increased the mean 25-OHD3 level from 20.5 ± 7.8 to 74.8 ± 30.2 nmol/l (P < 0.0001). In total, 76% of the patients achieved a serum 25-OHD3 level >50 nmol/l, and 48% of patients achieved a serum 25-OHD3 level >75 nmol/l. Hypercalcemia, defined as a serum calcium level >2.55 mmol/l, was not observed. The mean responses within the groups treated with 100 000, 150 000, or 200 000 IU are shown in Table 2. The individual changes in 25-OHD3 levels within each group are illustrated in Fig. 2. Baseline 25-OHD3 levels were similar in the three groups: 19.0 ± 7.4, 20.4 ± 9.7, and 20.7 ± 8.4 nmol/l respectively. The mean 25-OHD3 increased by 29 nmol/l in group A (95% confidence interval (CI): 23–35 nmol/l, P < 0.0001), by 43 nmol/l in group B (95% CI: 36–50 nmol/l, P < 0.0001), and by 69 nmol/l in group C (95% CI: 64–75 nmol/l, P < 0.0001). The mean rise in serum 25-OHD3 in group B was greater than that in group A (P < 0.05), and the rise in group C was greater than that in group B (P < 0.0001). The highest post-treatment 25-OHD3 levels were observed in the subjects receiving 200 000 IU in 2 months. The maximum level was 185 nmol/l (Fig. 2). A statistically significant decrease in serum PTH was observed only in group C (5.5 ± 5.3 –4.0 ± 2.8, P < 0.01), and complete suppression of serum PTH did not occur (data not shown). Serum creatinine rose from 73.8 ± 41.1 to 80.2 ± 51.2 μmol/l in group B (P < 0.05), and the small increases in groups A and C were statistically not significant.

The range in serum 25-OHD3 responses was large. This was in part attributed to the wide dose range that varied from 670 to 4348 IU/kg body weight. The variability in response to treatment was comparable for the three groups (Fig. 2). Multivariate regression analysis with all factors except the ‘dose per kg body weight’ showed that the rise in serum 25-OHD3 was inversely related to body weight and baseline 25-OHD3, and positively correlated with the total dose (variance of the model: 0.43, P < 0.0001). It was not related to age, sex, body height, BMI, or season in this study population. When the multivariate analysis was repeated, but now with inclusion of the ‘dose per kg body weight’, only two statistically significant determinants remained: baseline serum 25-OHD3 and the dose per kg body weight (variance of the model: 0.42, P < 0.0001). As the baseline serum 25-OHD3 explained only 4% of the total variance in this model, it was removed, leaving only the ‘dose per kg body weight’ as a clinically relevant parameter. The increase in 25-OHD3, expressed as a function of the dose/kg body weight, is shown in Fig. 3. On the left, the complete data set is shown, including the polynomial regression line with its 95% CI in red (R² = 0.40, P < 0.0001). The linear regression line is defined by the equation: Δ25-OHD3 = −1.15 + 0.024 × (dose per kg body weight), R² = 0.38, P < 0.0001. This line fell well within the 95% CI of the polynomial line.
To obtain a practical instrument, the linear equation was simplified. The intercept had a 95% CI of $K_{11.0-9.4}$, and thus did not differ from zero, which allows elimination from the equation. The 95% CI of slope of the line ranged from 0.020 to 0.028. This variability allows a minor deviation to a mean slope value of 0.025. Both measures resulted in a more simple equation that would be easy to use in general practice:

$$D_{25-OHD\text{3}} = 0.025 \times (\text{dose per kg body weight}).$$

This line also fell within the 95% CI of the polynomial curve (not shown). The dose required for correction of the serum $25$-$\text{OHD}_3$ level can be derived from Fig. 3 on the right. This figure focuses on the clinically relevant range for vitamin D correction, i.e. corrections of up to 100 nmol/l. For example, a patient with a $25$-$\text{OHD}_3$ level of 5 nmol/l will require a rise of 70 nmol/l to reach target level of 75 nmol/l ($\Delta 25$-$\text{OHD}_3 = 75 - 5 = 70$ nmol/l). To achieve this level, a cholecalciferol dose of 3000 IU/kg body weight will be required. The loading dose can also be calculated with the equation: loading dose (IU) = $40 \times (75 - \text{serum 250HD}_3$ $(\text{nmol/l})) \times (\text{body weight (kg)})$.

It is stressed that this equation should not be used for subjects with body weights > 125 kg. As shown in Fig. 1, only a few patients had body weights larger than 125 kg.

### Discussion

This study has formulated a loading dose guideline for rapid correction of vitamin D deficiency using solubilized cholecalciferol. The calculation of the dose required to normalize serum $25$-$\text{OHD}_3$ is based on the degree of vitamin D deficiency and body weight. It appears to be a safe procedure. In this study, no toxic effects were observed, hypercalcemia did not develop, vitamin D levels never reached the danger range, and complete suppression of PTH levels was not observed. The sampling was well distributed over all seasons, including summer, which further supports the safety of the proposed regimen. The study included young and elderly subjects, lean and obese, with a variety of underlying diagnoses such as those commonly seen in the outpatient clinic of internal medicine in a general teaching hospital. Therefore, the described approach is applicable in a broad array of subjects; however, it is not valid in the case of malabsorption. Once the target level is reached, a maintenance dose will be required to sustain the serum $25$-$\text{OHD}_3$ level around 75 nmol/l.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>100 000 IU</th>
<th>150 000 IU</th>
<th>200 000 IU</th>
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<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td><strong>Creatinine (μmol/l)</strong></td>
<td>65.7±14.6</td>
<td>66.2±13.6</td>
<td>73.8±41.1</td>
</tr>
<tr>
<td><strong>Total calcium (mmol/l)</strong></td>
<td>2.34±0.11</td>
<td>2.33±0.18</td>
<td>2.32±0.09</td>
</tr>
<tr>
<td><strong>Phosphorus (mmol/l)</strong></td>
<td>1.06±0.20</td>
<td>1.13±0.37</td>
<td>1.02±0.19</td>
</tr>
<tr>
<td><strong>Albumin (g/l)</strong></td>
<td>39.0±3.4</td>
<td>39.7±3.9</td>
<td>38.8±3.6</td>
</tr>
<tr>
<td><strong>PTH (pmol/l)</strong></td>
<td>4.3±1.5</td>
<td>3.5±1.9</td>
<td>5.7±4.1</td>
</tr>
<tr>
<td><strong>25-OHD₃ (nmol/l)</strong></td>
<td>19.0±7.4</td>
<td>48.3±13.0†</td>
<td>20.4±9.7</td>
</tr>
<tr>
<td><strong>Dose/kg (IU/kg)</strong></td>
<td>1495±378</td>
<td>2028±553</td>
<td>2701±748</td>
</tr>
<tr>
<td><strong>Δ25-OHD₃ (nmol/l)</strong></td>
<td>29.3±15.4</td>
<td>43.1±29.1</td>
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$\Delta 25$-$\text{OHD}_3$, change in $25$-$\text{OHD}_3$ level. For comparison within groups: *$P<0.05$; †$P<0.01$; ‡$P<0.0001$. 

**Figure 2** Changes in serum $25$-$\text{OHD}_3$ in response to the oral administration of solubilized cholecalciferol. Group A: 100 000 IU, group B: 150 000 IU, and group C: 200 000 IU.
The present study does not provide data on this. The optimal maintenance dose for solubilized cholecalciferol remains to be determined, and this is currently the subject of further study.

Until recently, vitamin D supplementation studies were mainly limited to elderly people, particularly those who lived in nursing homes where the prevalence of vitamin D deficiency is 75% or higher. Some studies evaluated the efficacy of high doses at monthly or 3-month intervals, but most studies have used the officially recommended cholecalciferol dose of 600–800 IU/day, or equivalents thereof (12–16). Most studies used predefined fixed doses, irrespective of the degree of vitamin D deficiency and body weight, and assessed the efficacy of the regimen after 4–6 months or longer. These studies were not designed to establish the requirements for a loading dose. A dose of 800 IU/day may help to improve vitamin D status in the long run, but it is not the optimal approach to achieve a rapid correction of severe vitamin D deficiency (16). According to our calculations, a 75-kg patient with a serum 25-OH-D3 level of 5 nmol/l will need a total loading dose of 210 000 IU to raise the serum level to 75 nmol/l. If, for simplicity, 100% absorption is assumed, a daily dose of 800 IU will take 3 months to raise the level to 30 nmol/l, 5.5 months to raise the level to 50 nmol/l, and almost 9 months to reach the target level of 75 nmol/l.

It is well established that the serum 25-OH-D3 levels achieved with a fixed dose of oral cholecalciferol are inversely related to body weight or BMI (17, 18). This is not unexpected since obese subjects have lower baseline 25-OH-D3 levels than the non-obese subjects. Also, the rise in serum 25-OH-D3 will be less in obese subjects because of the larger distribution volume due to the marked increase in body fat. In view of these aspects, we adjusted the loading dose for body weight. Multivariate regression analysis confirmed that the introduction of the factor ‘dose per kg body weight’ removed the relation between the rise in serum 25-OH-D3 and body weight. The study population demonstrated a wide range in body weights, and therefore, the equation is considered to be valid for lean, normal, and obese subjects. For the present, however, we advise against its use in morbidly obese subjects, i.e. subjects with a BMI > 40 kg/m². Experience with this subgroup is still too limited to allow firm conclusions.

Discussions on vitamin D requirements and daily allowances are not new. Over the years, the recommended daily dose for adults has gradually increased from 200 to 800 IU, and recently, doses of 1000 and even 2000 IU/day or higher have been proposed (19, 20). As discussed by Vieth in an excellent review on vitamin D supplementation and safety, many recommendations before 1997 lacked any scientific basis, and the more recent recommendations are often still too low (19). Vitamin D toxicity is often feared for, but usually this fear is unfounded. All published cases of vitamin D toxicity involved intakes of more than 40 000 IU/day for a prolonged period of time, with serum 25-OH-D3 levels exceeding > 250 nmol/l. To achieve these levels, daily doses of 15 000 IU or more for many months are needed. The lowest cumulative dose of cholecalciferol leading to toxic 25-OH-D3 levels was 3 600 000 IU given in 3 weeks (19). The loading doses that we have proposed are 10–20 times less, which is another argument to consider them as safe.

Data derived from a dose-escalation study in healthy men with a mean weight of 85 kg are of interest to compare with those derived from the present method (21). Based on the responses of oral cholecalciferol given in doses of 0, 1000, 5000, or 10 000 IU/day for a period of 20 weeks, it could be calculated that a rise in cholecalciferol intake of about 57 IU/day is needed to establish a 1 nmol/l rise in serum 25-OH-D3. Applied to our population, and given for 8 weeks, a total amount of 57×7×8×50 or 160 000 IU would be required to increase serum 25-OH-D3 by 50 nmol/l in an 85-kg subject. This compares well with the 170 000 IU that are required according to our calculation model. The advantages of our method are that it is applicable to a broad variety of subjects, and that it allows adjustment...
for body weight. The effect of body weight is considerable: a 50 nmol/l rise in a 60-kg subject will require a loading dose of 120 000 IU, whereas a 100-kg subject will require 200 000 IU.

In the present study, cholecalciferol was given in doses of 25 000 IU/week. Higher doses or a higher dose frequency might shorten the treatment period, but it is not exactly known how this will affect the 25-OHD3 response. It has been demonstrated that single doses of 50 000 or 100 000 IU are safe and effective. In non-obese subjects, these doses raised serum 25-OHD3 to peak levels of about 15 and 35 nmol/l within 1–2 weeks respectively (9, 22). The use of these higher doses may alter the vitamin D3 pharmacokinetics to an extent that an adjusted dose calculation might be required. There is evidence that very high doses may saturate hydroxylation reactions, thereby reducing the efficiency to generate 25-OHD3 within a given period of time (23).

Another aspect that is relevant to the assessment of loading doses is the ongoing debate concerning the bioequivalence of ergocalciferol and cholecalciferol (8, 9, 23–27). All studies using single boluses or brief treatment periods with relatively high doses of vitamin D indicate that cholecalciferol is more potent than ergocalciferol in raising serum 25-OHD3. This applies to the oral route as well as to the i.m. route (26). Estimates are that cholecalciferol is 2–3 times more potent than ergocalciferol in raising serum 25-OHD3. This lower potency has been attributed to a shorter half-life as a result of a lower binding affinity to vitamin D-binding protein (24). In contrast to these observations, ergocalciferol was found to be equally effective as cholecalciferol in maintaining serum 25-OHD3 status when treatment was given for a prolonged period of time, e.g. 3 months, and at a much lower dose (25). The magnitude of the dose may be the key to explaining the discrepancy between the studies. As long as this issue is not fully clarified, the recommended dose calculation procedure should only be relied upon to assess the cholecalciferol loading dose.

In conclusion, for rapid correction of vitamin D deficiency, we recommend a cholecalciferol loading dose regimen based on the equation: dose (IU) = 40 × (75 − serum 25-OHD3) × body weight. The equation is based on data derived from subjects weighing 125 kg or less, and thus, it should not be used in subjects with considerably larger body weights. Furthermore, it is important to note that the calculated loading dose should be given in portions of 25 000 IU/week. Higher doses per week or higher dosing frequencies might affect the vitamin D3 pharmacokinetics, and thus require an adjusted dose calculation.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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