Clinical Nutrition xxx (2017) 1–7

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Randomized control trials

Dose–response effects of supplementation with calcifediol on serum 25-hydroxyvitamin D status and its metabolites: A randomized controlled trial in older adults

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ARTICLE INFO

Article history:
Received 17 May 2016
Accepted 26 March 2017

Keywords:
Vitamin D
Calcifediol
Dose–response
Supplementation
25-Hydroxyvitamin D
PTH

SUMMARY

Background & aims: Oral supplementation with vitamin D is recommended for older adults to maintain a sufficient 25-hydroxyvitamin D (25(OH)D) status throughout the year. While supplementation with vitamin D_2 or D_3 is most common, alternative treatment regimens exist which require further investigation with respect to increasing 25(OH)D concentration. We investigated the dose–response effects of supplementation with calcifediol compared to vitamin D_3 and assessed the dose which results in mean serum 25(OH)D_3 concentrations between 75 and 100 nmol/L.

Methods: This randomized, double-blind intervention study included men and women aged ≥65 years ($n = 59$). Participants received either 5, 10 or 15 μg calcifediol or 20 μg vitamin D_3 per day, for a period of 24 weeks. Blood samples were collected every four weeks to assess response profiles of vitamin D related metabolites; serum vitamin D_3, 25(OH)D_3, 1,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$) and 24,25-dihydroxyvitamin D_3 (24,25$(OH)_2D_3$). Further, serum calcium, plasma parathyroid hormone, and urinary calcium were evaluated.

Results: Supplementation with 20 μg vitamin D_3 increased 25(OH)D_3 concentrations towards 70 nmol/L within 16 weeks. Supplementation with 10 or 15 μg calcifediol increased 25(OH)D_3 levels >75 nmol/L in 8 and 4 weeks, respectively. Steady state was achieved from week 12 onwards with serum 25(OH)D_3 levels stabilizing between 84 and 89 nmol/L in the 10 μg calcifediol group. A significant association was observed between the changes in 25(OH)D_3 and 24,25$(OH)_2D_3$ ($R^2 = 0.83$, $P < 0.01$), but not between 25(OH)D_3 and 1,25$(OH)_2D_3$ ($R^2 = 0.04$, $P = 0.18$). No cases of hypercalcemia occurred in any treatment during the study period.

Conclusions: Calcifediol supplementation rapidly and safely elevates serum 25(OH)D_3 concentrations to improve vitamin D status in older adults. A daily dose of 10 μg calcifediol allows serum 25(OH)D_3 concentrations to be maintained between 75 and 100 nmol/L.

Trial registration number: NCT01868945.

1. Introduction

Vitamin D deficiency is common worldwide, and particularly prevalent in the elderly [1–4]. A deficiency can be caused by environmental and age-related factors, affecting vitamin D uptake or metabolism. Vitamin D can be obtained from the diet as vitamin D_2 (ergocalciferol) or D_3 (cholecalciferol). However, relatively few foods contain vitamin D, and therefore the dietary intake is considered low. As such, vitamin D_3 is mainly acquired after sun exposure, as it can be synthesized from 7-dehydrocholestrol after cutaneous exposure to ultraviolet-B radiation [5]. However, production of 7-dehydrocholesterol is often limited to the summer months [6], and depends on many behavioral factors, such as outdoor activities and clothing [7], as well as the capacity of the

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http://dx.doi.org/10.1016/j.clnu.2017.03.029

skin to synthesize vitamin D_3, which is suggested to be decreased in older adults [8,9]. To be biologically active, vitamin D is hydroxylated by the liver into the prehormone 25-hydroxyvitamin D (25(OH)D) and converted primarily in the kidney to the active hormone 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), which acts upon a broad variety of cells in the body. These metabolites can be further hydroxylated in the kidney into the inactive metabolite 24,25-dihydroxyvitamin D ($24,25(OH)_2D$) and as such regulate the available pool and synthesis of 25(OH)D and $1,25(OH)_2D$. However, several factors such as a declined hepatic or renal function [10,11] can affect the metabolism of vitamin D and can increase the risk of vitamin D deficiency. Current recommendations show no consensus with regard to the optimal vitamin D status, with the Institute of Medicine (IOM) defining serum 25(OH)D levels of 50 nmol/L as adequate and others advocating a threshold of 75 nmol/L [12–14]. However, all agree that vitamin D supplements are needed to meet requirements in the older population. Supplementation with vitamin D_2 or D_3 is currently most common. However, supplementation with calcifediol, the $25(OH)D_3$ metabolite, might be considered as well. As calcifediol is more hydrophilic and already hydroxylated, it can present an effective supplementation strategy in cases of malabsorption or impaired hepatic function [15]. Previous studies have demonstrated that calcifediol is more potent in increasing serum $25(OH)D_3$ status compared to native vitamin D_3 [15–19]. This makes it an interesting alternative to be considered in the older population. However, additional clinical trials are needed to establish the appropriate dosing and safety of calcifediol supplementation in this population. Therefore, we investigated the dose–response effects of calcifediol compared to vitamin D_3 on serum $25(OH)D_3$ and its metabolites in people aged 65 years or older.

2. Materials and methods

2.1. Trial design

This study was a double-blind trial including subjects randomly assigned to either 5, 10 or 15 μg calcifediol or 20 μg vitamin D_3 per day. The full study covered a screening visit and a 24 week intervention period including monthly visits to measure vitamin D metabolites and to monitor safety parameters. Randomization was carried out by an independent researcher using SAS software 9.20, with stratification on BMI (20–29, 30–35 kg/m^2) and permuted blocks of 4. All subjects and researchers remained blinded to treatment assignment until data collection and analyses were completed. The study was carried out in Wageningen, the Netherlands (latitude 51°N), between 26th of August 2013 and 30th of April 2014. The study protocol was approved by the Medical Ethics Committee of Wageningen UR and written informed consent was provided by all participants. The study was registered at clinicaltrials.gov as NCT01868945 and was performed according to ICH-GCP.

2.2. Participants

Subjects were recruited via registries of municipalities and invited for a screening visit to measure eligibility according inclusion and exclusion criteria. Subjects were included if they were 65 years or older, had a serum $25(OH)D_3$ concentration between 25 and 50 nmol/L and a body mass index between 20 and 35 kg/m^2. Exclusion criteria were a serum calcium level >2.6 mmol/L, diagnosis with kidney stones in the past 10 years, renal insufficiency, liver failure, malabsorption syndromes, sarcoidosis and primary hyperparathyroidism. Use of medication that might interfere with vitamin D metabolism led to exclusion (e.g. thiazides, parathyroid hormone, bisphosphonates). In addition, subjects were excluded if they consumed >3 alcoholic beverages per day, used vitamin D supplements in the three months prior to the screening visit, were not willing to stop the use of multivitamins during the study, were expected to increase sun exposure (e.g. planned holiday to a sunny resort), were blood donor or had a surgery planned.

2.3. Intervention

Study supplements were hard gelatin capsules that were identical in appearance and taste. DSM Nutritional Products Ltd. provided calcifediol or vitamin D_3 in spray-dried form, and supplements were manufactured by Fisher Clinical Services GmbH. The Analytical Research Centre of DSM Nutritional Products tested the capsules using high performance liquid chromatography analysis (HPLC). The actual content of the capsules was: 5.1, 10.3 and 15.3 μg calcifediol or 22.3 μg vitamin D_3. At the start of the study, subjects were instructed to consume one capsule per day at breakfast. Compliance was assessed by capsule count every two months. Subjects were considered compliant when ≥80% of the supplements were taken during the intervention.

2.4. Measurements

2.4.1. Laboratory analyses

All blood samples were collected in a fasted state in the morning and stored at −80 °C until analysis. At screening, serum $25(OH)D_3$ samples were analyzed using isotope dilution-online solid phase extraction liquid chromatography-tandem mass spectrometry (ID-XLC-MS/MS) (VU Medical Centre, Amsterdam, The Netherlands) [20]. At baseline and every 4 weeks during the intervention period, a more comprehensive analysis was performed. Serum albumin and calcium were measured by colorimetric analysis to monitor albumin-corrected calcium [21]. EDTA blood samples were used to measure intact PTH by sandwich chemiluminescence immunoassay. In addition, morning spot-urine was collected to monitor urinary calcium levels (expressed as calcium/creatinine ratio) (SHO laboratory, Velp, the Netherlands). Vitamin D metabolites, i.e. serum vitamin D_3, $25(OH)D_3$, $1,25(OH)_2D_3$, $24,25(OH)_2D_3$ were analyzed at the end of the study using LC/MS/MS (Analytical Research Center, DSM Nutritional Products, Kaiseraugst, Switzerland). The inter-assay and intra-assay CVs were ≤15%. Due to sensitivity reasons, the Lower Limit of Quantitation (LLQ) for the baseline measurement of vitamin D_3 had to be increased from 1.3 to 2.6 nmol/L in 56 out of 59 baseline blood samples. Besides, analysis of vitamin D_3 and $1,25(OH)_2D_3$ showed several laboratory values below the calibration point, these values are set at the LLQ for data interpretation. The method of analysis lacked sensitivity to accurately measure low concentrations of $25(OH)D_2$ as 37 out of 59 samples were below the detection limit at baseline. Therefore, we restrict our analysis to the reporting of D_3-related metabolites. All laboratory analyses were performed blinded to treatment allocation.

2.4.2. Questionnaires

Participants filled out a comprehensive questionnaire during the screening visit. Medical history, medication, dietary supplement use, alcohol consumption (number of alcoholic drinks per week) and smoking habits (current, former, never) were assessed. During the intervention phase, subjects filled out a questionnaire every 4 weeks to monitor changes in health status or medication use. Dietary vitamin D and calcium intake were recorded using a Food Frequency Questionnaire (FFQ) at baseline. This FFQ was developed using validated FFQs that were updated to facilitate the reporting of habitual vitamin D and calcium intake [22–24].

Please cite this article in press as: Vaes AMM, et al., Dose–response effects of supplementation with calcifediol on serum 25-hydroxyvitamin D status and its metabolites: A randomized controlled trial in older adults, Clinical Nutrition (2017), http://dx.doi.org/10.1016/j.clnu.2017.03.029