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Poor bioavailability of vitamin D_2 from ultraviolet-irradiated D_2-rich yeast in rats

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ARTICLE INFO

Article history:
Received 23 March 2018
Revised 9 July 2018
Accepted 10 July 2018

Keywords:
Bioavailability
Biofortification
Rats
UV-irradiated yeast
Vitamin D_2
25-Hydroxyvitamin D

ABSTRACT

Ultraviolet-irradiated yeast (*Saccharomyces cerevisiae*) can be used to biofortify bakery products with vitamin D, but in bread, it was not effective in increasing serum 25-hydroxyvitamin D [25(OH)D] in humans, possibly because of the low digestibility of the yeast matrix. We investigated the effects of vitamin D_2–rich intact yeast cells and their separated fraction, yeast cell walls, which we hypothesized to provide vitamin D_2 in a more bioavailable form, on serum 25(OH)D and its metabolites in growing female Sprague-Dawley rats (n = 54) compared to vitamin D_2 and D_3 supplements (8 treatment groups: 300 or 600 IU vitamin D/d, and a control group, 8-week intervention). The D_3 supplement groups had the highest 25(OH)D concentrations, and the vitamin D_2 supplement at the 600-IU dose increased 25(OH)D better than any yeast form ($P < .001$ for all, analysis of covariance, adjusted for body weight). There were no significant differences between the yeast forms at the same dose ($P > .05$). Serum 24,25-dihydroxyvitamin D (a vitamin D catabolite) concentrations and the trend in the differences between the groups were in line with 25(OH)D ($P < .001$ for all). The 24,25-dihydroxyvitamin D to 25(OH)D ratio between the D_2 supplement and the yeast groups did not differ ($P > .05$). These findings do not support the hypothesis: the ability of the different ultraviolet-treated vitamin D_2–containing yeast forms to increase 25(OH)D did not differ, and the poor bioavailability of vitamin D_2 in the yeasts compared D_3 or D_2 supplements could not be explained by the increased vitamin D catabolism in the yeast-treated groups.

1. Introduction

Because of the limited selection of naturally vitamin D–rich food sources, food fortification with vitamin D has been considered a viable option to improve vitamin D intake in the population [1]. Although still debated, vitamin D_3 may be more potent than vitamin D_2 in terms of increasing serum 25-hydroxyvitamin D [25(OH)D, widely regarded as an index of vitamin D status] concentration [2]. Although most vitamin D–fortified foods are enriched with animal-originated vitamin

Abbreviations: BMC, bone mineral content; BMD, bone mineral density; D_2, vitamin D_2; D_3, vitamin D_3; LC-MS/MS, liquid chromatography–tandem mass spectrometry; S-PTH, serum parathyroid hormone; S-Ca, serum calcium; S-Pi, serum phosphate; UV, ultraviolet; 24,25$(OH)_2$D, 24,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D.

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https://doi.org/10.1016/j.nutres.2018.07.008
0271-5317/

D_3, vitamin D_2 may have other advantages such as its commercial production can be very cost-effective and it is suitable also for use in strict vegetarians [3]. For these reasons, vitamin D_2 has potential for broader utilization in food fortification.

In addition to the traditional mode of fortification, in which vitamin D is exogenously added to the product, the vitamin D contents of the foodstuffs can be increased by biofortification means [4]. Among others, this can include the ultraviolet (UV) B irradiation of fungi, that is, mushrooms or baker's yeast [5]. UV-irradiated yeast, with enhanced vitamin D_2 levels, can be used to enrich bakery products with vitamin D: irradiated yeast cells retain most of their gassing power, which makes them ideal for enrichment of yeast leavened products, such as bread [6]. Hohman et al [7] studied a bread made with UV-treated, vitamin D_2–rich yeast in vitamin D–deficient rats, and vitamin D_2 from yeast was shown to be bioavailable, although not as effective in increasing plasma 25 (OH)D concentrations as a vitamin D_3 supplement. Later, Itkonen and coworkers [8] investigated the bioavailability of vitamin D_2 from bread baked with UV-treated yeast in an 8-week winter-based randomized controlled trial in young healthy women. Consumption of bread produced with the UV-treated, vitamin D_2–rich yeast did not increase total 25 (OH)D concentration, whereas women given bread made with regular yeast and either a vitamin D_2 or D_3 supplement (providing the same level of vitamin D as D_2-enriched bread) had significant improvements in total 25(OH)D concentration. Interestingly, Lipkie et al [9] recently used their in vitro bioaccessibility model, which simulated digestion in the gastrointestinal tract after ingestion of UV-yeast fortified bread, and found that intact yeast cells were present in the digesta of the yeast-fortified bread. These findings suggest a low bioaccessibility of vitamin D_2 in this UV-treated yeast due to the lower digestibility of the yeast matrix. This may explain the findings of low bioavailability of the vitamin D_2 from the bread used in the study of Itkonen et al [8]. It is worth noting that UV-irradiated D_2-rich yeast is already used in some breads on the market and, despite its vitamin D content, could be a poor source of bioavailable vitamin D.

Because of the matrix effects, it is possible that the bioavailability of the D_2 in yeast, and thus the ability to increase serum 25(OH)D, may be better if a separate cell wall fraction of the yeast is used. In that case, the matrix is already broken because the yeast cell walls are separated from the intact yeast cells by autolysis or hydrolysis and centrifugation [10]. Therefore, we hypothesize that yeast cell wall fraction due to potentially better bioavailability of its D_2 may be more capable of improving vitamin D status than the intact yeast cells. Accordingly, we investigated the ability of vitamin D_2–rich intact yeast cells (*Saccharomyces cerevisiae*) and yeast cell walls to increase serum 25(OH)D in growing rats compared to vitamin D_2 and D_3 supplements. In addition, to discount the possibility that vitamin D_2 from the UV-treated yeast is metabolized more rapidly than supplemental vitamin D_2, the concentration of the 24-pathway degradation metabolite, 24,25-dihydroxyvitamin D [24,25$(OH)_2$D], was also assessed. The effects of the different forms of vitamin D on bone development within the growing rats were examined by measuring femoral bone mineral content (BMC), bone area, and bone mineral density (BMD). Finally, additional information about vitamin D–related metabolism and safety of the different vitamin D sources was obtained by measuring calcium metabolism biomarkers in serum.

2. Methods and materials

2.1. *Ethical approval*

The project was approved by the Animal Experiment Board in Finland (Laboratory Centre of the University of Helsinki permit number KEK15-009).

2.2. *Rats and diets*

Three-week old, Sprague-Dawley Outbred female rats (n = 54) were obtained from Harlan Laboratories (Madison, WI, USA). They were placed in the Laboratory Animal Centre of the University of Helsinki and cared for by its personnel. The light/dark cycle was 12 hours, and water was provided ad libitum. The animals were allowed to acclimatize 1 week prior the study. During this acclimatization period, the rats were fed with Teklad global 16% protein rodent diet (2916C-031015MA; Harlan Laboratories, Madison, WI, USA). For the experimental period, the rats were stratified into 9 groups of 6 animals each. All diets were obtained from Harlan Laboratories (Madison, WI, USA). The basis of each diet was the AIN-93G diet (recommended for growing rodents) with vitamin-free tested casein (TD. 07669). The diets of the 9 groups varied by the amount and source of vitamin D: control diet and diets containing 1 of 2 different doses of vitamin D (300 or 600 IU/d) and as vitamin D_3 supplement, vitamin D_2 supplement, vitamin D_2–rich intact yeast cells, or vitamin D_2–rich yeast cell walls. The diets are described in detail in Table 1. The maximum food consumption was estimated to be 20 g/d which was provided daily, and the excess food was weighted after the experiment.

Supplemental vitamin D_3 was provided by Harlan Laboratories, whereas supplemental vitamin D_2 was obtained from Sigma-Aldrich (ergocalciferol, 40 000 000 USP U/g, E5750, CAS 50-14-6, EC 200-014-9; St Louis, MO, USA). The yeasts were provided by LALLEMAND Inc (Montreal, Canada). The production process for the UV-irradiated D_2-rich yeast preparations is described elsewhere [6,10]. The D_2 supplements and the yeasts preparations were shipped to Harlan Laboratories where the diets were produced. The exact vitamin D contents of LALLEMAND yeasts were analyzed by Covance (Princeton, NJ, USA) by high-performance liquid chromatography [11]. The amount of D_3 in the D_2 diets was below the detection limit of 20 μg/100 g. The vitamin D_2 contents of the spray-dried intact yeast cells and yeast cell walls were 39 250 μg/100 g (1 570 000 IU) and 138 500 μg/100 g (5 540 000 IU), respectively.

2.3. *Experiments*

The experimental period was for 8 weeks during which the rats were allowed to feed ad libitum. Each rat was weighted every 2 weeks (ie, at 0, 2, 4, 6, and 8 weeks) within the experimental period, when the rats were 4, 6, 8, 10, and 12 weeks of age, respectively. After 8 weeks, the rats were euthanized with CO_2, and their necks were broken. Blood was drawn by cardiac