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Research Article

Direct Exposure to Sunlight Accelerates Vitamin D Biodegradation in Milk

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ABSTRACT

Background: Vitamin D is essential for the gastrointestinal absorption of calcium and mechanical integrity of the musculoskeletal system. It can be ingested and is also produced in the skin by the action of ultraviolet (UV) sunlight on 7-dehydro-cholesterol, converting it to cholecalciferol. Given the risks of exposure to sunlight and convenient availability of sunblock lotions, many people tend to rely on the oral intake of vitamin D. In the United States milk is routinely fortified with vitamin D and is one of the primary dietary sources of the vitamin. Hypovitaminosis D is nevertheless quite prevalent and has been associated with a number of pathologies.

Objective: The purpose of our study is to determine whether the rate of cholecalciferol biodegradation in fortified milk is accelerated by exposure to direct sunlight.

Methods: This study was conducted on a sunny day when the temperature was 72°F. Commercially available skim milk was used. Three clear glass containers of milk were placed under direct sunlight, 3 in an adjacent shaded area, and 3 in a refrigerator. Samples from each container were obtained at 0, 5, 10, 30, 60, and 90 minutes. The modified AOAC Official Method 992.26 and high performance liquid chromatography (HPLC) were utilized to extract and analyze cholecalciferol, and the Vitamin D content (mg/L) was calculated using the Beer-Lambert law.

Results: Our data show that the vitamin D content of fortified milk decreases significantly (to about 80% of its original value) over a period of 60 minutes when exposed to direct sunlight. The vitamin D content of fortified milk kept unrefrigerated in shade also decreases over a period of 60 minutes (by about 10%), but the vitamin D content of samples kept refrigerated remains largely unchanged.

Conclusions: Vitamin D degradation in milk exposed to sunshine occurs steadily over a period of 10 minutes to 60 minutes, leveling off between 60 and 90 minutes. Vitamin D content of fortified milk stored in a refrigerator remains consistent throughout the time period tested.

Introduction

Vitamin D is a misnomer because it can be formed in the skin when ultraviolet light interacts with 7-dehydro-cholesterol, converting it to cholecalciferol. By definition a vitamin cannot be formed within the human body. Vitamin D also can be ingested through the consumption of animal-based food (cholecalciferol) or plant-based food (ergocalciferol). Vitamin D enhances the intestinal absorption of calcium by stimulating the formation of calcium transport proteins in the small intestine [1]. It plays a pivotal, essential role in maintaining calcium homeostasis and musculoskeletal health.

Primarily in the liver, but also in several other body organs, both cholecalciferol and ergocalciferol follow the same metabolic pathway: first hydroxylation at the 25-position to form 25-hydroxyvitamin D which is then stored mostly in fat tissue and is a readily available source of vitamin D. Further hydroxylation of 25-hydroxyvitamin D occurs in the kidneys and is governed by the circulating serum

parathyroid hormone (PTH) levels: an elevated serum PTH levels activates the 1-alpha-hydroxylase enzyme in the kidneys to attach a hydroxyl group at the 1-position to form 1,25-di-hydroxy-vitamin D: the most active vitamin D metabolite which increases the active gastro-intestinal absorption of calcium. Once the serum calcium normalizes, the hydroxylation of vitamin D in the kidneys is shifted to other positions, such as the 22, 23 or 24 positions yielding less active vitamin D metabolites [2]. The hydroxylation of 25-hydroxy vitamin D also occurs in a number of cells to maintain local cellular functions.

Although severe vitamin D deficiency may lead to rickets in children and osteomalacia in adults, there are no specific, easily recognizable, clinical features of early vitamin D deficiency. Signs and symptoms of vitamin D deficiency include ill-defined generalized aches and pains, bone tenderness, muscle weakness (especially proximal), unsteadiness, repeated falls, as well as neuropsychiatric symptoms including lethargy, fatigue, and depression [3]. Vitamin D deficiency also may predispose to a number of other conditions including immune dysfunction, type 1 and 2 diabetes mellitus, cardiovascular diseases, neurocognitive deficits, and various neoplasia such as prostate, breast, and colorectal cancers [4,5].

Vitamin D deficiency is quite prevalent, estimated to affect about one billion people world-wide [4]. It is due to a relatively low oral vitamin D intake and the present tendency to avoid direct exposure to sunlight and widespread use of suntan lotions to protect the skin from the potential nefarious effects of direct exposure to sunlight, especially an increased risk of skin neoplasia. It is indeed ironic that sunlight is so essential for the skin to produce vitamin D and yet has to be restricted to avoid skin cancer.

The 25-hydroxyvitamin D that occurs naturally in some animal and plant food products is more easily absorbed and utilized by the human body than that obtained through separately formulated pill or chewable vitamin D supplements [6]. Unless enriched with the vitamin, very few foods naturally contain vitamin D. These include: fatty fish such as salmon, herring, sardines, oysters and shrimps; some mushrooms, turnips and dark leafy vegetables such as spinach, kale, and collard greens.

Given the paucity of foods that naturally contain vitamin D, vitamin D intake through food is limited. Several foods, however, are now commonly fortified with vitamin D, such as milk, yogurt, cereals and orange juice. Though vitamin D supplementation is often prescribed clinically in order to address individual cases of hypovitaminosis D in the short-term, fortification of staple foods such as milk and bread is widely considered to be the best method of rectifying vitamin D deficiency on a broad population level [7]. Populations of countries that maintain national vitamin D fortification policies (such as the United States, Canada, and Finland) obtain 28-63% of their total vitamin D intake via milk products, whereas people living in nations that have only partial or no fortification policies receive little-to-no vitamin D through consumption of dairy foods [8].

In the 1930s in the United States, population-wide efforts to provide vitamin D supplementation of milk and milk products nearly extinguished rickets and osteomalacia [9]. Unfortunately, however, since then dietary intake of milk has steadily decreased, sparking multiple vitamin D deficiency-related health concerns. In order to combat this decline, the United States Department of Health and Human Services issued Dietary Guidelines recommending that vitamin D fortification be increased not only in milk but also in other dairy food products such as yogurt and processed cheese [9]. The U.S. National Osteoporosis Foundation recommends a daily intake of 800-1,000 IU cholecaciferol for postmenopausal women and men over the age of 50 [10]. Recent studies show that dietary intake of fortified dairy products improves bone metabolism more than calcium supplementation alone [11]. Sustained regular consumption of fortified milk has been found to increase bone mineral density at the femoral neck of the hip in different populations of postmenopausal women, and even possibly to positively affect glucose and lipid profiles [12,13].

Direct addition of vitamin concentrates to food products has long been considered a more dependable method than supplementation of animal feed or irradiation. Vitamin D concentrates are added to dairy foods prior to pasteurization. In the 1990s the U.S. Food and Drug Administration mandated that vitamin D fortification in milk amount to 100-150% of the listed value on the label to ensure that sufficient fortification is achieved in spite of variability in vitamin content [14]. In the U.S., all nonfat and low fat milk must contain at least 400 IU (but no more than 800 IU of vitamin D) per quart [14,15].

A major concern is that vitamin D in food products degrades with time and with exposure to air and light, which not only affects the vitamin D content but also the scent and flavor of fortified milk products [9]. Currently little research is available on the effect of vitamin D degradation on the taste and smell of dairy products [9]. Several studies have shown that added vitamin D in food products tends to remain stable throughout processing and storage prior to consumer purchase [9]. Although it may be presumed that vitamin D will not degrade significantly during the relatively short time period between purchase and consumption of milk products, this area is not thoroughly studied and is potentially problematic, especially as a 2001 report showed that 53-55% of fortified milk products available for purchase in the United States did not comply with their label claims for vitamin D content [16].

The purpose of our study is to determine whether exposure to direct or indirect sunlight affects the biodegradation of vitamin D in vitamin D fortified milk commercially available for general consumption.

Materials and Methods

Commercially available skim milk (Kroger brand) within the recommended period of its 'Best used by date' was used. Three clear glass containers of the milk were stored in a refrigerator, 3 were kept unrefrigerated in the shade, and adjacent to these, 3 were kept in full sunlight. Prior to sample collection each container was shaken well to ensure homogeneity. Lids were placed on the containers to avoid contamination by particulate matter. Then 20-mL aliquots from each container were taken at 0, 5, 10, 30, 60, and 90 minutes. These time points were selected based on the results of a pilot study conducted over a period of several hours which showed that most of the biodegradation occurs within the first 60 minutes of exposure to direct sunlight.

The modified AOAC Official Method 992.26 was utilized to extract vitamin D_3 (cholecalciferol) as described in AOAC Method 2002.05 [17,18]. A 20 mL sample of milk was mixed and ethanol: propanol (95:5, v:v) and 0.20 g of L-ascorbic acid were added to the

mixture. The content was shaken for 30 seconds, followed by the addition of 4 g of potassium hydroxide pellets. The content was placed in a 75°C water bath for 30 minutes. The supernatant was mixed with equal portion (65 mL) of each ethyl ether and petroleum ether. The solution was mixed well, then the organic layer was subjected to rotary evaporation and then transferred to a glass vial using acetone. After the evaporation of acetone, the sample was suspended in 5 mL of ethyl ether twice. The solvent was then allowed to go to dryness under a stream of nitrogen gas.

The solidified sample extracts were refrigerated at 4°C overnight, then dissolved in 1 mL of ethanol and filtered using a 0.45 µm syringe filter prior to HPLC analysis. The HPLC was performed using Shimadzu LC-20AD dual pumps equipped with a SPD-20A UV detector and SIL-20A Prominence autosampler. An ES Industries Chromosorb WR C_{18}column (120 A, 5 µm and 15 cmx4.6 mm) was used at room temperature. The mobile phase of methanol, ethanol (17:3, v:v), was pumped at a flow rate of 0.40 mL/min. A 100-µL of the extract was injected and the chromatograms were obtained using Shimadzu LC Solution software at the UV wavelength setting of 265 nm.

To validate the method, calibration curves were examined before and after analyzing the extracts. A sample of milk was also spiked with vitamin D_3 standard to validate the extraction procedure. The vitamin D content (mg/L) was calculated using the Beer-Lambert law [19,20].

Individual data were plotted (Figure 1) and analyzed. The importance of time and location were later assessed using a mixed ANOVA model for repeated measures since each sample (A, B, C) was measured 6 times over the time period observed. The analysis was done using Minitab 18 using the option STAT > ANOVA > Balanced Analysis of Variance and choosing the option of restricted model as it is done in Minitab for the repeated measures case.

Results

This study was conducted on a sunny spring day at 12:00 noon: cloudless sky, temperature 72°F, no significant wind. The results are shown in the accompanying table and plot. The variability in vitamin D among samples under the same conditions of place and time were minimal. A clear pattern emerged.

During the first 10 minutes the vitamin D content was similar for the 3 places—refrigerator, shade, and sunlight.

At 30 minutes whereas there was still little difference in vitamin D content between the milk kept in the shade and in the fridge, the vitamin D content of the milk kept in direct sunshine dropped and continued to drop at the same rate throughout the remaining 30 minutes of the first 60 minutes. In the period between 60 minutes and 90 minutes the vitamin D content of samples kept in direct sunlight did not exhibit any further degradation and remained steady at the level to which it dropped at the end of 60 minutes.

After 60 minutes the vitamin D content in milk kept unrefrigerated in the shade dropped to a level similar to that of the milk kept in the sunshine at 30 minutes, and there was no further degradation. The rate of decline in vitamin D content is similar for the milk placed in sunshine and in shade, but the decline in the sunshine starts after 10 minutes whereas the decline in the shade starts after 30 minutes. In both cases the decline in vitamin D stops at 60 minutes. The rate of decline in vitamin D, assumed a linear decline and based on the available data, is approximately 0.0008 units per minute.

The output indicates that the difference among samples (A,B,C) kept in the same location is not significant (p-value 0.672). It also indicates that both the place of storage and the time make a difference in the vitamin D content and that the way time affects the vitamin D content depends on the location (the interaction between time and place is significant). The p-values for place, time, and interaction between time and place were all less than 0.001.

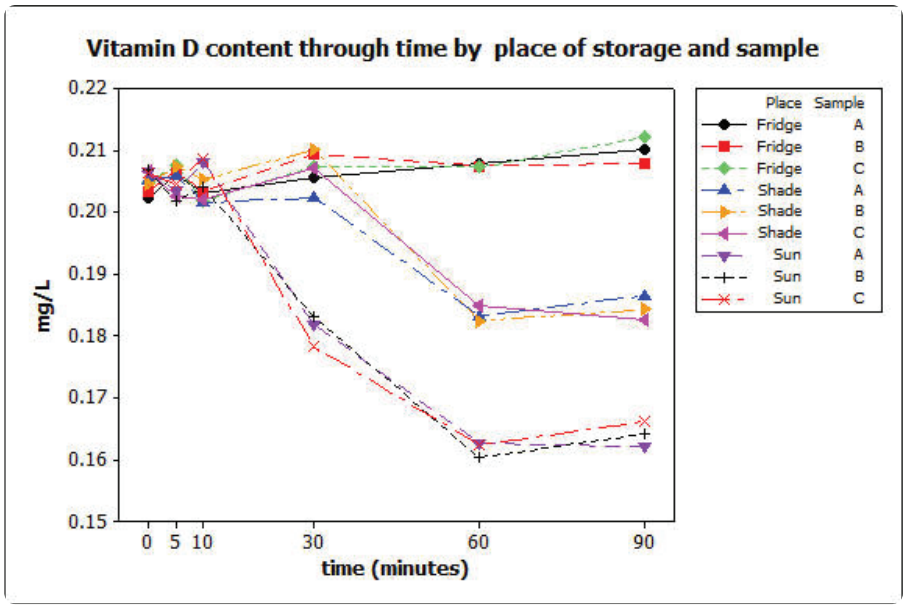

Figure 1: Concentration of Vitamin D (mg/L) at Various Times of the Study.

Table 1: Concentration (mg/L) of Vitamin D and Standard Deviation at Various Times of the Study.

Time	Fridge		Shade		Sun	
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev
0	0.203489	0.0012783	0.205411	0.0010533	0.206656	0.0002480
5	0.206802	0.0007704	0.205362	0.0024394	0.203272	0.0014755
10	0.202772	0.0008413	0.202951	0.0019930	0.206931	0.0025394
30	0.207487	0.0018807	0.206580	0.0039233	0.181035	0.0025400
60	0.207547	0.0003304	0.183535	0.0012924	0.161759	0.0012683
90	0.210098	0.0021610	0.184473	0.0018797	0.164126	0.0020174

Our data show that the vitamin D content of fortified milk decreases significantly (to about 80% of its original value) over a period of 60 minutes when exposed to direct sunlight. The vitamin D content of fortified milk kept unrefrigerated in shade also decreases over the same period of time, but to a lesser extent: about 90%. On the other hand the vitamin D content of samples kept refrigerated remains largely unchanged throughout the test period. Glass containers do not appear to protect vitamin D from degradation.

Discussion

Whereas there is some reassurance about vitamin D concentration in milk from production to sale time [9], there is little information about the vitamin D concentration in milk from sale to consumption time. This is particularly relevant as nowadays increasing numbers of people rely on vitamin supplementation in food to obtain adequate amounts of vitamin D while at the same time avoiding direct exposure of unprotected skin to sunshine. There is a need to anticipate potential reduction of the potency of vitamin D levels due to biodegradation.

Our results indicate that the vitamin D content of milk exposed to direct sunlight decreases over a period of 60 minutes by about 20% of its original content and the degradation process starts after just 10 minutes of exposure. On the other hand, only 10% of vitamin D content is degraded when milk is kept unrefrigerated in the shade, and the degradation process starts after 30 minutes. There is no evidence of vitamin D degradation in samples kept refrigerated throughout the study period.

Although it may be argued that milk will seldom be left exposed to direct sunlight for a full hour, it is not uncommon to leave milk exposed to direct sunlight for 10-30 minutes. Furthermore, sometimes in restaurants milk jugs are left unrefrigerated and exposed for a few hours prior to its addition to coffee or tea. This may lull consumers into a false sense of security, as they may assume that even after exposure to sunlight they are getting the full vitamin D content of the fortified milk.

In retrospect, the temperature of the samples should have been noted at the various time points, as temperature may affect the degradation process. However, our findings are so marked as to have significant implications if milk is left exposed to direct sunlight for more than 10 minutes. More work is needed to study the effect of vitamin D degradation in milk products.

Conclusion

Our results show that vitamin D in fortified milk biodegrades when it is left exposed to direct sunlight for longer than 10 minutes and to a lesser extent when milk if kept unrefrigerated. Glass containers do not protect against cholecalciferol biodegradation.

Author Contributions

RCH, RMM, and WAC developed the protocol. RMM did laboratory analysis. ES performed statistical analysis. RCH, RMM, ES, and JC wrote the manuscript. All authors reviewed the manuscript and edited and approved the final version.

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