Short communication: Artificial ultraviolet B light exposure increases vitamin D levels in cow plasma and milk

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ABSTRACT

The number of dairy cows without access to pasture or sunlight is increasing; therefore, the content of vitamin D in dairy products is decreasing. Ultimately, declining vitamin D levels in dairy products will mean that dairy products are a negligible source of natural vitamin D for humans. We tested the ability of a specially designed UVB lamp to enhance the vitamin D₃ content in milk from dairy cows housed indoors. This study included 16 cows divided into 4 groups. Each group was exposed daily to artificial UVB light simulating 1, 2, 3, or 4 h of summer sun at 56°N for 24 d, and the group with simulated exposure to 2 h of summer sun daily continued to be monitored for 73 d. We found a significant increase in 25-hydroxyvitamin D₃ (25OHD₃) levels in plasma as well as vitamin D₃ and 25OHD₃ levels in milk after daily exposure for 24 d in all treatment groups. Extending daily exposure to artificial UVB light to 73 d did not lead to an increase of vitamin D₃ or 25OHD₃ level in the milk. In conclusion, the change in production facilities for dairy cows providing cows with no access to pasture and sunlight causes a decrease of vitamin D levels in dairy products. This decrease may be prevented by exposing cows to artificial UVB light in the stable.

Key words: vitamin D, artificial UVB exposure, cow milk

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The metabolic active form of vitamin D, 1,25-dihydroxyvitamin D, is essential for regulating the levels of calcium and phosphorus in vertebrates; in addition, vitamin D insufficiency has been linked to an increased risk of hypertension, autoimmune diseases, diabetes,

and cancer (DeLuca, 2004). Cutaneous synthesis of vitamin D₃ is considered the major source of vitamin D, but sun exposure is limited during winter at northern latitudes and intake from food becomes essential (Madsen et al., 2013).

Generally, fatty fish have the highest natural content of vitamin D and are regarded as an important source of vitamin D. Meat and dairy products have lower contents of vitamin D than fish, but a high intake of meat and dairy products is typical in a Western diet; consequently, their contribution to the total vitamin D intake is significant despite their low content of vitamin D. In Denmark, cow milk contributes approximately 12% of the total vitamin D intake via consumption of milk, cheese, and other dairy products (Pedersen et al., 2010). Mandatory or voluntary fortification of, for example, milk and margarine are implemented in some countries, but in other countries (e.g., Denmark), fortification with vitamin D is negligible. Two primary sources of vitamin D exist for cows: cholecalciferol (vitamin D₃) provided in the feed or produced endogenously in animals by UVB exposure of 7-dehydrocholesterol in the skin. Bio-fortification strategies for vitamin D optimize these natural processes to increase the content of vitamin D in food products eaten by consumers.

Several factors influence the synthesis of vitamin D₃ in the skin. In cows, vitamin D₃ synthesis in the skin derived from sun exposure has been shown to be independent of hair coverage but does depend on the duration of light exposure (Hymøller and Jensen, 2010, 2012). In addition, season and latitude influence the synthesis of vitamin D₃ in the skin. In dairy cows, vitamin D₃ supplementation increases vitamin D₃ status during the winter but has no effect during the summer if the cows have access to pasture (Hymøller et al., 2009). Observational studies have shown seasonal variations, with the maximum level of vitamin D in milk being detected during the summer (Chick and Roscoe, 1926; Bechtel and Hoppert, 1936). Similarly, vitamin D₃ levels in milk from cows in New Zealand grazing on pastures

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year round were highest in the summer (Kurmann and Indyk, 1994). Whole milk derived from organic cows in the winter, when cows are housed in stables, bought on the Danish market, showed a vitamin D content that was 6 times lower compared with that of whole milk bought during the summer, when the cows are, by regulation, on pasture (Jakobsen and Saxholt, 2009). Studies of human breast milk have provided further evidence for the association between sunlight exposure and vitamin D₃ content in milk (Greer et al., 1984; Ala-Houhala et al., 1988).

In Denmark, the number of dairy cows without access to pasture has increased as dairy farming has become more efficient (Knowledge Center for Agriculture, 2014). However, the current vitamin D recommendation is based on access to pasture. Ultimately, declining vitamin D levels in dairy products will transform dairy products to a negligible source of natural vitamin D for consumers. In this study, we tested the efficacy of a lamp specifically constructed to increase vitamin D₃ synthesis. The lamp was developed to mimic the sun to enhance the natural synthesis of vitamin D₃ in cows with no access to sunlight. Our objective was to produce milk that had levels of vitamin D similar to or higher than that of milk derived from dairy cows with access to pasture. In addition, our intention was to benefit the cows, so we included measurements of vitamin D₃ status. The determination of other endpoints used to analyze the health of the dairy cows was outside the scope of this study due to economic constraints.

The current study complied with the Danish Ministry of Justice Law No. 1306 (November 23, 2007) concerning experiments with animals and the care of animals used for experimental purposes. This study was designed to assess the effect of daily exposure to an artificial UVB light source for 30, 60, 90, and 120 min, which imitated sun exposure comparable to approximately 1, 2, 3, and 4 h of summer sun at 56°N, respectively, on vitamin D₃, 25-hydroxyvitamin D₃ (25OHD₃), and fat contents in cow milk as well as on the 25OHD₃ level in plasma. Sixteen dairy cows of the black and white Danish Holstein breed were included in this study and divided into 4 treatment groups, with 4 cows in each group.

Coat color of the cows was assessed as the percentage of black coat area visually estimated from photographic silhouettes of the cows from different angles (Sneddon et al., 2004). Before inclusion in the treatment groups, cows were deprived of vitamin D₃ in their diet for 6 mo and had no access to sun exposure during this period. The exception, for practical reasons, was that cows exposed to artificial UVB light for 60 min daily, simulating 2 h of summer sun, had been provided feed with vitamin D₃ until a couple of months before the start of the experiment.

During the study, all cows were housed in a tie stable with slatted floors and fed ad libitum with a maize-, clover grass-, and rye silage-based TMR void of vitamin D₃. Feeding took place once a day at 0900 h, and milking was carried out twice a day at 0600 and 1700 h. The study was carried out between July and November 2011 at Aarhus University, Department of Animal Science (Tjele, Denmark).

The lamp applied in this study was developed specifically to simulate the sun and to provide the wavelengths necessary for conversion of 7-dehydrocholesterol to vitamin D₃ in the skin of the dairy cows; wavelengths ranged from 280 to 415 nm. An OL 756 spectroradiometer (Optronics Laboratories, Orlando, FL) was used to scan the light emitted from the lamp from 250 to 400 nm with 1-nm increments to calculate the erythema dose according to the Erythema Reference Action Spectrum and Standard Erythema Dose (SED; ISO/CIE Standard ISO 17166:1999/CIE S 007-1998; ISO, 1999). One SED was defined as 100 J/m² (Diffey et al., 1997). The lamp was situated at a distance of 3.5 m from the cows, and 30, 60, 90, and 120 min of artificial UVB light exposure was calculated to equal 1, 2, 3, and 4 h, respectively, of full body exposure of midday summer sun at 56°N (Bogh et al., 2012).

The duration of the study was 24 d for the groups simulating 1, 3, and 4 h of sunlight, whereas it was extended to 73 d for the group exposed daily to artificial UVB light simulating 2 h of sunlight. Every day, each of the cows was positioned in front of the lamp at a distance of approximately 3.5 m for the designated duration of exposure. The cows' heads were facing opposite the lamp.

Throughout the study period, milk and blood were collected from the cows. Milk samples were collected in vacuum buckets, and the milk was stirred before sampling an aliquot of a minimum of 250 mL. Sampling was done before the first artificial UVB light exposure (d 0) and at d 24 for all groups; sample collection was repeated on d 3, 10, 17, and weekly thereafter until d 73 for the cows exposed to the artificial UVB light daily for 60 min. Plasma samples were obtained from blood collected from the tail vein in sodium heparin-coated Vacutainer tubes (Becton, Dickinson Co., Franklin Lakes, NJ) between 0830 and 0930 h on d 0 before artificial UVB light treatment and subsequently every 3 to 4 d. All samples of milk and plasma were stored at -18°C or below until analysis.

The analyses of vitamin D₃ and the metabolite 25OHD₃ were performed by using a method including saponification and liquid/liquid extraction (Jakobsen et al., 2004; Jakobsen and Saxholt, 2009), followed by quantification by electrospray ionization tandem mass spectrometry as described previously (Jäpelt et al.,

2013). Aliquots of 25 g (± 1 g) of milk were taken for analyses. The content of fat in the milk was determined by the gravimetric method, according to a modified Schmid-Bondzynski-Ratslaff procedure (NMKL, 1989). In the laboratory at the Department of Animal Science, Aarhus University (Tjele, Denmark), the content of 25OHD₃ in plasma was assessed in 1.5 mL of plasma by a method described elsewhere (Hymøller and Jensen, 2011).

Statistical analysis was performed with SAS software (SAS Institute Inc., Cary, NC). To examine the effect of the artificial UVB light exposure, paired *t*-tests were used, whereas one-way ANOVA was used to test the differences between the treatment groups. A repeated-measures model was fitted to compare the estimated level at d 24 to the estimated maximum level attained. The number of days of artificial UVB light exposure and coat color were included as factors in the model. Observations from the same cow could not be assumed to be independent, and they were modeled using a first-order autoregressive moving-average correlation structure. Finally, linear normal regression models were fitted to test the association between 25OHD₃ in plasma and vitamin D metabolites in the milk. The vitamin D metabolite and fat levels are given as mean $\pm$ standard error of the mean (SEM).

The effects of 30, 90, or 120 min of daily exposure to a UVB lamp for 24 d on levels of vitamin D₃, 25OHD₃, and percentage fat in milk are shown in Figure 1. From d 0 to 24, the artificial UVB light exposure was found to generate a significant increase in vitamin D₃ and 25OHD₃ levels in milk for all treatment groups. For fat content, no effect was found in the 30- and 90-min groups, but a slight decrease was seen in the 120-min group from d 0 until 24.

For the level of 25OHD₃ in milk compared with that in plasma at d 24, the regression of 25OHD₃ in milk on 25OHD₃ in plasma was significant ($P = 0.03$), with a slope of 0.11 (95% CI: 0.01–0.20), whereas no association was found at d 0 ($P = 0.99$). For the level of vitamin D₃ in milk versus that in plasma, no statistically significant association on d 0 or 24 was found ($P = 0.28$ and 0.08, respectively).

As shown in Figure 2, the levels of 25OHD₃ in plasma at d 0 and 24 revealed a significant effect of the artificial UVB light exposure in all treatment groups ($P = 0.0073$ for 30 min, $P = 0.0105$ for 90 min, and $P = 0.0064$ for 120 min). A significant difference was shown between the groups, as the cows exposed to the UVB lamp for 90 and 120 min daily showed higher vitamin D levels than the cows exposed for 30 min daily ($P = 0.001$).

The effects of 60-min daily lamp exposure for 73 d on the levels of vitamin D₃, 25OHD₃, and percentage fat

Figure 1. Content of vitamin D₃, 25-hydroxyvitamin D₃, and fat in milk sampled at d 0 and 24 (mean $\pm$ SEM) from cows ($n = 4$ in each group) exposed to a UVB lamp daily for 30, 90, or 120 min. The *P*-values originate from paired *t*-tests comparing d 0 with d 24.

in milk are shown in Figure 3. In milk, a statistically significant increase from d 0 to 24 was shown for vitamin D₃ ($P = 0.029$), but not for 25OHD₃ ($P = 0.089$) or percentage fat ($P = 0.971$). A repeated-measures model was fitted to compare the estimated level at d 24 to the estimated maximum level attained during 73 d of exposure. No significant difference was found between the estimated maximum level and the level on d 24 in milk for vitamin D₃ ($P = 0.40$), 25OHD₃ ($P = 0.087$), or fat ($P = 0.074$). However, we did detect a statistically significant increase in plasma 25OHD₃ from d 0 to 24 ($P = 0.0017$) and a further increase until d 73 ($P < 0.001$), with an estimated maximum at d 64 (Figure 4).

In commercial milk, levels of vitamin D₃ and 25OHD₃ have been shown to be correlated with fat content (Jakobsen and Saxholt, 2009). However, in this study, the percentage of fat in the milk was not a determinant for the content of vitamin D₃. In another study using exclusively pasture-grazed cows, vitamin D₃ levels were also shown to be independent of the proportion of fat in the milk (Kurmman and Indyk, 1994). This difference in fat content is probably due to the cow's natural variation in milk production and not to the production and separation of the milk into skimmed and whole milk.

The UVB lamp used in this study was developed to stimulate cutaneous synthesis of vitamin D₃ in vertebrate animals. Cutaneous vitamin D₃ synthesis can be stimulated by irradiation of the skin to artificial UVB light, which has been shown previously in pigs (Cooper et al., 1997) and humans (Greer et al., 1984; Bogh et al., 2012). The maximum synthesis of the vitamin D₃ precursor previtamin D₃ in the skin occurs at 295 to 300 nm. Monochromatic UV light at 295 nm and sunlight are reported to cause different degradation profiles of previtamin D₃ to lumisterol and tachysterol, probably because of the different UV absorption spectra of these compounds (MacLaughlin et al., 1982). To mimic solar synthesis, we developed and selected a broadband UVB light source.

We showed a significant increase in the vitamin D₃ and 25OHD₃ levels in the milk from cows exposed to UVB light for 24 d compared with d 0 (Figure 1). The artificial UVB light exposure, which simulated summer sun at 56°N for approximately 4 h daily for 24 d, resulted in 18 ± 6 ng of vitamin D₃/100 g of milk and 5 ± 2 ng of 25OHD₃/100 g of milk. These levels are similar to the contents reported by Jakobsen and Saxholt (2009) in Danish milk, which showed a maximum content of 19 ng of vitamin D₃ and 8 ng of 25OHD₃ per 100 g of

Figure 2. Vitamin D (mean $\pm$ SEM) in plasma of cows ($n = 4$ in each group) exposed daily to the UVB lamp for 30, 90, or 120 min. We detected a significant difference between d 0 and 24 ($P = 0.0073$, 0.0105 , and 0.0064 , respectively).

whole milk (containing 3.5% fat). Kurmann and Indyk (1994) found a maximum of 27 ng of vitamin D₃/100 mL in milk from New Zealand, but they did not quantify 25OHD₃. We sought to determine if we had reached a steady-state vitamin D₃ level after 24 d of artificial UVB light exposure by extending the daily exposure of 60 min (similar to 2 h of summer sunlight) from 24 to 73 d. We observed no significant increase in the vitamin D₃ and 25OHD₃ levels in the milk (Figure 3). This finding underlines that exposing cows to artificial UVB light for 24 d is sufficient to obtain a maximum level of vitamin D in the milk.

Furthermore, we showed a significant increase in the plasma vitamin D₃ level in cows exposed to artificial UVB light. By simulating 3 or 4 h of daily sun exposure with the lamp for 24 d, the vitamin D₃ level in the plasma increased to 30 ng of 25OHD₃/mL (Figure 2). This level was similar to that of cows grazing at pasture for 2.5 or 5 h daily during 24 d of Danish summer, which achieved vitamin D levels of 22 and 36 ng/mL, respectively (Hymøller and Jensen, 2012). This comparison is strong because the 2 studies were performed in the same breed as used in this study—Danish Holstein—and both sets of analyses were run by the same laboratory.

However, we did not observe a steady state for vitamin D level in plasma after 24 d of daily artificial UVB light exposure for 60 min; the data in Figure 4 show convergence toward a steady state after 24 d. A maximum vitamin D level in plasma has been observed in humans and minipigs. In intervention studies of humans exposed to UV light or treated orally with different levels of vitamin D₃, data have shown a similar maximum level of vitamin D that depends on the UV exposure period or oral dose (Heaney et al., 2003; Bogh et al., 2012); similar results have been found in minipigs (Burild et al., 2015).

In this study, we showed an association between plasma vitamin D level and milk 25OHD₃ level. However, we did not identify an association between the level of vitamin D in plasma and that in milk. This finding is in contrast to studies in sows that were fed different levels of vitamin D₃, in which an association was shown between plasma vitamin D level and milk vitamin D₃ level in sow milk (Flohr et al., 2014; Weber et al., 2014).

The limited number of cows in each group was a limitation of this study. However, we showed the efficacy of artificial UVB exposure to increase vitamin D₃ levels in milk and plasma to levels similar to those of cows grazing at pasture during the summer. Potentially, this process could be used in organic and conventional farming to ensure a high vitamin D level in cows raised indoors year round. In particular, this process will not

Figure 3. Vitamin D₃, 25-hydroxyvitamin D₃, and fat in milk sampled regularly from d 0 to 73 (mean $\pm$ SEM) from cows ($n = 4$ in this group) exposed daily to the UVB lamp for 60 min. Between d 0 and 24, a significant increase was shown for vitamin D₃ in milk ($P = 0.029$), but not for 25-hydroxyvitamin D₃ and fat ($P = 0.089$ and 0.971 , respectively). We detected no further increase between d 24 and 73 for all compounds ($P < 0.05$).

Figure 4. The vitamin D (mean $\pm$ SEM) in the plasma of cows ($n = 4$ in this group) exposed daily to the UVB lamp for 60 min. We detected a significant increase between d 0 and 24 ($P = 0.0017$) and a further increase to d 73 ($P < 0.001$).

cause any toxicity for the cows if we presume that the production of vitamin D₃ in the cow's skin has similar metabolism as that in human skin (Webb and Holick, 1988).

We developed a light source for use in stables that emitted vitamin D-producing wavelengths together with light at higher wavelengths for normal lighting. At this stage, further development of the lamp prototype is necessary to make it suitable for industrial production of dairy products, thus enabling the cows to produce milk with the same high level of vitamin D₃ in the winter as in the summer. Further optimization of the artificial UVB light exposure is needed to secure a constant and high level of vitamin D₃ in dairy products year round. We did not evaluate health benefits for the cows in this study, but a higher vitamin D₃ status might reduce the requirement for phosphorous and reduce the risk of mastitis (Lippolis et al., 2011). Based on the findings of this study, exposing dairy cows to artificial UVB light would contribute to the health of people consuming dairy products and could improve the health of the cows by contributing to a general increase of the vitamin D₃ level in the plasma. The production system would be in line with bio-fortification, as the increase of the content of vitamin D₃ in dairy products is part of a natural production system. Because of the risk of skin cancer, humans are advised not to stay in the midday sun; therefore, it is essential to state that the artificial lighting system should be turned off during work hours. Dairy cows should not be exposed to a higher dose than cows grazing in the pasture during a summer day.

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