J. Dairy Sci. 93:2025–2029
doi:10.3168/jds.2009-2991

Vitamin D_3 synthesis in the entire skin surface of dairy cows despite hair coverage

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ABSTRACT

How hair-coated animals such as dairy cows synthesize endogenous vitamin D_3 during exposure to summer sunlight has been unclear since vitamin D_3 and its relation to sunlight was discovered. The fur of fur-bearing animals is thought to be comparable to clothing in humans, which prevents vitamin D_3 synthesis in the skin during exposure to sunlight. Different scenarios have been suggested but never tested in cows; for example, that vitamin D_3 is synthesized from sebum on the hair and ingested by cows during grooming or that body areas such as the udder and muzzle that have scant hair exclusively produce the vitamin. To test different scenarios, 16 Danish Holstein dairy cows were subjected to 4 degrees of coverage of their bodies with fabric that prevented vitamin D_3 synthesis in the covered skin areas. The treatments were horse blanket (cows fitted with horse blankets), udder cover (cows fitted with udder covers, horse blanket + udder cover (cows fitted with both horse blankets and udder covers), and natural (cows without any coverage fitted). The cows were let out to pasture daily between 1000 and 1500 h for 4 wk in July and August 2009. Blood samples were collected 15 times during the study and analyzed for content of 25-hydroxyvitamin D_3 [25(OH)D_3] indicative of the animals' vitamin D_3 status. Results showed that uncovered cows had a higher 25(OH)D_3 concentration in plasma after 28 d of access to sunlight compared with covered cows and that the plasma concentration of 25(OH)D_3 was strongly inversely correlated to the body surface area covered. These results are consistent with findings in humans, wherein the vitamin D_3 status of different individuals was inversely proportional to the amount of clothing worn during exposure to artificial sunlight. Hence, it appears that human clothing and cow hair are not comparable with respect to prevention of vitamin D_3 synthesis and that cows, like humans, synthesize vitamin D_3 evenly over their body surface. That vitamin D_3 should be synthesized from sebum on the hair and obtained by cows as a result of grooming is not supported by the findings in the present study either, because large differences were found between the treatment groups. If grooming were the source of vitamin D_3, then a relatively even 25(OH)D_3 concentration between treatments would be expected, because covered cows would obtain vitamin D_3 by grooming uncovered herdmates.

Key words: vitamin D_3 synthesis, skin, hair and coat, dairy cow

INTRODUCTION

How hair-coated animals such as dairy cows synthesize endogenous vitamin D_3 during exposure to summer sunlight has been unclear since vitamin D_3 and its relation to sunlight was discovered (Carpenter and Zhao, 1999). Synthesis of vitamin D_3 in the skin is facilitated by sunlight or more precisely by incident UVB light (280–315 nm) that reaches the surface of the earth during the summer months in the Northern Hemisphere (Engelsen et al., 2005). The UV light cleaves 7-dehydrocholesterol in the skin and produces pre-vitamin D_3, which spontaneously isomerizes into vitamin D_3 at body temperature (MacLaughlin et al., 1982; Stryer, 1995). This process takes place in the stratum spinosum and stratum basale layers of human skin (Norman, 1998), and its contribution to the vitamin D_3 status of an individual has been shown to be inversely proportional to the amount of clothing worn by human test subjects during exposure to UV light (Matsuoka et al., 1992).

The hair of fur-bearing animals is thought to compare with clothing in humans, and it is heavily debated how hair- or fur-coated animals synthesize their vitamin D_3 (Carpenter and Zhao, 1999). Fur-bearing animals such as rats and rabbits do have 7-dehydrocholesterol in their skin, and isolated shaved skin from these species does produce vitamin D_3 (Bekemeier, 1959); however, Gaylor and Sault (1964) showed that 7-dehydrocholesterol in rat skin was mainly associated with sebaceous glands. Therefore, it was speculated that 7-dehydrocholesterol in sebum might be translocated onto the rat's fur and skin surface, where it would be irradiated and turn into

Received December 11, 2009.
Accepted January 14, 2010.
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Figure 1. Cows fitted with udder cover (left) and horse blanket (right). Photo courtesy of Information Officer Janne Hansen, Aarhus University, Faculty of Agricultural Sciences, Tjele, Denmark.

vitamin D_3, to be ingested when the animal groomed itself or other animals (Carpenter and Zhao, 1999).

In cattle, 3 hypotheses on vitamin D_3 synthesis during exposure to summer sunlight can be put forward: 1) the self-grooming theory based on rat studies; 2) the hypothesis that skin areas with no or scant hair coverage (e.g., udder and muzzle) are sufficient to synthesize the vitamin D_3 found in cattle during summer; and 3) the hypothesis that the physical properties of hair are different from those of human clothing and that cattle are able to synthesize vitamin D_3 all over the skin surface. The aim of the present study was to test if any of the suggested hypotheses explained how cows synthesize endogenous vitamin D_3 during exposure to summer sunlight.

MATERIALS AND METHODS

Animals and Housing

The present experiment complied with the Danish Ministry of Justice Law No. 1306 (November 23, 2007) concerning experiments with animals and care of experimental animals. Sixteen dairy cows of the Danish Holstein breed with an average milk yield of 34.6 ± 1.6 (mean ± SEM) kg of ECM per day were deprived of vitamin D_3 for 6 mo by omitting vitamin D_3 from their feed and housing them without access to sunlight. The cows were divided into 4 treatment groups according to milk yield and parity. For the duration of the study, the cows were let out to pasture from 1000 to 1500 h every day and spent the remaining time housed in a tie-stable out of the sunlight. Cows were fed ad libitum with a maize and clover grass based TMR without added vitamin D_3. The TMR was fed once a day at 0900 h, and milking was carried out twice a day at 0600 and 1700 h. The study was conducted from July 20 to August 17, 2009, at Aarhus University, Faculty of Agricultural Sciences (Tjele, Denmark; 9.3°E/56.3°N). The average daily amount of sunshine at the study site was 5.4 h, which was slightly higher than during a typical summer in the area (DMI, 2009).

Treatments

The treatments consisted of 4 different degrees of coverage of the cows' bodies with fabric that prevented vitamin D_3 synthesis in covered skin areas. The treatments were 1) horse blanket: cows were fitted with Horze Eczema UV protection blankets from Horze (Hollola, Finland; Figure 1); 2) udder cover: cows were fitted with udder covers (Figure 1); 3) horse blanket + udder cover: cows were fitted with both horse blankets and udder covers; and 4) natural: cows had no coverage fitted.

Plasma Samples

Blood was collected from the tail vein in sodium heparin-coated Vacutainer tubes between 0830 and 0930 h on d 1, 2, 3, 4, 5, 7, 9, 11, 14, 16, 18, 21, 25, and 28 of the study and centrifuged for 10 min at 1,500 × *g*. Plasma was transferred to sample tubes and stored at −18°C until analysis.

Plasma Analysis

In the laboratories at Aarhus University, Faculty of Agricultural Sciences (Tjele, Denmark), the plasma samples were analyzed for content of the liver-derived 25-hydroxyvitamin D_3, 25(OH)D_3, which is indicative of the vitamin D_3 status of the animals. After saponification and extraction, separation was carried out by reverse phase gradient HPLC on a C_{30} column from YMC (Dinslagen, Germany) and UV detection at 265 nm using 1α-hydroxyvitamin D_3 as an internal standard for quantification. The maximum uncertainty of the analytical method within day was 6.2%, and the day-to-day error was 1.8%. The method is described in detail by Hymøller et al. (2009).

Statistical Analysis

Repeated measures of 25(OH)D_3 in plasma of the cows were analyzed in R (R Development Core Team, 2009) using the Linear and Nonlinear Mixed Effects Models package NLME (Pinheiro et al., 2009) for fitting the following nonlinear model:

$$Y_{ij} = A_i + (B_i - A_i) \exp [-\exp (K_i) \times d] + e_{ij},$$

where d is day of sampling (0, 1, 2, 3, 4, 5, 7, 9, 11, 14, 16, 18, 21, 25, 28; input variable), and, as functions of treatment and cow, A_i is a numeric parameter representing the right hand asymptote as $d \rightarrow \infty$; B_i is a numeric parameter representing the response when $d = 0$; K_i is a numeric parameter representing the natural logarithm of the rate constant; and e_{ij} is the random error with mean value zero and variance proportional to the expected values according to the power of the mean. Multiple comparisons were carried out using the Simultaneous Inference in General Parametric Models package MULTCOMP (Hothorn et al., 2008). Differences were considered statistically significant when $P \leq 0.05$. Exposed body surface areas as a percentage of total body surface area within each treatment group were graphically estimated from photographic silhouettes of cows from different angles (Sneddon et al., 2004).

RESULTS

The average concentration of 25(OH)D_3 in plasma at the beginning of the study after vitamin D_3 deprivation was 2.8 ± 0.2 ng/mL (mean ± SEM). At the end of the study (d 28), the 25(OH)D_3 concentrations were 28.6 ± 3.1 ng/mL in the natural group, 23.2 ± 1.5 ng/mL in the udder cover group, 8.9 ± 1.8 ng/mL in the horse blanket group, and 6.0 ± 0.5 ng/mL in the horse blanket + udder cover group. The development in plasma concentrations of 25(OH)D_3 within the 4 different treatments during the 28 d of study are shown in Figure 2. Curve parameter estimates for the peak concentration of 25(OH)D_3 showed a significant difference between the natural treatment and the horse blanket treatment (29.3 ± 4.3 ng/mL; $P \leq 0.01$) and between the horse blanket + udder cover treatment (33.7 ± 4.1 ng/mL; $P \leq 0.01$). The udder cover treatment differed significantly from both the horse blanket treatment (27.8 ± 5.7 ng/mL; $P \leq 0.05$) and the horse blanket + udder cover treatment (32.2 ± 5.6 ng/mL; $P \leq 0.05$). There was, however, no significant difference between the natural treatment and the udder cover treatment or between the horse blanket treatment and the horse blanket + udder cover treatment (Table 1). Furthermore, there was no substantial difference between treatments in the initial 25(OH)D_3 concentration in plasma at d 0.

The 25(OH)D_3 concentration peak estimates (A_i) and the cows' exposed body surface area as a percentage of their total body surface area are shown in Table 2. Peak estimates for the horse blanket and the horse blanket + udder cover treatments were significantly different from the peak estimate for the natural treatment ($P \leq 0.001$), whereas there was no significant difference between the natural and the udder cover treatments (Table 2). The 25(OH)D_3 concentration peak estimates relative to the natural treatment were almost proportional to the exposed body surface area as a percentage of the total body area (natural treatment) in the cows on the udder cover and horse blanket treatments (Table 2). In cows on the horse blanket + udder cover treatment, the 25(OH)D_3 concentration was only 17% of the concentration in the natural treatment, despite 24% of their body area being exposed (Table 2). The correlation between exposed body surface area and relative peak estimate was $r^2 = 0.996$ ($P \leq 0.01$).

DISCUSSION

Dairy cows respond to exposure to summer sunlight or UV light by an increased 25(OH)D_3 concentration in their plasma (Hidiroglou et al., 1985; Hymøller et al., 2009). One of the early discoveries in vitamin D_3 research was that white rats fed a rachitogenic diet failed to develop rickets when exposed to direct sunlight or UV light (Hess, 1922; Hess et al., 1922). However, so did white rats not exposed to sunlight or UV light as long as they were caged with an irradiated cage mate (Nelson and Steenbock, 1925, as cited in Carpenter and Zhao, 1999). This phenomenon was assumed to be the result of nonirradiated rats grooming irradiated rats, thereby obtaining vitamin D_3 from their fur. This finding was partly supported by recent findings by Kalueff et al. (2004), who showed that mice lacking vitamin D

Table 1. Difference in peak 25-hydroxyvitamin D_3 estimates (ng/mL) between treatments (mean ± SE)

	Treatment		
Treatment	Udder cover	Horse blanket	Horse blanket + udder cover
Natural (no cover)	1.5 ± 6.2	29.3 ± 4.3**	33.7 ± 4.1**
Udder cover		27.8 ± 5.7*	32.2 ± 5.6*
Horse blanket			4.4 ± 3.3

*$P \leq 0.05$; **$P \leq 0.01$.

receptors (**VDR**) showed increased grooming behavior. However, it is unknown whether the increased grooming was related to a physiological vitamin D_3 deficiency through lack of VDR or if changes in the nervous system, caused by vitamin D_3 deficiency or lack of VDR, gave rise to the abnormal grooming behavior. In contrast, black rats were shown to develop rickets when fed a rachitogenic diet despite being irradiated with UV light, which indicates that the finding was unrelated to vitamin D_3 synthesis from sebum on the fur (Hess, 1922). The results of the present study do not support the grooming theory in cattle. If grooming is the source of vitamin D_3 in cattle, relatively equal 25(OH) D_3 concentrations between treatment groups would be expected, because covered cows would groom uncovered herd mates and thereby obtain vitamin D_3.

From the results of the present study, vitamin D_3 synthesis in the skin of dairy cows appears to take place across the entire body surface and not just in areas like the udder and muzzle where hair coverage is scarce or lacking. The 25(OH)D_3 status of the cows after 4 wk of access to summer sunlight depended heavily on the size of the exposed body surface area. The larger the body surface area of a given cow that was exposed to the sun during the 4-wk study, the higher the plasma 25(OH)D_3 concentration of the cow at the end of the study.

Matsuoka et al. (1992) showed that in humans exposed to UV light during winter, the increase in vitamin D_3 concentration was inversely proportional to the amount of clothing worn by the test subjects during UV light exposure. The largest increase in vitamin D_3 concentration in serum was found in undressed subjects; the increase in subjects dressed in summer and autumn clothing, respectively, was only 33 and 10% of that in undressed subjects ($P < 0.05$). Hence, vitamin D_3 is synthesized in all areas of the skin of dairy cattle despite their hair coverage. However, it does appear that vitamin D_3 synthesis is less effective when only relatively heavy coated body parts such as the legs and head are exposed to the sun than when the rest of the body surface is exposed. This finding is in agreement with results from studies in sheep, where Quarterman

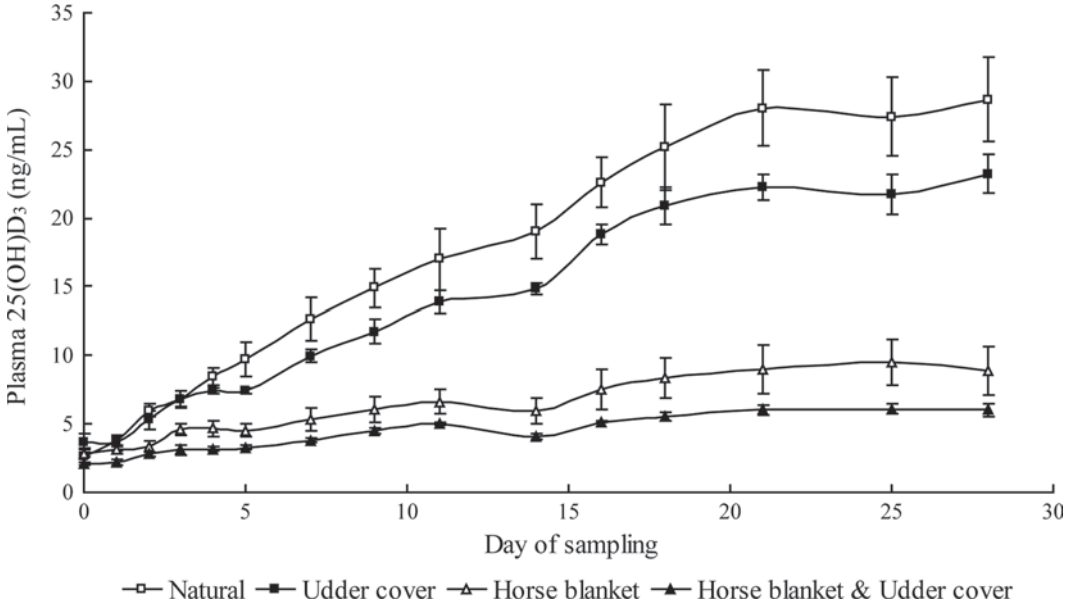

Figure 2. Plasma concentrations of 25-hydroxyvitamin D_3 (ng/mL; mean ± SEM) in dairy cows during 28 d in July and August 2009 under the following treatments: natural (n = 4), udder cover (n = 4), horse blanket (n = 4), and horse blanket + udder cover (n = 4).

Table 2. Estimated 25-hydroxyvitamin D_3 peak concentrations (ng/mL) of the horse blanket, udder cover, and horse blanket + udder cover treatments relative to the natural (no cover) treatment and exposed body surface area (mean ± SE)

Treatment	Exposed body surface area[1] (%)	Peak estimate (ng/mL)	Relative peak estimate[1] (natural = 100)
Natural	100	40.7 ± 3.6	100
Udder cover	94	39.2 ± 6.4	96
Horse blanket	28	11.4 ± 4.4***	28
Horse blanket + udder cover	24	7.0 ± 4.3***	17

[1]Correlation between exposed body surface area and relative peak estimate $r^2 = 0.996$ ($P \leq 0.01$).

***$P \leq 0.001$ compared with the natural treatment.

et al. (1964) and Hidiroglou and Karpinski (1989) showed that shorn sheep had significantly more vitamin D_3 in their blood than unshorn sheep during exposure to sunlight or light from UV lamps.

CONCLUSIONS

Vitamin D_3 synthesis in dairy cattle takes place in all areas of the skin and is not exclusively associated with skin areas where hair coverage is scant or lacking (e.g., udder and muzzle). The theory that vitamin D_3 should be synthesized from sebum on the hair and obtained by cows as a result of self-grooming or grooming of herdmates is not supported by the findings of this study.

ACKNOWLEDGMENTS

The authors gratefully acknowledge A. Strathe (Aarhus University) for his assistance with the statistical modeling and analysis of the data. The study was financed by the Ministry of Food, Agriculture, and Fisheries (Copenhagen, Denmark) and the Danish Cattle Association (Aarhus, Denmark).

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