Institut für Tierernährung

der Vetsuisse-Fakultät Universität Zürich

Direktorin: Prof. Dr. med. vet. Annette Liesegang

Influence of UVB exposure on the vitamin D-status and calcium homeostasis of growing sheep and goats

Inaugural-Dissertation

zur Erlangung der Doktorwürde der

Vetsuisse-Fakultät Universität Zürich

vorgelegt von

Sandra Kovács

Tierärztin

aus Gütersloh, Deutschland

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Für

Peter,

meine Eltern,

sowie Csilla und Corinna

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Danksagung

Curriculum Vitae

Vetsuisse-Fakultät Universität Zürich (2015)
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Influence of UVB exposure on the vitamin D-status and calcium homeostasis of growing sheep and goats

Not all animals are able to build vitamin D (VitD) in skin and are dependent on dietary VitD supply. Studies about VitD synthesis in goats are hardly known, whereas VitD synthesis in sheep is discussed controversial. The purpose of this study was to investigate the influence of exposure to ultraviolet radiation (UVB) on VitD status, intestinal calcium absorption and bone metabolism in growing sheep and goats. The hypothesis was that growing sheep and goats synthesize VitD in skin as a result of UVB exposure and the respective consequences for their VitD blood levels and the associated parameters can be shown. Fourteen lambs and goat kids were kept in an UVB-free environment and randomly assigned to two groups. One group was daily exposed to UVB for twelve weeks, the other group served as control group. Before the start and during the experiment blood samples were taken and bone mineral status was tested. After slaughtering samples were taken from skin, gastrointestinal tract and kidney for further analyses. Sample analysis showed similarities as well as differences between sheep and goats which probably indicates a species-specific difference in VitD metabolism. In both species UVB exposed animals showed lower 7-dehydrocholesterol values in skin than their respective control groups. These and other results indicate that both species synthesize VitD in skin when being exposed to UVB.

Keywords

Vitamin D, vitamin D receptor, ruminants, bone mineral density, calcium absorption

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Influence of UVB exposure on the vitamin D-status and calcium homeostasis of growing sheep and goats

Nicht alle Tiere sind in der Lage Vitamin D (VitD) in der Haut zu bilden und sind auf VitD-Zufuhr über die Nahrung angewiesen. Studien über VitD-Synthese bei Ziegen sind kaum bekannt, während diese bei Schafen kontrovers diskutiert wird. Ziel der Arbeit war es, den Einfluss ultravioletter Strahlung (UVB) auf den VitD-Status, die intestinale Kalziumabsorption und den Knochenmetabolismus heranwachsender Schafe und Ziegen zu untersuchen. Die Hypothese bestand darin zu zeigen, dass Schaf- bzw. Ziegenlämmer UVB-expositionsbedingt VitD in der Haut bilden, welches entsprechende Auswirkungen auf ihre VitD-Blutwerte und die damit assoziierten Parameter hat. Vierzehn Schaf- bzw. Ziegenlämmer wurden in UVB-freier Umgebung untergebracht und in zwei Gruppen eingeteilt. Eine Gruppe wurde zwölf Wochen lang mit UVB bestrahlt, die andere fungierte als Kontrollgruppe. Vor und während des Versuchs wurden Blutproben entnommen und der Knochenmineralstatus getestet. Nach der Schlachtung wurden Proben der Haut, des Verdauungstrakts und der Niere entnommen. Die Probenanalysen zeigten sowohl Gemeinsamkeiten als auch Unterschiede zwischen Schafen und Ziegen auf, was möglicherweise auf einen Unterschied im VitD-Metabolismus zurückzuführen ist. Die UVB-exponierten Tiere beider Spezies zeigten im Vergleich zur jeweiligen Kontrollgruppe niedrigere 7-Dehydrocholesterolwerte in der Haut. Diese und weitere Ergebnisse deuten darauf hin, dass beide Spezies VitD bilden, wenn sie UVB-Strahlung ausgesetzt sind.

Schlüsselwörter

Vitamin D, Vitamin D-Rezeptor, Wiederkäuer, Knochenmineraldichte, Kalziumabsorption

Influence of UVB exposure on the vitamin D status and calcium homeostasis of growing sheep and goats

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Running head

UVB effect on vitamin D and Ca in ruminants

Summary

The purpose of this study was to investigate the influence of exposure to ultraviolet radiation (UVB) on vitamin D status, intestinal calcium absorption and bone metabolism in growing sheep and goats. The hypothesis was that growing sheep and goats are able to synthesize vitamin D within their skin as a result of UVB exposure and that respective consequences for their vitamin D blood levels and the associated parameters can be shown. Fourteen 18-weeks-old lambs and goat kids were kept in an UVB-free environment and randomly assigned to two groups. One group was daily exposed to UVB (300 Watt) for twelve weeks, the other group served as control group. Except for the exposure to UVB all animals were kept under the same conditions and fed according to their requirements. Before the start of the experiment

and every second week blood samples were taken. Also the left metatarsus of each animal was analysed by quantitative computer tomography to test for bone mineral status before the start, in week 7 and at the end of the experiment. After twelve weeks the animals were slaughtered and samples were taken from skin, gastrointestinal tract and kidney for further analyses. In the present study, exposure to UVB led to increased serum 1,25-dihydroxyvitamin D (1,25VitD) levels in goat kids, whereas in lambs serum 25-hydroxyvitamin D (25VitD) levels were increased. In both species UVB exposed animals showed lower 7-dehydrocholesterol (7DHC) values in skin than their respective control groups. These results indicate that growing goat kids and lambs are able to synthesize vitamin D in the skin when being exposed to UVB.

Keywords

25-hydroxyvitamin D, 1.25-dihydroxyvitamin D, vitamin D receptor, growing ruminants, bone mineral density, calcium absorption

Introduction

There are two ways to meet vitamin D requirements in mammals: via nutrition and via synthesis in skin by ultraviolet light (UVB, wavelength between 290 und 315 nm) from the sun or other UVB sources (MacLaughlin et al., 1982; Hidiroglou and Karpinski., 1989). Exposure to UVB radiation causes photolysis in the epidermis converting 7-dehydrocholesterol (7DHC) into previtamin D3 (pre-D3), which then undergoes a temperature-dependent thermal isomerization to vitamin D. During continual UVB exposure pre-D3 photoisomerises to biologically inactive photoproducts lumisterol and tachysterol (storage form). This process is reversible. When pre-D3 storage in skin is depleted, UVB radiation will photoismerise lumisterol and tachysterol back into pre-D3 (Holick, 1990). Once formed, vitamin D is exported out of the plasma membrane of the keratinocytes into the blood where it is bound to the vitamin D binding protein (VDBP) (Holick, 2008; Haddad et al., 1993). Circulation transports vitamin D to the liver where it is converted by the enzyme vitamin-D-25-hydroxylase (25-OHase) to 25-hydroxyvitamin D (25VitD) (Holick, 2008). As this process is not strictly regulated serum-25VitD serves as functional indicator for vitamin D status (Shahriari et al., 2010). The following hydroxylation of 25VitD to the biologically most active vitamin D metabolite, 1,25-dihydroxyvitamin D (1,25VitD), by the renal 25-hydroxyvitamin D-1α-hydroxylase (1-OHase) is tightly regulated (Holick, 2008). As it has been shown for monogastric animals, 1,25VitD stimulates intestinal calcium (Ca) absorption and inhibits renal Ca excretion (Hoenderop et al., 2005). Under conditions of adequate dietary Ca intake, 1,25VitD stimulates bone mineralisation und formation of bone matrix. In contrast, when Ca supply is restricted, 1,25VitD has a catabolic effect on bone (Wilkens et al., 2010; Boyce and Weisbrode, 1983) thus affects bone mineral density (BMD).

Not all species are able to synthesize vitamin D in skin, for example cats and dogs depend on dietary intake to meet their vitamin D demand (How et al., 1994). While it has been shown that cattle can synthesize vitamin D in their skin (Hymøller and Jensen, 2010), studies about

vitamin D synthesis in goats are hardly known (Kohler et al., 2013). Regarding cutaneous synthesis of vitamin D in ovines, the available data are conflicting (Hidiroglou and Karpinski, 1989; Kohler et al., 2013). Physiological processes in ruminants differ (Wilkens et al., 2014). Therefore, cattle, sheep and goats should not be considered as identical species. Thus, in this study we also expected to observe species-specific specialities during the experiment.

The objective of the present study was to investigate the influence of UVB exposure on vitamin D status, intestinal calcium absorption and bone metabolism in growing sheep and goats. The hypothesis was that like cattle growing sheep and goats are able to synthesize vitamin D within their skin as a result of exposure to UVB light with all the respective consequences for their vitamin D blood levels and the associated parameters.

Materials and methods

Animals and measurements

Fourteen shorn East Frisian milk sheep lambs (six males and eight females) aged 18 ± 1.2 weeks were randomly assigned to two groups after balancing for gender (three males and four females in each group). Additionally, fourteen Saanen dairy goat kids (fourteen males, 18 ± 2.0 weeks old) were involved in this study; randomly assigned to two groups. The mean body weight before the start of the experiment was 22.8 ± 3.34 kg for both, goat kids and lambs. The animals were kept in groups in an UVB-free environment and fed individually twice daily for 12 weeks and were slaughtered at the end of the experiment. One lamb group (LUV, lambs UVB-exposed) and one goat kid group (GUV, goat kids UVB-exposed) were daily irradiated for 30 minutes with UV lamps (OSRAM ultra vitalux, 300 Watt, OSRAM GmbH, München, Germany) in the same way. The remaining lambs and goats kids (LC, lambs control, and GC, goat kids control, respectively) were not exposed to UVB radiation and served as control groups.

The feeding covered all nutrient requirements and consisted of hay and concentrate (Melior

2921C Combifloc, Meliofeed AG, Herzogenbuchsee, Switzerland: VitD3: 5'000 I.U/kg organic matter (OM); Ca: 9.5 g/kg OM). The diet for goat kids at 20 kg body weight was 600 g hay and 200 g concentrate daily, increasing till 30 kg body weight where the diet consisted of 700 g hay and 350 g concentrate daily. For lambs at 20 kg body weight the diet consisted of 700 g hay and 250 g concentrate daily, increasing till 30 kg body weight, where the diet consisted of 800 g hay and 400 g concentrate daily. To evaluate individual feed intake, refusals were weighed 45 minutes after serving.

Blood samples were taken from the jugular vein (in the morning 2 hours after feeding or feeding and irradiation, and 6 hours after feeding or feeding and irradiation, respectively, the mean concentration of both samples were used) (Greiner Bio-One VACUETTE® Z Serum Clot Activator, 6 ml, St. Gallen, Switzerland) before the start of the experiment and in weeks 3, 5, 7, 9, and 11 on treatment. Blood was centrifuged (3000 x *g*, 15 min, 4º C) within 30 min after sampling and serum was stored at -20 ºC and -80 ºC until analyses were performed. Urine samples (spontaneous urination) were collected before morning feeding and stored at -20 ºC.

Bone mineral density (BMD) and cortical bone thickness (CBT) of the left metatarsus of each animal was measured using pQCT (XCT 2000 bone scanner; Stratec Medizinaltechnik, Pforzheim, Germany) before the start, in week 7 and at the end of the experiment (after slaughtering). Thereby the length of the metatarsus, cortical BMD and trabecular BMD as well as cortical thickness (cortical mode 2; threshold for cortical bone >640 mg/cm^3) were measured (Liesegang and Risteli, 2005) in the middle of the diaphysis (50% of metatarsus length) as well as distal in the metaphysis (10% of length). In addition, all animals were weighed biweekly.

After slaughtering, skin samples were taken from forehead, neck, antebrachium, back and kneefold. In addition, kidney samples and mucosa samples from rumen, duodenum and jejunum were collected. The experiment was approved by the respective Swiss authority for

animal welfare under the approval number 162/2012.

Blood and urine sample analyses

Determination of serum-25VitD was done using a commercially available RIA kit (25-hydroxyvitamin D RIA, sheep-anti 25VitD antibody, Immunodiagnostics Systems GmbH, Frankfurt am Main, Germany) as described previously (Kohler et al, 2013, Sidler-Lauff et al, 2010, Liesegang and Risteli, 2005). The intra- and interassay CVs were 5.3% (n=10) and 8.1% (n=15), respectively and the sensitivity was <3 nmol/l. Determination of 1,25VitD levels in serum was also carried out using another RIA kit (1,25VitD RIA, sheep-anti 1,25VitD antibody, Immunodiagnostics Systems GmbH, Frankfurt am Main, Germany). The respective intra- and interassay CVs were 9.1% and 9.6%, respectively and the sensitivity was <8 pmol/l. Serum and urine levels of Ca and phosphorous (P) were determined using commercial testkits (Ca, testkit DIA00460B, Cresolphthalein complexone; P, testkit DIA00620C, ammonium molybdate; Diatools AG, Villmergen, Switzerland) by colorimetry using an autoanalyzer (Cobas Mira Roche-autoanalyzer, F. Hoffman-La Roche Ltd., Basel, Switzerland). The intra- and interassay CVs were 0.62% and 1.66% for Ca, and 0.86% and 1.07% for P. The sensitivities for Ca and P were 0.050 mmol/L and 0.065 mmol/l, respectively. Creatinine concentration was determined by a kinetic color-test using a respective kit (Crea Jaffe, DIA00540, Diatools AG, Vilmergen, Switzerland).

Bone turnover was calculated as the ratio of serum crosslaps (SCL) and serum osteocalcin (OC) (Liesegang et al., 2000). Osteocalcin was analysed using a commercial EIA testkit (Metra Osteocalcin, Quidel Corporation, San Diego, California, USA) with a murine monoclonal anti-OC-antibody. The intra- and interassay CVs were 4.8% and 4.8%, respectively and the sensitivity was 0.45 ng/ml. Determination of SCL was performed using an ELISA (Serum CrossLaps ELISA, Immunodiagnostic Systems GmbH, Frankfurt am Main, Germany). The intra- and interassay CVs were 1.7% and 2.5% and the sensitivity was 0.020

ng/ml.

Determination of growth hormone (GH) was analysed using an ELISA (Sheep Somatotropin (GH1) ELISA Kit, Goat growth hormone (GH) ELISA Kit, respectively, LuBioScience GmbH, Luzern, Switzerland). The intra- and interassay CVs both were 1.5% and 1.5% and the sensitivity was 0.675 ng/ml for sheep and 3.120 ng/ml for goats respectively. Determination of insulin like growth factor I (IGF-I) was performed using a commercial ELISA (IGF-I ELISA of human insulin-like growth factor-I - IGFBP-blocked, Mediagnost®/TECOmedical AG, Sissach, Switzerland). The intra- and interassay CVs were 6.8% and 6.7% and the sensitivity was 0.09 ng/ml.

Analysis of cutaneous samples

The skin of forehead, neck, antebrachium, back and kneefold was shaved before slaughtering. Within 5 minutes after slaughtering the skin samples were frozen in liquid nitrogen and stored at -80 ºC until being analysed as described by Morris (1999) with minor modifications. The samples were chromatographed at 254 nm using a HPLC (High Performance Liquid Chromatography). A Nucleosil silica column (EC250/4.6 Nucleosil 100-5C18, Macherey-Nagel, Düren, Germany) was used to analyse 7DHC and Lumisterol.

Ussing chamber experiments

Directions of active Ca net fluxes in rumen and duodenum were measured using modified Ussing chamber technique (Ussing and Zerhan, 1951) as described by Sidler-Lauff et al. (2010). Samples of rumen (from the base) and duodenum (directly after pancreatic duct entrance) were taken within 10 minutes after slaughtering. From the determined unidirectional fluxes (Jms, fluxes from mucosal side to serosal side; Jsm, fluxes from serosal side to mucosal side) net ion fluxes (Jnet) were calculated according to Jnet = Jms – Jsm (nmol/h/cm^2) from the mean unidirectional fluxes.

Western blot analysis

To determine mucosal vitamin D receptor (VDR) expression of rumen (stratum basale), duodenum (after pancreatic duct entrance), jejunum (central part) and kidney (cross section) were collected within 10 min after slaughtering. Mucosal layers were mechanically removed, frozen in liquid nitrogen and stored until being analysed at -80 ºC. Protein preparation for VDR detection was done as described by Sprekeler et al. (2011) with modifications. The antibody directed against VDR (Abcam AB54387, Sigma-Aldrich ChemieGmbH, Buchs, Switzerland) was diluted 1:200 in TBST containing 3% nonfat dry milk and 3% BSA. After subsequent washes in TBST, the membranes were incubated with HRP-conjugated polyclonal anti-rat IgG (A9037, Sigma-Aldrich Chemie GmbH, Buchs, Switzerland), 1:20,000 in TBST with 5% nonfat dry milk. Visualisation of bound antibody was achieved using enhanced chemiluminescence (Pierce; Thermo Scientific, Bonn, Germany) and a ChemiDoc (Bio-Rad, München, Germany) system. The Western blot analyses for calbindin-D_{9K} (Calb9k) were carried out as described by Schröder et al. (2001) and Wilkens et al. (2012).

For normalisation to the housekeeping gene GAPDH, monoclonal Anti-GAPDH (G8795, Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) was used with the polyclonal anti-mouse IgG-specific peroxidase antibody (A2304, Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) as second antibody. VDR- and Calb9K-expression was calculated as the ratio of VDR or Calb9K, respectively, to GAPDH.

Statistical analysis

All data are presented as means ± standard errors (SE). To test the differences of the time-dependent patterns in the groups, a multivariate analysis of variance for repeated measurements (MANOVA) was performed with group (with or without UVB) as a cofactor included in the model. A Wilcoxon signed rank test for paired samples was used to analyze

the statistical differences between the sampling days within each group. To limit the influence of extreme values, the differences between the groups were tested with the non-parametric Kruskal-Wallis test. The differences between the different sites of the skin samples in the different groups were tested using a one-way ANOVA with group as factor. If there were significant differences, a Kruskal-Wallis-Test was performed.

Results of Western blot analyses were compared by Student´s t-test (UVB-radiated versus control) for each species. Data are given in relation to the respective control group.

All statistical analyses were performed with the statistical software SYSTAT for Windows (Version 8.0, SPSS Inc. Chicago, IL). The level of significance was set to $P < 0.05$ for all tests.

Results

Body weight gain of the animals was not affected by UVB exposure. No significant species-related differences were observed (Figure 1). The mean body weight at week 7 was 28.1 ± 3.5 kg for both goat kids and lambs, and 31.6 ± 3.7 kg at the end of the observation period. The mean biweekly body weight gain was 1.8 ± 0.3 kg for both, goat kids and lambs.

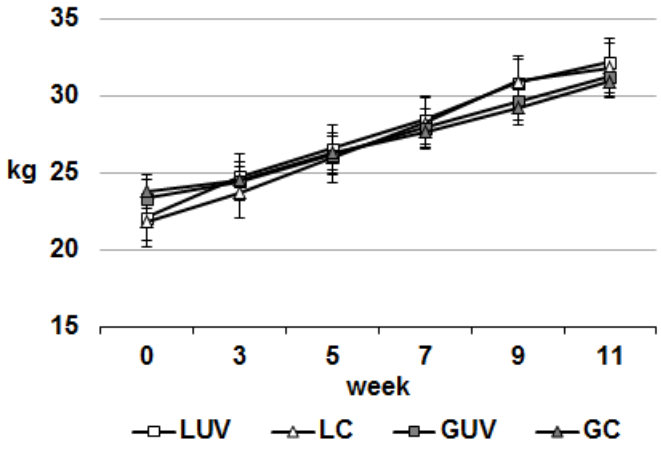

Figure 1 Effect of daily UVB exposure on means (with SE) of weight gain (kg) in goat kids and lambs (n = 7). GUV, UVB exposed goat kids, GC, control goat kids, LUV, UVB exposed lambs, LC, control lambs.

In goat kids, no effect of time was found for serum 25VitD irrespective of the treatment group (Table 1), but mean 25VitD blood levels over time were lower compared to lambs. An

increase in 25VitD level in the blood serum of both lamb groups was observed from week 0 to 5 with the LUV group showing higher levels from week 0 to 7 compared to LC ($p < 0.05$).

A species-specific effect on 1,25VitD showed that goat kids had higher 1,25VitD levels ($p < 0.05$) compared to lambs. In all four groups, a time-dependent increase in blood serum 1,25VitD was demonstrated: Levels in week 0 were lower ($p < 0.05$) compared to week 11. UVB exposure resulted in higher blood serum 1,25VitD levels over the entire experimental period in goat kids ($p < 0.05$), but not in lambs which showed no difference between LUV and LC.

Table 1 Effect of daily UVB exposure on means (with SE) of blood serum vitamin D in goat kids and lambs (n = 7)

	GUV	GC	LUV	LC	p-value group*
25 Vitamin D (nmol/l)					
Week 0	108 ± 14	93.0 ± 10	108 ± 4.3^{y}	92.0 ± 4.5^{yz}	0.447
Week 3	106 ± 8.8^{abc}	87.3 ± 4.5^{c}	117 ± 6^{ay}	104 ± 3.9^{by}	0.017
Week 5	100 ± 6.3^{abc}	87.6 ± 5.7^{c}	129 ± 6.8^{ax}	110 ± 3.8^{bxy}	<0.001
Week 7	99.8 ± 5.8^{abc}	83.8 ± 6^{c}	132 ± 9.2^{ax}	107 ± 4.6^{bxy}	<0.001
Week 9	89.5 ± 10^{ab}	87.7 ± 7.7^{b}	129 ± 8.6^{ax}	109 ± 4.3^{axy}	0.009
Week 11	101 ± 12^{ab}	86.9 ± 8.4^{b}	126 ± 7.7^{ax}	114 ± 4.1^{ax}	0.020
p-value time†	0.596	0.493	<0.001	<0.001	
1.25 Vitamin D (pmol/l)					
Week 0	180 ± 8.7^{ax}	122 ± 11^{bz}	56.5 ± 6.3^{cy}	51.3 ± 4.1^{cy}	<0.001
Week 3	195 ± 19^{ax}	146 ± 5.4^{byz}	91.9 ± 11^{cx}	97.2 ± 9.5^{cx}	<0.001
Week 5	200 ± 20^{ax}	136 ± 4.2^{byz}	92.4 ± 10^{cx}	97.6 ± 17^{cx}	<0.001
Week 7	224 ± 21^{axy}	168 ± 10^{bx}	106 ± 14^{cx}	92.9 ± 12^{cx}	<0.001
Week 9	237 ± 14^{ay}	149 ± 9.3^{byz}	98.4 ± 9.1^{cx}	99.2 ± 18^{cx}	<0.001
Week 11	234 ± 20^{ay}	150 ± 12^{bxy}	92.2 ± 9^{cx}	90.7 ± 12^{cx}	<0.001
p-value time	0.006	0.011	<0.001	<0.001	

GUV, UVB exposed goat kids, GC, control goat kids, LUV, UVB exposed lambs, LC, control lambs.
[abc] mean values within a row with different superscripts differ significantly ($p < 0.05$).
[xyz] mean values within a column with different superscripts differ significantly ($p < 0.05$).
* Differences in groups reflect both differences between species as well as irradiation treatment.
† Differences in time points reflect the differences to previous measurements only.

During the entire experimental period blood serum Ca in GUV was higher compared to GC ($p < 0.01$, data not shown). Time-related effects were observed in none of the groups. The range

of levels for all animals over the experimental period was 2.2-3.0 mmol/l. In lambs, no irradiation-related effect was seen. A species-specific difference was observed in blood serum P where the goat kids reached higher levels ($p < 0.05$, data not shown) compared to the lambs. There was no effect due to UVB exposure in both species. The P levels for all groups during the experiment ranged between 1.4-2.9 mmol/l.

Bone turnover in lambs showed a time-dependent increase ($p < 0.05$, Table 2).

Table 2 Effect of daily UVB exposure on means (with SE) serum bone formation and resorption markers in goat kids and lambs (n = 7)

	GUV	GC	LUV	LC	p-value group*
Serum Osteocalcin (ng/ml)					
Week 0	152.9 ± 20.4	136.7 ± 23.1^{y}	136.2 ± 12.4	118.5 ± 10.9	0.593
Week 3	140.9 ± 17.9	147.0 ± 22.9^{y}	136.8 ± 15.1	128.8 ± 12.7	0.904
Week 5	150.5 ± 25.9	110.1 ± 14.9^{z}	116.5 ± 16.8	123.4 ± 12.8	0.435
Week 7	134.4 ± 24.8	116.9 ± 19.8^{z}	127.6 ± 19.8	110.5 ± 14.9	0.838
Week 9	144.2 ± 25.0	133.0 ± 20.8^{y}	112.2 ± 17.4	101.4 ± 10.1	0.396
Week 11	141.0 ± 26.8	131.4 ± 18.3^{y}	118.2 ± 12.3	108.1 ±10.9	0.602
p-value time	0.479	0.045	0.104	0.214	
Serum Crosslaps (ng/ml)					
Week 0	1.07 ± 0.09^{a}	1.17 ± 0.12^{a}	0.73 ± 0.06^{bz}	0.71 ± 0.06^{bz}	0.001
Week 3	1.14 ± 0.11^{ab}	1.24 ± 0.12^{a}	0.83 ± 0.07^{cy}	0.90 ± 0.08^{bcxy}	0.019
Week 5	1.20 ± 0.17	1.17 ± 0.12	0.83 ± 0.07^{y}	0.96 ± 0.08^{x}	0.091
Week 7	1.10 ± 0.15	1.12 ± 0.16	0.80 ± 0.06^{yz}	0.86 ± 0.09^{yz}	0.164
Week 9	1.14 ± 0.11^{a}	1.16 ± 0.09^{a}	0.80 ± 0.06^{byz}	0.75 ± 0.05^{bz}	0.001
Week 11	1.22 ± 0.10^{a}	1.31 ± 0.15^{a}	0.93 ± 0.07^{by}	0.94 ± 0.09^{abxy}	0.032
p-value time	0.603	0.451	0.021	<0.001	
Bone turnover (x 10^{-3}) ‡					
Week 0	0.75 ± 0.08^{ab}	1.00 ± 0.19^{a}	0.55 ± 0.05^{bz}	0.61 ± 0.05^{aby}	0.034
Week 3	0.84 ± 0.05	0.90 ± 0.08	0.64 ± 0.08^{y}	0.72 ± 0.06^{xy}	0.051
Week 5	0.88 ± 0.12^{ab}	1.11 ± 0.09^{a}	0.75 ± 0.07^{bx}	0.79 ± 0.05^{bx}	0.026
Week 7	0.89 ± 0.09	1.02 ± 0.11	0.68 ± 0.07^{y}	0.80 ± 0.07^{x}	0.056
Week 9	0.87 ± 0.09	0.94 ± 0.08	0.77 ± 0.08^{x}	0.77 ± 0.07^{xy}	0.413
Week 11	0.95 ± 0.09	1.04 ± 0.08	0.82 ± 0.07^{x}	0.89 ± 0.05^{x}	0.177
p-value time	0.168	0.331	0.001	0.004	

GUV, UVB exposed goat kids, GC, control goat kids, LUV, UVB exposed lambs, LC, control lambs.
^{ab} mean values within a row with different superscripts differ significantly ($p < 0.05$).
^{xyz} mean values within a column with different superscripts differ significantly ($p < 0.05$).
* Differences in groups reflect both differences between species as well as irradiation treatment.
† Differences in time points reflect the differences to previous measurements only.
‡ Bone turnover was calculated as serum crosslaps-osteocalcin-ratio.

Generally, bone turnover rates were lower in lambs compared to goat kids. However, this could not be verified statistically.

Serum GH concentrations in goat kids were three to four times higher than those found in lambs ($p < 0.05$) (Table 3). While there was a decrease over time in goat kids, serum GH levels in lambs remained constant. In both species, no irradiation-related effects were observed. The mean IGF-I serum levels increased over time in all four groups ($p < 0.05$) while no irradiation-related effect was observed. Goat kids showed slightly higher values compared to lambs, although statistical significance was not reached.

Table 3 Effect of daily UVB exposure on means (with SE) of blood serum growth hormone (GH) and insulin-like growth factor-I (IGF-I) in goat kids and lambs (n = 7).

	GUV	GC	LUV	LC	p-value group*
GH (ng/ml)					
Week 0	29.6 ± 3.9^{ax}	35.0 ± 5.1^{aw}	6.91 ± 0.5^{b}	8.59 ± 0.8^{b}	<0.001
Week 3	33.6 ± 4.9^{ax}	29.3 ± 5.2^{ax}	7.34 ± 0.5^{b}	7.34 ± 0.5^{b}	<0.001
Week 5	29.4 ± 4.4^{axy}	26.8 ± 4.1^{xy}	7.60 ± 0.5^{b}	7.99 ± 0.8^{b}	<0.001
Week 7	30.1 ± 3.4^{axy}	23.6 ± 3.6^{axyz}	7.11 ± 0.6^{b}	7.19 ± 0.5^{b}	<0.001
Week 9	21.3 ± 2.8^{az}	28.5 ± 6.0^{axyz}	6.99 ± 0.4^{b}	8.95 ± 0.4^{b}	0.001
Week 11	24.9 ± 3.0^{ay}	23.3 ± 3.7^{az}	7.03 ± 0.6^{b}	8.18 ± 0.6^{b}	<0.001
p-value time†	0.004	0.020	0.704	0.117	
IGF-I (ng/ml)					
Week 0	168 ± 18^{x}	158 ± 16^{z}	140 ± 9.4^{z}	120 ± 12^{y}	0.152
Week 3	217 ± 35^{xy}	210 ± 16^{y}	173 ± 17^{yz}	157 ± 15^{x}	0.255
Week 5	254 ± 45^{yz}	201 ± 25^{yz}	176 ± 25^{yz}	167 ± 21^{x}	0.264
Week 7	274 ± 47^{z}	215 ± 24^{y}	185 ± 23^{yz}	165 ± 17^{x}	0.121
Week 9	291 ± 40^{az}	307 ± 43^{ax}	215 ± 30^{abxy}	170 ± 16^{bx}	0.044
Week 11	342 ± 56^{z}	317 ± 35^{x}	231 ± 31^{x}	212 ± 32^{x}	0.112
p-value time	<0.001	<0.001	<0.001	0.007	

GUV, UVB exposed goat kids, GC, control goat kids, LUV, UVB exposed lambs, LC, control lambs.
^{abc} mean values within a row with different superscripts differ significantly ($p < 0.05$).
^{wxyz} mean values within a column with different superscripts differ significantly ($p < 0.05$).
* Differences in groups reflect both differences between species as well as irradiation treatment.
† Differences in time points reflect the differences to previous measurements only.

In week 0 total mean BMD was smaller in lambs compared to goat kids. But while values of the former ones increased significantly over time, BMD in goat kids remained more or less stable (Table 4). Total cortical density (CD) and cortical thickness (CT) increased during the

experimental period in all groups, although statistical significance was not reached for CT in GUV. In week 12 both parameters were greater in lambs compared to goat kids ($p < 0.001$; $p < 0.05$). A time-dependent increase was also revealed for trabecular density in lambs as well as in the GUV ($p < 0.05$). However, the animals of the GC group showed significantly higher levels in week 0 compared to all other groups but their trabecular density level did not vary over the experimental period. Irradiation-specific effects could not be observed for any of the parameters investigated.

Table 4 Effect of daily UVB exposure on means (with SE) metatarsus bone density in goat kids and lambs (n = 7)

	GUV	GC	LUV	LC	p-value group*
Total bone mineral density‡					
Week 0	567.5 ± 24.8^{a}	545.8 ± 23.4^{a}	468.9 ± 23.7^{bz}	456.9 ± 31.7^{bz}	0.013
Week 7	566.7 ± 22.9	553.3 ± 19.7	588.8 ± 20.5^{y}	607.6 ± 18.5^{y}	0.275
Week 12	578.1 ± 17.5^{b}	600.1 ± 10.9^{b}	652.6 ± 8.3^{bx}	671.6 ± 11.6^{bx}	<0.001
p-value time†	0.715	0.114	<0.001	<0.001	
Cortical density§					
Week 0	921 ± 29^{z}	947 ± 22^{y}	911 ± 23^{y}	823 ± 52^{y}	0.087
Week 7	956 ± 27^{y}	927 ± 10^{y}	962 ± 15^{y}	966 ± 31^{y}	0.617
Week 12	1004 ± 9^{bx}	1010 ± 8^{bx}	1066 ± 10^{ax}	1075 ± 8^{ax}	<0.001
p-value time†	0.041	0.002	<0.001	0.002	
Cortical thickness¶					
Week 0	2.68 ± 0.1	2.85 ± 0.07^{y}	2.45 ± 0.1^{z}	2.56 ± 0.1^{y}	0.557
Week 7	3.12 ± 0.1	2.99 ± 0.1^{xy}	3.02 ± 0.1^{y}	3.22 ± 0.1^{x}	0.556
Week 12	3.00 ± 0.1^{b}	3.11 ± 0.04^{bx}	3.26 ± 0.04^{ax}	3.23 ± 0.1^{abx}	0.044
p-value time	0.185	0.037	<0.001	<0.001	
*Trabecular density***					
Week 0	381 ± 21^{by}	481 ± 25^{a}	371 ± 22^{by}	324 ± 42^{bz}	0.011
Week 7	503 ± 25^{x}	500 ± 29	512 ± 18^{x}	516 ± 32^{y}	0.977
Week 12	498 ± 29^{x}	500 ± 20	513 ± 15^{x}	561 ± 33^{x}	0.343
p-value time	0.001	0.821	<0.001	0.001	

GUV, UVB exposed goat kids, GC, control goat kids, LUV, UVB exposed lambs, LC, control lambs.
[ab] mean values within a row with different superscripts differ significantly ($p < 0.05$).
[xyz] mean values within a column with different superscripts differ significantly ($p < 0.05$).
* Differences in groups reflect both differences between species as well as irradiation treatment.
† Differences in time points reflect the differences to previous measurements only.
‡ Total bone mineral density are shown as means (Total density of measuring point at 50% and total density of measuring point at 10% of metatarsus length) (i.e., distal 0%, proximal 100%).
§Measuring point at 50% of metatarsus length.
¶Measuring point at 50% of metatarsus length.
** Measuring point at 10% of metatarsus length.

The concentration of 7DHC in skin taken from the forehead showed no significant differences between irradiated animals and their respective control groups (Table 5). Neck, antebrachium, back and kneefold 7DHC skin levels were all smaller in UVB exposed animals of both species. But due to great variances this could only be verified statistically for 7DHC concentration determined in samples from antebrachium, back and kneefold of goat kids. Generally, the highest levels of 7DHC were observed in forehead skin followed by antebrachium and the lowest in back skin. However, significance was not reached in LC. Lumisterol in lambs was below detection limit in all samples. In goats higher levels of lumisterol were observed in neck, antebrachium and back of GUV compared to GC. However, this was not statistically significant. But the observation that significant differences in lumisterol content of different body parts were found only in UVB exposed goats, but not in control animals, might also point to an effect of irradiation.

Table 5 Effect of daily UVB exposure on means (with SE) of vitamin D-synthesis in skin of goat kids and lambs after slaughtering (n = 7)

	GUV	GC	LUV	LC	p-value group*
7 DHC (µg/g)					
Forehead	22.9 ± 3.1^{w}	16.3 ± 0.4^{w}	17.1 ± 1.3^{w}	23.1 ± 2.5	0.100
Neck	5.63 ± 0.6^{z}	6.45 ± 0.6^{y}	12.8 ± 3.1^{wx}	16.2 ± 4.8	0.074
Antebrachium	9.01 ± 0.9^{bx}	13.3 ± 0.8^{ax}	14.1 ± 0.9^{awx}	19.6 ± 2.6^{a}	0.001
Back	3.8 ± 0.4^{bz}	6.16 ± 0.5^{ay}	7.04 ± 2.6^{ay}	13.7 ± 6.1^{a}	0.009
Kneefold	8.69 ± 1.1^{by}	11.0 ± 0.8^{abx}	12.6 ± 0.9^{axy}	15.6 ± 2.6^{a}	0.046
p-value region†	<0.001	<0.001	0.012	0.369	
Lumisterol (µg/g)					
Forehead	0.37 ± 0.1^{y}	0.72 ± 0.35	-‡	-	0.397
Neck	1.11 ± 0.59^{y}	0.66 ± 0.11	-	-	0.539
Antebrachium	1.56 ± 0.7^{x}	1.07 ± 0.25	-	-	0.537
Back	2.83 ± 0.95^{w}	1.31 ± 0.15	-	-	0.169
Kneefold	1.98 ± 0.42^{w}	2.28 ± 0.34	-	-	0.617
p-value region	0.043	0.434			

GUV, UVB exposed goat kids, GC, control goat kids, LUV, UVB exposed lambs, LC, control lambs.
^{ab} mean values within a row with different superscripts differ significantly ($p < 0.05$).
^{wxyz} mean values within a column with different superscripts differ significantly ($p < 0.05$).
* Differences in groups reflect both differences between species as well as irradiation treatment.
† Differences in region reflect differences between the sampled body regions.
‡ Lumisterol levels of LUV and LC were below detection limit.

In rumen, active Ca net absorption for all animals (Table 6) was shown. Neither a species-specific difference nor an irradiation-dependent effect was detected. In duodenum, active Ca net secretion was observed in all groups with Ca net secretion being lower ($p < 0.005$) in GUV than in the other three groups.

Table 6 Effect of daily UVB exposure on means (with SE) on calcium net fluxes in the gastrointestinal tract of goat kids and lambs after slaughtering (n = 7)

	GUV	GC	LUV	LC	p-value group*
Calcium net fluxes (nmol/h/cm²)†					
Rumen	4.60 ± 1.5	5.34 ± 2.2	4.84 ± 1.8	5.51 ± 2.3	0.812
Duodenum	-6.29 ± 3.1[a]	-10.2 ± 1.9[b]	-10.0 ± 2.6[b]	-11.3 ± 4.3[b]	0.034

GUV, UVB exposed goat kids, GC, control goat kids, LUV, UVB exposed lambs, LC, control lambs.
[ab] mean values within a row with different superscripts differ significantly ($p < 0.05$).
* Differences in groups reflect both differences between species as well as irradiation treatment.
† Positive values refer to Ca absorption from lumen, negative values to Ca secretion into lumen.

VDR protein expression was found in rumen, duodenum, jejunum and kidney in goat kids (Figure 2) as well as in lambs (Figure 3).

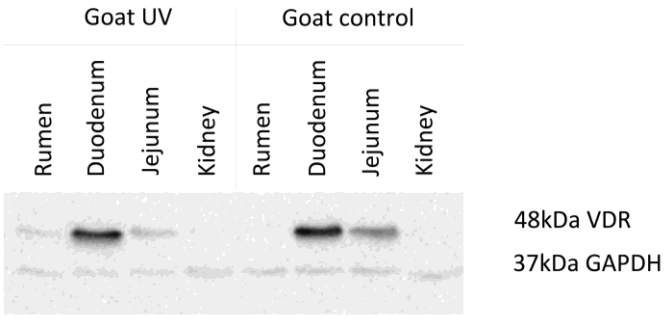

Figure 2 Example Western blot results for goat kids. The proteins were measured by densitometry.
Sample tissues are rumen, duodenum, jejunum and kidney.
Upper bars represent the respective VDR proteins of the individual sample tissues.
Lower bars represent the respective housekeeping gene (here: GAPDH).
GUV, one UVB exposed goat kid, GC, one goat kid from control group.

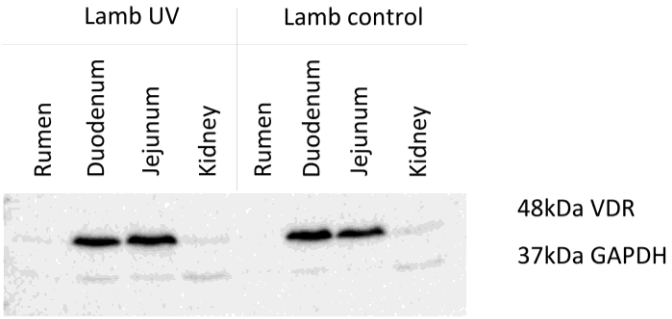

Figure 3 Example Western blot results for lambs. The proteins were measured by densitometry.
Sample tissues are rumen, duodenum, jejunum and kidney.
Upper bars represent the respective VDR proteins of the individual sample tissues.
Lower bars represent the respective housekeeping gene (here: GAPDH).
LUV, one UVB exposed lamb, GC, one lamb from control group.

Although expression of VDR was numerically smaller in the rumen and greater in the small intestine of irradiated animals of both species and also in the kidney of UVB exposed lambs, due to high variances no statistical significance could be found. Duodenal Calb9k-expression seemed to be greater in GUV compared to GC although again values did not reach significant level (Figure 4). In lambs no irradiation-dependent effect was observed. The highest ratio of VDR to GAPDH expression in all four groups were detected in jejunum, followed by duodenum.

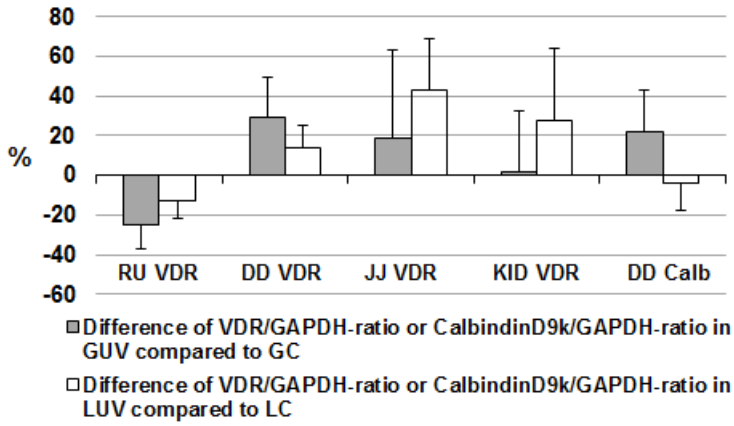

Figure 4 Effect of daily UVB exposure on ratio of VDR to GAPDH or Calbindin-D_{9k} to GAPDH expression in GUV and LUV compared to GC and LC respectively as percentages (on means + SE) in the gastrointestinal tract and the kidneys of goat kids and lambs after slaughtering (n = 7).
GUV, UVB exposed goat kids, GC, control goat kids, LUV, UVB exposed lambs, LC, control lambs.
RU, rumen, DD, duodenum, JJ, jejunum, KID, kidney.
VDR, vitamin D receptor to GAPDH expression, Calb, Calbindin-D_{9k} to GAPDH expression.

Discussion

The present study was conducted to test the hypothesis that growing sheep and goats are able to synthesize vitamin D within their skin when being exposed to UVB.

While in goat kids UVB exposure had no effect on serum 25VitD, lambs (LUV) showed higher 25VitD levels than LC. Differences were also shown between lambs and goat kids. Although basal values obtained in week 0 were identical, at the end of the observation period even lambs without UVB exposure showed higher 25VitD levels compared to goat kids. As serum 25VitD serves as an indicator for vitamin D status (Shahriari et al., 2010), this difference must have been caused by either differences in absorption from the gastrointestinal tract, differences in cutaneous synthesis or differences in vitamin D metabolism. A possible explanation for higher serum 25VitD levels in lambs might be the VitD intake via concentrate in this study. Lambs got on average 20% more concentrate to cover all of their nutrient requirements. Therefore, lambs ingested relatively more VitD than goat kids. In further studies VitD supplementation should be optimized to enable better comparisons.

But even although dietary VitD supply might have been a bit too high in sheep, the increase over time found in UVB-exposed lambs was greater than the respective rise seen in LC. This indicates cutaneous synthesis in irradiated lambs. In addition, cutaneous concentrations of the VitD precursor, 7DHC, determined for several sites all over the body were numerically smaller in LUV than in LC in all locations investigated.

With exception of the forehead skin, this observation could also be made in samples obtained from goats. Furthermore, concentrations of the inactive VitD metabolite lumisterol were numerically higher in those parts of the body where the concurrent decrease in 7DHC was greatest: the antebrachium and the back. An explanation for the missing irradiation-effect in GUV in forehead may be that animals were exposed to UVB during hay feeding on ground. In contrast to sheep, goat kids needed the whole 30 minutes of daily UVB exposure-time to finish their diet. Consequently GUV animals were only exposed to UVB with lowered heads

so direct irradiation on forehead was therefore probably low. However, frequent UVB exposure leads to skin alteration such as thickening of the epidermis at least in white merino sheep (Forrest and Fleet, 1985). Thickened skin as well as more pigmented skin let pass less UV rays which results in less 7DHC conversion. These high 7DHC amounts found in GUV forehead skin may therefore be related to physiological skin protection mechanisms. In the future determination of individual minimal erythemal doses (MED; measure of skin sensitivity to UV irradiation) could be included to measure skin alteration and the ability for cutaneous vitamin D synthesis to adapt UVB radiation to particular skin alteration.

No lumisterol could be detected in ovine skin. One possible explanation might be that sheep do not store vitamin D precursors in skin but convert them to vitamin D to store it in 25VitD form in blood. On the other hand, the contribution of other storage forms of transformed 7DHC such as Tachysterol, Suprasterol I, Suprasterol II or 5,6-Transvitamin D3 could be more important in lambs. In both cases, the interesting species specific difference should be investigated in further studies.

Thus, the absence of a treatment-specific effect on 25VitD in goats does not mean that cutaneous synthesis of VitD does not occur in this species. Interestingly, the more active metabolite 1,25VitD was not only generally higher in goat kids than in lambs, there was also a stimulating effect of UVB exposure in goats, but not in lambs.

The question which cannot be answered satisfactory at this stage of our studies is why a hypothetically surplus of irradiation-derived 25VitD is converted into 1,25VitD in goats, but not in sheep. Species differences in respect to VitD metabolism have been reported earlier. Kohler et al. (2013) found higher concentrations of serum 1,25VitD in lactating goats compared to lactating sheep and the increase in serum 1,25VitD in response to dietary calcium restriction is more pronounced in goats, too (Wilkens et al., 2012). Hence, the higher levels of 1,25VitD observed in the current study in goat kids suggest that in comparison to lambs either more 25VitD is transformed to 1,25VitD or less 1,25VitD is inactivated. As 25VitD remains

stable in GUV while 1,25VitD increases over time, the goat kids probably converted more 25VitD to 1,25VitD than the lambs.

The enzyme responsible for the second hydroxylation, the renal 1α-hydroxylase CYP27B1 is regulated by several factors. The most important one is the plasma concentration of parathyroid hormone (PTH) which is secreted to maintain normocalcemia in times of enhanced demand or decreased availability of Ca (Holick, 2004; Horst et al., 2005). Dietary intake met always the Ca demands in both, sheep and goat kids and serum Ca levels measured were within normal ranges in all animals (Pugh, 2002). If PTH would have been increased in the goat kids not only serum 1,25VitD would have been increased, but also the bone resorption marker CrossLaps in goats (Wilkens et al., 2012). In the current study, serum concentration of the bone resorption marker remained stable in both goat groups. This observation argues against an effect of the lower dietary intake of Ca in goats compared to sheep, although serum Ca levels in GC were the lowest of all groups.

It cannot be excluded that species-specific differences in the plasma concentration of vitamin binding protein (VDBP), whose presence influences both, 25VitD and 1,25VitD blood levels, contribute to the differences in respect to vitamin D metabolism (Zella et al., 2008). This should be considered in further studies.

Renal hydroxylation of 25VitD to 1,25VitD is also influenced by growth hormone (GH) and insulin-like growth factor I (IGF-I) (Nesbitt and Drenzer, 1993; Tryfonidou and Hazewinkel, 2004). Enhanced hydroxylation independent of PTH in goat kids resulting in lower 25VitD levels but higher 1,25VitD concentrations might be related to the higher GH/IGF-I values found in goat kids. To reveal the underlying mechanisms, further studies should address the major metabolites of vitamin D catabolism, 24,25VitD and 1,24,25VitD, too. Serum GH levels in goat kids were three to four times higher compared to lambs. Since goats come up with high serum GH levels the ELISA test kit sensitivity is adjusted accordingly. In this study all observed goat kid serum GH levels were in the specified detection range and well above

the specified minimum detectable dose. The GH/IGF-I system is associated with bone remodelling (Žofková, 2003) and IGF-I is, at least in young men, positively correlated with bone turnover (Fatayerji and Eastell, 1999) and therefore in line with the higher bone turnover rates found in goat kids.

Interestingly, Tryfonidou et al. (2003) observed similar results in a study which showed that high bone turnover and respective serum concentrations of GH were accompanied by high circulating 1,25VitD levels in growing dogs which were fed according to their requirements. So one might speculate that the interfering effect of growth is more pronounced in goat kids compared to lambs.

As a consequence of greater 1,25VitD concentrations, intestinal Ca absorption should be increased in goat kids (Hoenderop et al., 2005; Wilkens et al., 2012) resulting in higher plasma Ca concentrations. In fact, significant differences could neither be found in respect to functional data on ruminal and intestinal Ca transport obtained by Ussing chamber technique, nor for expression of VDR and the cytosolic Ca transport protein Calb9k. But against the background of very high variations it should be mentioned that abundance of intestinal VDR and Calb9k was slightly affected by UVB exposure. And although active duodenal Ca secretion was detected by Ussing chamber technique *in vitro*, this does not necessarily mean that *in vivo*, when Ca concentrations are higher on the luminal side, Ca is secreted, too. The observed differences *in vitro* indicate an effect on Ca transporting structures which might result in higher Ca absorption *in vivo*. The complete lack of alterations in ruminal tissues is in line with former investigations that showed that active Ca absorption from the rumen is most probably not regulated by vitamin D metabolites (Sidler-Lauff et al., 2010, Wilkens et al., 2011, Wilkens et al., 2012).

Goat kids showed higher bone turnover levels compared to lambs. The time patterns of parameters related to BMD are in accordance with former results obtained in growing sheep in the studies of Liesegang et al. (2010, 2013) thus indicating a normal physiological process

of aging. Significant species-differences in BMD between the two species were found in week 12 where lambs showed higher BMD levels compared to goat kids. This agrees with Liesegang and Risteli (2005) where higher BMD were measured in growing sheep than in growing goats. Liesegang and Risteli suggested that sheep are able to embed excessive amounts of Ca in bone while goats need longer for this process.

Another explanation for the higher BMD found in lambs compared to goat kids may be circulating 24,25VitD levels. This product of vitamin D catabolism influences endochondral ossification by promoting chondrozyte maturation (Boyan et al., 2001). Tryfonidou and Hazewinkel (2004) found increased 1,25VitD and decreased 24,25VitD plasma levels related to increased plasma GH/IGF-I levels in growing dogs. They suggested a decreasing 1,25VitD clearance under physiologically GH excess. Considered reversely, as lambs showed higher 25Vitd and lower 1,25VitD levels than goat kids, higher 24,25VitD levels are expected in lambs which were in line with their higher BMD and should therefore be observed in further studies.

In summary, our results indicate that both growing species synthesize vitamin D in skin when being exposed to UVB, although the species-specific differences seem to occur afterwards in respect to VitD metabolism. From the data on intestinal Ca transport it can be concluded that goat kids seem to profit more from UVB than lambs. In the future studies, animals should be tested without any supplementation of vitamin D.

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