Vitamin D status in growing dairy goats and sheep: Influence of ultraviolet B radiation on bone metabolism and calcium homeostasis

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ABSTRACT

The aim of this study was to investigate how controlled UVB irradiation in combination with reduced nutritional vitamin D (vitD) supply affects vitD status and Ca metabolism of growing goats and sheep. The hypothesis was that, like dairy cows, goats and sheep are able to compensate for the missing nutritional supply of vitD through endogenous production in the skin, with the consequence of a high vitD status and a balanced Ca homeostasis. Sixteen lambs and 14 goat kids aged 3 and a half months were housed in an UVB free environment and fed hay and a vitD-free concentrate over a period of 13 wk. One group of each species was exposed to UVB lamps daily during individual feeding; the other groups served as controls. Serum, urine, and feces samples were taken at the start and at a monthly interval. Serum was analyzed for vitD metabolites, bone markers, growth hormone, insulin-like growth factor I, Ca, and P. Apparent digestibility and urinary excretion of Ca and P were determined. The left metatarsus was analyzed by peripheral quantitative computer tomography for bone mineral density before starting and at the end of the trial. In wk 13, all animals were slaughtered and samples of skin, rumen, duodenum, kidney, and bone (metatarsus) were collected. Content of sterols of vitD synthesis in the skin, Ca flux rates in rumen and duodenum, expression of vitD receptor in duodenum and kidney, renal and intestinal gene expression of Ca transport proteins, and renal enzymes related to vitD metabolism were determined. The UVB exposure led to lower 7-dehydrocholesterol content in the skin and a better vitD status (higher serum 25-hydroxyvitamin D), but no signs of vitD deficiency were seen in the control groups and no effect of irradiation was detected in the analyzed parameters of Ca homeostasis. Differences between the 2 species were detected: lambs had

a higher increase of bone mineral density, lower values of bone markers, growth hormone, and insulin-like growth factor I in serum and higher tachysterol and lower lumisterol content in skin compared with goat kids. The results indicated that growing lambs and goat kids are able to compensate for a vitD-reduced diet by cutaneous vitD synthesis when exposed to UVB irradiation and therefore to keep a high vitD status. In contrast, when a reduced vitD diet is combined with missing UVB exposure, the vitD status drops, but the experimental time was probably too short to induce a vitD deficiency or an effect on Ca homeostasis.

Key words: bone metabolism, calcium, skin, vitamin D

INTRODUCTION

Endogenous synthesis of vitamin D (**vitD**) in the skin or nutritional supply is needed to meet vitD requirements of humans and animals. In human skin, when UV radiation (UVB, wavelengths 290–315 nm) is absorbed, 7-dehydrocholesterol (**7DHC**) is photoisomerized to pre-vitamin D₃ (**pre-D3**). This unstable product can be thermally isomerized to vitamin D₃ (**vitD3**, cholecalciferol) or reversibly photoisomerized to the inactive storage forms lumisterol and tachysterol (MacLaughlin et al., 1982; Webb and Holick, 1988; Holick, 2004). Afterward, vitD3 is transferred into the blood circulation and transported by vitD binding protein to the liver where, together with the vitamin D₂ (**vitD2**, ergocalciferol) coming from nutrition, the enzyme vitamin-D-25-hydroxylase (**25-OHase**) transforms them into 25-hydroxyvitamin D (**25vitD**; Haddad et al., 1993; Holick, 2008; Jones, 2013). The 25vitD can be stored in tissues as well as transported to the kidney where it is converted to the active product 1,25-dihydroxyvitamin D (**1,25vitD**, calcitriol) by the enzyme 25-hydroxyvitamin-D-1 α -hydroxylase (**1 α -OHase**; Horst and Reinhardt, 1983; Holick, 2008; Dittmer and Thompson, 2011). The amount of 1,25vitD is controlled by its strictly regulated production by 1 α -OHase and

Received April 21, 2017.

Accepted July 4, 2017.

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by the degradation through the enzyme 24-hydroxylase (**24-OHase**; Horst and Reinhardt, 1983; Holick, 2008). High Ca and P serum levels decrease serum 1,25vitD, whereas parathyroid hormone, which is secreted when Ca serum level is low, as well as growth hormone (**GH**) and IGF-I, increase 1,25vitD in serum. (Horst and Reinhardt, 1983; Nesbitt and Drezner, 1993; Tryfonidou et al., 2003; Dittmer and Thompson, 2011).

In ruminants, with sufficient dietary Ca supply, vitD stimulates bone formation and mineralization, whereas if Ca serum level drops, vitD enhances mobilization from the bones and intestinal Ca absorption (Liesegang and Risteli, 2005; Schröder and Breves, 2006; Dittmer and Thompson, 2011).

It could be demonstrated that humans as well as several different species (including cows) are able to produce vitD in their skin (Holick et al., 1979; Hymøller and Jensen, 2010), in contrast to other species like cats and dogs who depend entirely on dietary supply (How et al., 1994; Morris, 1999). With the experiment of Kovács et al. (2015), it could be demonstrated that goats and sheep are able to produce vitD within their skin, but its importance compared with nutritional supply is still unclear and available data are conflicting (Hidioglou and Karpinski, 1989; Kohler et al., 2013).

A continuous effort has been made to optimize the dairy production and composition of animal sources for human nutrition, for example, the increase of vitD content in milk (Kohler et al., 2013; Jakobsen et al., 2015; Weiss et al., 2015). The actual need for the common use of diets highly supplemented with vitD in ruminant nutrition needs to be questioned. Indeed, if it can be proven that ruminants are capable of covering their vitD demand by endogenous production through UVB exposure, even in the situation of challenged Ca

homeostasis because of the growing process, livestock husbandry can be optimized.

The aim of our study was to investigate, based on the results of Kovács et al. (2015), how controlled UVB irradiation in combination with reduced nutritional vitD supply affects the vitD status and Ca regulation (including intestinal Ca absorption, renal excretion, and bone metabolism) of healthy growing goats and sheep of dairy breeds with special attention on species-specific differences. Our hypothesis was that they are able to fully compensate the missing nutritional vitD supply through endogenous production in the skin while exposed to UV light. This will lead to a vitD status comparable to a status when vitD is supplemented, and consequently to a well-balanced Ca homeostasis and healthy growing process.

MATERIALS AND METHODS

Sixteen East Friesian milk sheep lambs (8 females and 8 males) and 14 Saanen dairy goat kids (all males) were included in this study. At the beginning (wk 0), the mean age was 3 and a half months and the mean BW was 20.8 ± 0.7 kg. All lambs were shorn before starting the experiment.

Over the trial time of 13 wk, the animals were housed in groups, in pens, on wood shavings, in a UVB-free environment. At the end, wk 13, all animals were slaughtered.

Animals were fed individually twice a day with hay of the second cut (at the beginning for growing, stage 3) and a concentrate specially designed for these trials without vitD (Food 2921 without vitamin D addition, Kliba Nafag, Kaiseraugst, Switzerland, Table 1). They had access to NaCl licking bowl [UFA Salzleckstein, sodium chloride (38.5% sodium) and iodine (100 mg/kg), UFA AG, Herzogenbuchsee, Switzerland] and water ad libitum. Weighing was performed every week and the feed ration was individually adapted. The ration started at daily 700 g of hay and 250 g of concentrate for lambs with 20 kg of BW and 600 g of hay and 200 g of concentrate for goat kids with 20 kg of BW, and was increased continuously until daily 900 g of hay and 450 g of concentrate for lambs and 750 g of hay and 375 g of concentrate for goat kids, respectively, when reaching 35 kg of BW. To evaluate the intake of each animal, refusals were weighed 60 min after feeding. All nutrient requirements were covered except for vitD (Agroscope, 2013).

The lambs and goat kids were randomly assigned to 2 groups for each species, after balancing for sex and weight. Half of the animals, UVB-exposed lambs (**LUV**) and UVB-exposed goat kids (**GUUV**), were ex-

Table 1. Food composition

Item	Hay	Concentrate
DM (%)	93.6	91.9
Crude fiber (% of DM)	21.8	6.46
CP (% of DM)	13.5	12.1
Crude fat (% of DM)	2.09	3.73
ADF (% of DM)	28.4	10.3
ADL (% of DM)	3.74	1.99
NDF (% of DM)	50.2	30.2
Crude ash (% of DM)	8.22	6.72
HCl insoluble ash (% of DM)	1.43	0.58
Ca (g/kg of OM)	8.93	12.3
P (g/kg of OM)	2.66	3.9
Mg (g/kg of OM)	2.73	3.26
Vitamin A ¹ (IU/kg)	—	24,000
Vitamin D ¹ (IU/kg)	—	0

¹Amount of additives according to the manufacturer's declaration (KLIBA NAFAG, Kaiseraugst, Switzerland).

posed daily to UVB irradiation of UV lamps (Osram Ultra Vitalux, 300 Watt, Osram GmbH, München, Germany) for 30 min during feeding. Irradiation was monitored each morning on the animals' back with a UVB-Radiometer (Solarmeter 6.2, Solartech, Glenside, PN; response range of 280–320 nm) to ensure a minimum irradiation of 20 $\mu\text{W}/\text{cm}^2$. With this dosage, the 30 min of lamps irradiation corresponded to 55 min of sun exposure in summer near Zürich (calculated with the mean daily UVB dose measurements of Kohler et al., 2013). The rest of the animals served as controls and were assigned to the lamb control (**LC**) and goat kid control (**GC**) groups.

All the procedures in this experiment were approved by the respective Swiss authority for animal welfare (approval number 162/2012, Kantonales Veterinäramt Zürich, Switzerland) in accordance with the animal welfare law of Switzerland.

Sample Collection

Samples of urine, blood, and feces were collected before the start of the experiment (wk 0), and afterward in wk 3, 7, and 11. Spontaneous morning urine was collected on the sampling day before feeding and immediately cooled at 5°C. Before the samples were stored at –20°C, pH was measured (827 pH Lab, Metrohm AG, Herisau, Switzerland). Venal blood was taken from a jugular vein (Greiner Bio-One Vacuette Z Serum Clot Activator, 5 mL, St. Gallen, Switzerland) 2 and 6 h after morning feeding or feeding with irradiation and was centrifuged ($3,000 \times g$, 15 min, room temperature) within 30 min after sampling. Serum was then stored at –20 and –80°C. Feces samples were collected directly from the anus, pooled over 1 wk per animal, and also stored at –20°C.

Finally after slaughtering different samples were taken. Within 10 min, samples of the rumen (ventral ruminal sac) and duodenum (directly after pancreatic duct entrance) were taken to perform modified Ussing chamber technique as described by Sidler-Lauff et al. (2010). The net Ca ion fluxes (**J_{net}**) were calculated from the mean detected unidirectional fluxes (**J_{ms}** fluxes from mucosal to serosal side of the epithelium, **J_{sm}** fluxes from serosal to mucosal) with the formula $J_{\text{net}} = J_{\text{ms}} - J_{\text{sm}}$ (nmol/h per cm^2). Additionally, mucosa samples from duodenum as well as kidney (cross-section) samples were collected as described in Kovács et al. (2015) and stored at –80°C.

Skin samples of 5 different localizations (forehead, neck, antebrachium, back, and kneefold), shaved in advance, were taken within 5 min after slaughter, frozen in liquid nitrogen, and stored at –80°C. The left meta-

tarsal bones were cleaned from adherent tissues and stored at –20°C. Additionally, random food samples of hay and concentrate were taken during the experiment, pooled over time, and analyzed.

Serum and Urine Sample Analyses

Serum levels of following parameters were analyzed in samples taken 2 h after morning feeding or feeding with irradiation using commercial kits as described by Kovács et al. (2015): 25vitD (25-hydroxyvitamin D RIA, Immunodiagnosics Systems GmbH, Frankfurt am Main, Germany; intra- and interassay coefficients of variation 5.3 and 8.1%, respectively sensitivity <3 nmol/L; cross reactivity with 25-hydroxyvitamin D₃ and 24,25-dihydroxyvitamin D₃ 100%, with 25-hydroxyvitamin D₂ 75%, with vitD3 <0.01%, and with vitD2 <0.3%), 1,25vitD (1,25-Dihydroxy VitD RIA, Immunodiagnosics Systems GmbH; intra- and interassay coefficients of variation 9.1 and 9.6%, respectively, sensitivity <8 pmol/L; cross reactivity with 1,25-dihydroxyvitamin D₃ 100%, with 1,25-dihydroxyvitamin D₂ 97%, with 24,25-dihydroxyvitamin D₃ and 25-hydroxyvitamin D₃ <0.01%), GH [sheep somatotropin (GH1) ELISA Kit and goat GH ELISA Kit, LuBioScience GmbH, Lucerne, Switzerland; intra- and interassay coefficients of variation 15 and 15%, respectively, sensitivity 0.675 ng/mL for sheep and 3.120 ng/mL for goats], IGF-I (IGF-I ELISA of human IGF-I-IGFBP-blocked, Mediagnost/TECOmedical AG, Sissach, Switzerland; intra- and interassay coefficients of variation 6.8 and 6.7%, respectively, sensitivity 0.09 ng/mL), serum crosslaps (**SCL**, Serum CrossLaps ELISA, Immunodiagnostic Systems GmbH; intra- and interassay coefficients of variation 1.7 and 2.5%, respectively, sensitivity 0.020 ng/mL), and osteocalcin (**OC**, MikroVue Osteocalcin EIA Kit; Quidel Corporation, San Diego, CA; intra- and interassay coefficients of variation 4.8 and 4.8%, respectively, sensitivity 0.45 ng/mL).

In serum and urine samples, Ca and P levels were determined by colorimetry using an autoanalyzer (Cobas Mira Roche-autoanalyzer, F. Hoffman-La Roche Ltd., Basel, Switzerland) and commercial test kits (Ca, test kit DIA00460, Cresolphthalein complexone; P, test kit DIA00620, ammonium molybdate; Diatools AG, Villmergen, Switzerland, intra- and interassay coefficients of variation 0.62 and 1.66% for Ca, respectively, and 0.86 and 1.07% for P, respectively, sensitivity 0.050 mmol/L for Ca and 0.065 mmol/L for P). In serum samples, the mean values of the 2 measurements 2 and 6 h after morning feeding or feeding with irradiation were calculated. Urine creatinine (**Crea**) was also analyzed with the same method using another testkit (Crea

Jaffe, DIA00540; Diatools AG, intra- and interassay coefficients of variation 0.83 and 0.85%, respectively, sensitivity 18 $\mu\text{mol/L}$), and the results of Ca and P were related to these values.

Quantitative RT-PCR

In samples of the kidney, mRNA expression of vitD receptor (**VDR**), calbindin D_{28k} (**Calb-D28k**), $1\alpha\text{-OHase}$ (**CYP27B1**), and 24-OHase (**CYP24A1**) was analyzed. The RNA extraction was performed following the TRI Reagent protocol (TRI Reagent Solution, Ambion, Rotkreuz, Switzerland) and RNA concentration was measured by spectrophotometry using NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA). The RNA samples were then reverse transcribed in cDNA using a commercial kit (QuantiTect Reverse Transcription Kit, Qiagen, Hilden, Germany), according to the manufacturer's instructions using 1 μg of RNA in a reaction volume of 20 μL .

For quantification of RNA expression of VDR, Calb-D28k, CYP27B1, and the housekeeping genes $\beta\text{-actin}$ (for VDR) and GAPDH (for Calb-D28k, CYP27B1), we used specific primers and probes (Table 2). A reaction mixture (20 μL) containing TaqMan Universal PCR Master Mix (Applied Biosystems, Darmstadt, Germany), specific primers (1 μM), specific probe (0.75 μM), and reverse transcribed RNA (50 ng) was prepared. After the PCR products were amplified (95°C, 2 s, 45 cycles of 95°C, 3 s and 60°C, 30 s), they were analyzed on a 7500 Fast Real Time PCR System (ABI 7500Fast Sequence Detection System, Life Technologies, Waltham, MA). Cycle threshold values were determined with a threshold of 0.1.

Expression of CYP24A1 was determined using SYBR Green PCR assays (with a specific primer, Table 2) using a real-time PCR cycler (CFX96TM, Bio-Rad, Munich, Germany) as described by Herm et al. (2015).

Absolute copy numbers were determined using calibration curves generated with cloned PCR fragment standards as described by Wilkens et al. (2009).

Efficiency of the different PCR assays tested in advance for dilutions of intestinal or renal cDNA and cloned standard ranged from 90 to 120%. Specificity of the amplicons was verified using the National Center for Biotechnology Information Blast (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Parallel PCR assays for each gene were performed with cDNA samples, plasmid standards, and a no-template control containing water. Each series of experiments was carried out twice.

Western Blot Analysis

In samples of the duodenum, VDR expression on the protein level as well as GAPDH as the housekeeping protein were analyzed by Western blot analysis as described by Kovács et al. (2015). The antibodies used for VDR were an antibody against VDR (Abcam AB54387, Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) and a horseradish peroxidase-conjugated polyclonal anti-rat IgG (A9037, Sigma-Aldrich Chemie GmbH) and those for GAPDH were monoclonal anti-GAPDH (G8795, Sigma-Aldrich Chemie GmbH) and polyclonal anti-mouse IgG-specific peroxidase antibody (A2304, Sigma-Aldrich Chemie GmbH). Signal visualization was performed using enhanced chemiluminescence (Pierce, Thermo Scientific, Bonn, Germany) and a ChemiDoc system (Bio-Rad, München, Germany). For normalization, the ratio of protein expression of VDR to the GAPDH was calculated.

Skin Sample Analysis

Skin samples were analyzed by HPLC at 254 nm after being prepared as described by Morris (1999) with small modifications (Kovács et al., 2015). Using a Nu-

Table 2. Primers and probes used for TaqMan (Applied Biosystems, Darmstadt, Germany) assays [vitamin D receptor (*VDR*), calbindin D_{28k} (*Calb-D28k*), $1\alpha\text{-hydroxylase}$ (*CYP27B1*), $\beta\text{-actin}$, and *GAPDH*], synthesized by Mycosynth AG (Balgach, Switzerland), and for SYBR Green PCR assays (24-hydroxylase , *CYP24A1*, source: Herm et al., 2015), synthesized by TIB Molbiol (Berlin, Germany)

Gene	Sense and antisense primers (5'→3')	Probe
<i>VDR</i>	CAGATCGTGCTGCTGAAGTC ACCTGGAACCTTGATGAGGGG	FAM-CGACATGTCCTGGACCTGTG-BHQ-1
<i>Calb-D28k</i>	CAGAATCCACCTGCAATCA TCAAGTTCTGAAGCTCCTTTC	FAM-CTGGCTTCATTTTCGACGCTGA-BHQ-1
<i>CYP27B1</i>	CCTGGTCACTCTGTGTCACCT CGCTTGCCAAAGCCAAAGG	FAM-ACTTCAAGGGACCCTGCCCA-BHQ-1
$\beta\text{-Actin}$	ACTGGGACGACATGGAGAAG GTCATCTTCTCACGGTTGGC	FAM-CACCTTCTACAACGAGCTGC-BHQ-1
<i>GAPDH</i>	GTCACCAGGGCTGCTTTTAA ATCTCGCTCCTGGAAGATGG	FAM-AAGTTCCACGGCACAGTCAA-BHQ-1
<i>CYP24A1</i>	GAGGCCTCAAGAAACAGCAC CTGACCCTCTGCCAGTCTTC	

cleosil silica column (EC250/4.6 Nucleosil 100–5C18, Macherey-Nagel, Düren, Germany), 7DHC, pre-D3, lumisterol, tachysterol, and vitD3 were determined.

Peripheral Quantitative Computed Tomography

Peripheral quantitative computed tomography (pQCT; XCT 960 A Knochen Scanner; Stratec Medizintechnik, Pforzheim, Germany) of the left metatarsus was performed before starting the experiment (wk 0) and after slaughter (wk 13). The length of the metatarsus was measured, and total, cortical, and trabecular bone mineral density (BMD) as well as cortical bone thickness (CBT) were determined (cortical mode 2, threshold for cortical bone >640 mg/cm³) distal in the metaphysis (10% of the metatarsus lengths) and in the middle of the diaphysis (50% of the metatarsus lengths) as described by Liesegang and Risteli (2005).

Feed and Feces Analysis

The DM content of feed and feces (%) was determined by drying of 3 to 5 g feed samples and 3 to 5 g pooled feces samples at 103°C up to weight constancy. Afterward, samples were ashed at 550°C in a muffle furnace, hydrolyzed in hydrochloric acid 0.8%, and analyzed for content of Ca and P by colorimetry using the same test kits and autoanalyzer as for urine and serum samples. The amount of crude ash, HCl insoluble ash, crude fat, CP, and crude fiber in feed was determined by proximate analysis. The ADL and ADF in feed and feces, as well as the NDF in feed, were analyzed as described by Van Soest et al. (1991).

Apparent digestibility (AD) of Ca and P were calculated by the indicator method using ADL as the indicator and the formula by Stangl (2014):

$$AD(\%) = 100 - \frac{\% \text{ indicator in feed}}{\% \text{ indicator in feces}} \times \frac{\% \text{ mineral in feces}}{\% \text{ mineral in feed}} \times 100.$$

Statistical Analysis

The experimental design was a 2-factorial design with the factors irradiation (UV or C) and species (L or G) that built the 4 groups LUV, LC, GUV, and GC.

The statistical analyses were performed with SYSTAT (Version 13 for Windows, Systat Software Inc., San Jose, CA). To test the differences between the time-dependent patterns (for blood and urine samples, apparent digestibility, bone densities, and BW) and between the localization-dependent patterns (skin samples) in the groups, a multivariate ANOVA for repeated measurements (MANOVA) was performed, after testing

the data for normal distribution by Shapiro-Wilk test. If the data were not normally distributed, results were verified by nonparametric tests: Kruskal-Wallis was used to investigate the difference between the groups, and a Wilcoxon signed-rank test for paired samples was used to compare the time dependent effect within each group. To exclude a possible influence of the sex on the irradiation treatment within the lambs, a 2-way ANOVA (sex, irradiation, and sex × irradiation) was performed for all analyzed parameters. No interaction between irradiation and sex was found in any of the investigated parameters.

Additionally, Pearson's correlation coefficient between serum 25vitD levels and sterol contents in the skin was calculated and significance was evaluated by *t*-test (zero correlation).

All data are reported as means ± standard error. Differences were considered significant at *P* < 0.05 for all tests.

RESULTS

BW

Body weight increased gradually, indicating normal growth, in lambs from 21.2 ± 0.58 kg in wk 0 to 34.2 ± 0.85 kg in wk 13, and in goat kids from 20.2 ± 1.25 kg in wk 0 to 29.7 ± 1.53 kg in wk 13. The mean weekly increase was higher (*P* < 0.001) in lambs (0.996 ± 0.032 kg/wk) compared with goat kids (0.748 ± 0.036 kg/wk), but we observed no influence of the irradiation on the mean weekly increase and no significant difference in the BW between the groups at any time point.

Skin

Concentration of 7DHC in skin was influenced by irradiation (Table 3). In lambs, 7DHC values were significantly lower in LUV than in LC in skin samples from the back (*P* = 0.013) and the neck (*P* = 0.027), whereas in goat kids it was lower in GUV than in GC in samples from the back (*P* = 0.012), neck (*P* = 0.003), and antebrachium (*P* = 0.001). In both species, the lowest concentrations overall were discovered in samples of the back, followed by the neck, and the highest were in the skin of the forehead (*P* < 0.001). The 7DHC concentration of the back and the neck correlated negatively with the 25vitD serum concentration in wk 11 (*r* = −0.547, *P* = 0.012 and *r* = −0.461, *P* = 0.004, respectively; Figure 1). No species difference could be detected.

The concentration of pre-D3 in the skin was not influenced by irradiation, species, or localization.

Figure 1. Correlation between 25-hydroxyvitamin D (25vitD) in serum (wk 11) and 7-dehydrocholesterol (7DHC) in the back skin of lambs and goat kids. LUV = UVB-exposed lambs; LC = lambs control; GUV = UVB-exposed goat kids; GC = goat kids control. Pearson $r = -0.547$, $P = 0.012$.

Vitamin D₃ could not always be detected in each skin sample of the different locations in lambs or in the samples of forehead of goat kids, whereas in samples of all other localizations of the goat kids, vitD₃ was detectable. Again, no influence of irradiation, species, or location was found.

We observed a species difference in concentrations of tachysterol and lumisterol in the skin. Lumisterol was generally lower in lambs than in goats kids (significant only in samples of the back and the kneefold, with $P = 0.002$ and $P = 0.010$, respectively), and again was not detected in all lamb samples. On the other hand, tachysterol was generally lower in goats kids than in lambs (significant in samples of the head, antebrachium, and kneefold with $P = 0.004$, $P = 0.004$, and $P < 0.001$, respectively) and was not always detected in goat kid samples. An irradiation effect was not detected for tachysterol or lumisterol.

Table 3. Effect of irradiation on mean ($\pm$ SE) content of sterols in skin of lambs and goat kids after slaughter (μ g/g of skin)

	Group ¹				
Item	LUV	LC	GUV	GC	P-value group ²
7-Dehydrocholesterol					
Forehead	64.9 ± 5.46	60.2 ± 1.75	47.9 ± 7.74	47.5 ± 6.31	NS ³
Neck	13 ± 1.66 ^a	22.8 ± 3.4 ^b	18.1 ± 2.66 ^a	30.0 ± 1.82 ^b	0.001
Antebrachium	31.5 ± 4.33	37.3 ± 4.03	20.5 ± 2.3 ^a	34.0 ± 1.88 ^b	0.013
Back	9.64 ± 1.05 ^a	17.1 ± 2.32 ^b	10.7 ± 1.19 ^a	18.6 ± 2.25 ^b	0.004
Kneefold	28.5 ± 2.91	30.7 ± 3.78	20.3 ± 2.21	31.9 ± 3.34	NS
Pre-vitamin D ₃					
Forehead	2.71 ± 1.28	2.83 ± 1.3	0.8 ± 0.05	0.86 ± 0.1	NS
Neck	1.17 ± 0.21	1.37 ± 0.73	1.03 ± 0.18	0.9 ± 0.13	NS
Antebrachium	1.46 ± 0.34	1.61 ± 0.62	0.96 ± 0.17	1.01 ± 0.18	NS
Back	1.16 ± 0.24	1.47 ± 0.56	2.11 ± 0.56	2.53 ± 0.62	NS
Kneefold	2.59 ± 0.54	2.39 ± 0.82	1.47 ± 0.21	1.45 ± 0.35	NS
Vitamin D ₃					
Forehead	1.11 ± 0.86	1.67 ± 0.86	0.13 ± 0.06	0.45 ± 0.31	NS
Neck	0.17 ± 0.04	0.15 ± 0.05	0.31 ± 0.09	0.32 ± 0.11	NS
Antebrachium	0.41 ± 0.25	0.68 ± 0.26	0.37 ± 0.14	0.16 ± 0.05	NS
Back	0.18 ± 0.08	0.29 ± 0.06	0.7 ± 0.27	0.93 ± 0.39	NS
Kneefold	0.25 ± 0.04	0.31 ± 0.11	0.5 ± 0.13	0.24 ± 0.05	NS
Lumisterol					
Forehead	1.45 ± 0.81	0.53 ± 0.08	1.32 ± 0.23	1.35 ± 0.21	NS
Neck	0.52 ± 0.05	0.9 ± 0.27	1.14 ± 0.21	1.03 ± 0.18	NS
Antebrachium	0.98 ± 0.16	0.55 ± 0.1	1.39 ± 0.3	1.3 ± 0.24	NS
Back	0.46 ± 0.06 ^m	0.5 ± 0.14 ^m	3.35 ± 1.21 ⁿ	2.73 ± 0.52 ⁿ	0.021
Kneefold	0.56 ± 0.16 ^m	1.01 ± 0.36	2.43 ± 0.67 ⁿ	1.54 ± 0.2	0.028
Tachysterol					
Forehead	0.41 ± 0.07	0.34 ± 0.07 ^m	0.2 ± 0.06	0.12 ± 0.03 ⁿ	0.031
Neck	0.23 ± 0.06	0.24 ± 0.09	0.1 ± 0.03	0.09 ± 0.02	NS
Antebrachium	0.33 ± 0.06 ^m	0.31 ± 0.09	0.13 ± 0.02 ⁿ	0.09 ± 0.01	0.045
Back	0.3 ± 0.07	0.23 ± 0.09	0.17 ± 0.06	0.3 ± 0.12	NS
Kneefold	0.37 ± 0.06 ^m	0.24 ± 0.06 ^m	0.12 ± 0.03 ⁿ	0.08 ± 0.01 ⁿ	0.001

^{a,b}Different superscripts within a row and the same species indicate a significant influence by irradiation treatment ($P < 0.05$).

^{m,n}Different superscripts within a row indicate a significant influence by species ($P < 0.05$).

¹LUV = UVB-exposed lambs; LC = lambs control; GUV = UVB-exposed goat kids; GC = goat kids control.

²Differences in groups reflect both differences between species as well as irradiation treatment.

³NS: $P > 0.05$.

Serum

Serum 25vitD levels were significantly higher in LUV than LC over the whole experiment (Table 4). In goat kids the difference of GUV having higher levels than GC was significant from wk 3 until the end of the experiment. Only in wk 0 was there a species-specific difference, with goat kids having higher 25vitD levels compared with lambs. Over time, serum 25vitD levels increased significantly in the first 3 wk in lambs ($P = 0.012$ for LUV and $P = 0.017$ for LC), and decreased significantly in both control groups from wk 3 to 11

(LC, $P = 0.012$; and GC, $P = 0.018$), whereas in irradiated groups they remained higher with fluctuations.

Serum 1,25vitD levels decreased significantly over time only in the control groups (Table 4), in LC from wk 0 to 7 ($P = 0.036$), and in GC from wk 0 to 11 ($P = 0.018$). This decrease was not enough to create a significant difference between the groups at any time point.

In addition, no irradiation-dependent effect was detected in serum for the analyzed minerals (Ca, P), bone parameters (OC, SCL), and IGF-I. Serum Ca levels were higher ($P < 0.001$) in lambs (range 2.47–3.46

Table 4. Effect of irradiation on mean ($\pm$ SE) serum levels of 25-hydroxyvitamin D (25vitD), 1,25-dihydroxyvitamin D (1,25vitD), serum crosslaps (SCL), osteocalcin (OC), growth hormone (GH), and IGF-I in lambs and goat kids

Item	Group ¹				<i>P</i> -value group ²
	LUV	LC	GUV	GC	
25vitD (nmol/L)					
wk 0	92.9 $\pm$ 2.37 ^{a,m,x}	81.5 $\pm$ 4.21 ^{b,m,x}	127 $\pm$ 7.77 ^{n,x}	109 $\pm$ 8.16 ^{n,x}	<0.001
wk 3	130 $\pm$ 3.76 ^{a,y}	103 $\pm$ 5.5 ^{b,y}	126 $\pm$ 8.4 ^{a,xz}	100 $\pm$ 5.96 ^{b,x}	0.002
wk 7	100 $\pm$ 4.27 ^{a,x}	61.9 $\pm$ 2.47 ^{b,z}	87.8 $\pm$ 8.13 ^{a,y}	54.5 $\pm$ 3.29 ^{b,y}	<0.001
wk 11	110 $\pm$ 9.16 ^a	69.4 $\pm$ 3.9 ^{b,z}	107 $\pm$ 6.52 ^{a,z}	60.9 $\pm$ 3.85 ^{b,y}	<0.001
<i>P</i> -value, time ³	0.006	<0.001	<0.001	<0.001	
1,25vitD (pmol/L)					
wk 0	143 $\pm$ 15.2	142 $\pm$ 13.9 ^x	176 $\pm$ 29.8	157 $\pm$ 17.3 ^x	NS ⁴
wk 3	109 $\pm$ 13.4	115 $\pm$ 9.48 ^y	115 $\pm$ 11.4	121 $\pm$ 14.3 ^y	NS
wk 7	114 $\pm$ 21	108 $\pm$ 13.1 ^y	140 $\pm$ 4.11	139 $\pm$ 21.8	NS
wk 11	117 $\pm$ 25.5	119 $\pm$ 10.9	137 $\pm$ 13.2	102 $\pm$ 12.9 ^y	NS
<i>P</i> -value, time	NS	0.035	NS	0.008	
SCL (ng/mL)					
wk 0	1.11 $\pm$ 0.21	1.35 $\pm$ 0.31	0.92 $\pm$ 0.09 ^{xy}	0.87 $\pm$ 0.06 ^x	NS
wk 3	0.62 $\pm$ 0.06	0.89 $\pm$ 0.11	0.88 $\pm$ 0.1 ^x	0.82 $\pm$ 0.06 ^x	NS
wk 7	0.68 $\pm$ 0.05 ^m	0.7 $\pm$ 0.06 ^m	1.18 $\pm$ 0.1 ^{n,y}	1.15 $\pm$ 0.08 ^{n,y}	<0.001
wk 11	0.8 $\pm$ 0.06 ^m	0.91 $\pm$ 0.07 ^m	1.52 $\pm$ 0.08 ^{n,z}	1.61 $\pm$ 0.08 ^{n,z}	<0.001
<i>P</i> -value, time	NS	NS	<0.001	<0.001	
OC (ng/mL)					
wk 0	197 $\pm$ 8.86 ^{m,w}	169 $\pm$ 12.5 ^{m,x}	290 $\pm$ 25.3 ⁿ	271 $\pm$ 21.1 ⁿ	<0.001
wk 3	157 $\pm$ 6.71 ^{m,xy}	160 $\pm$ 6.28 ^{m,x}	222 $\pm$ 18.3 ⁿ	228 $\pm$ 22.8 ^{n,x}	0.001
wk 7	129 $\pm$ 4.41 ^{m,y}	124 $\pm$ 8.09 ^{m,y}	232 $\pm$ 27.7 ⁿ	231 $\pm$ 13.1 ^{n,x}	<0.001
wk 11	147 $\pm$ 8.97 ^{m,z}	128 $\pm$ 4.39 ^{m,y}	273 $\pm$ 16.7 ⁿ	307 $\pm$ 20.1 ^{n,y}	<0.001
<i>P</i> -value, time	<0.001	<0.001	NS	0.002	
GH (ng/mL)					
wk 0	2.97 $\pm$ 0.13 ^{m,x}	2.91 $\pm$ 0.03 ^{m,x}	6.64 $\pm$ 0.17 ^{n,x}	6.63 $\pm$ 0.18 ^{n,x}	<0.001
wk 3	2.83 $\pm$ 0.07 ^{m,x}	2.77 $\pm$ 0.07 ^{m,xz}	7.08 $\pm$ 0.2 ^{a,n,y}	6.26 $\pm$ 0.23 ^{b,n,x}	<0.001
wk 7	2.5 $\pm$ 0.05 ^{m,y}	2.52 $\pm$ 0.05 ^{m,y}	6.41 $\pm$ 0.13 ^{a,n,x}	5.84 $\pm$ 0.09 ^{b,n,y}	<0.001
wk 11	2.73 $\pm$ 0.1 ^{m,x}	2.64 $\pm$ 0.06 ^{m,z}	6.06 $\pm$ 0.16 ^{n,x}	5.89 $\pm$ 0.14 ^{n,y}	<0.001
<i>P</i> -value, time	0.002	<0.001	0.001	0.001	
IGF-I (ng/mL)					
wk 0	145 $\pm$ 11.6 ^{m,x}	135 $\pm$ 18.3 ^{m,x}	280 $\pm$ 50.9 ^{n,x}	263 $\pm$ 27.6 ^{n,x}	0.002
wk 3	190 $\pm$ 7.81 ^y	186 $\pm$ 26.9 ^y	240 $\pm$ 37.5 ^x	240 $\pm$ 37.5 ^x	NS
wk 7	218 $\pm$ 13.1 ^y	207 $\pm$ 41.2 ^{m,y}	240 $\pm$ 37.5 ^x	346 $\pm$ 36.9 ^{n,x}	0.032
wk 11	335 $\pm$ 20.7 ^{m,z}	285 $\pm$ 50 ^{m,z}	443 $\pm$ 45.8 ^{n,y}	476 $\pm$ 69.6 ^{n,y}	0.031
<i>P</i> -value, time	<0.001	0.005	<0.001	0.015	

^{a,b}Different superscripts within a row and the same species indicate a significant influence by irradiation treatment ($P < 0.05$).

^{m,n}Different superscripts within a row indicate a significant influence by species ($P < 0.05$).

^{w-z}Different superscripts within a column (group) indicate a significant influence by time ($P < 0.05$).

¹LUV = UVB-exposed lambs; LC = lambs control; GUV = UVB-exposed goat kids; GC = goat kids control.

²Differences in groups reflect both differences between species as well as irradiation treatment.

³Differences in time points reflect the differences between measurements in wk 0, 3, 7, and 11.

⁴NS: $P > 0.05$.

mmol/L) than in goat kids (range 2.28–2.82 mmol/L) during the entire experiment. No time-dependent effect was detected. Serum P level of goat kids increased over time with a significant increase from wk 7 to 11 ($P = 0.003$), whereas in lambs it did not vary significantly. At the last sampling in wk 11, goat kids had significant higher levels of serum P levels than lambs ($P < 0.001$). The range during the whole experiment was 1.47 to 2.49 mmol/L for lambs and 1.49 to 2.65 mmol/L for goat kids. Serum OC levels (Table 4) in lambs decreased significantly over time ($P = 0.001$) and were lower than in goat kids over the whole experiment ($P < 0.001$). Also, SCL levels (Table 4) were lower in lambs than in goat kids through the second half of the experiment (wk 7 and 11, $P < 0.001$) because of a time-dependent increase from wk 0 to 11 in goat kids ($P = 0.001$). Serum IGF-I levels increased significantly from wk 0 to 11 in all groups (lambs, $P = 0.012$; LC, $P = 0.012$; GUV, $P = 0.018$; and GC, $P = 0.018$, Table 4). Over the whole time, goat kids had higher serum levels than lambs, but it reached statistical significance only in wk 0, 7, and 11 ($P < 0.001$, $P = 0.025$, and $P = 0.005$, respectively).

Also, values of serum GH were always significantly higher in goat kids than in lambs ($P < 0.001$, Table 4), but showed different time courses. Whereas in lambs and GC the significant decrease until wk 7 ($P < 0.001$ and $P = 0.028$, respectively) was followed by an increase until wk 11 (significant only in lambs, $P = 0.002$), in GUV a peak occurred in wk 3 ($P = 0.028$) followed by a decrease until wk 11 ($P = 0.028$). Consequently, we saw an irradiation effect in wk 3 and 7 ($P = 0.021$ and $P = 0.003$, respectively), where GC had significantly lower GH levels in serum than GUV.

Urine

Urinary Ca and P content was not influenced by irradiation. Over time, values of Ca/Crea content in lambs increased from 0.051 ± 0.007 mmol/mmol of Crea in wk 0 to 0.153 ± 0.020 mmol/mmol of Crea in wk 11 ($P < 0.001$), whereas in goat kids it increased from 0.178 ± 0.028 mmol/mmol of Crea in wk 0 until 0.389 ± 0.076 mmol/mmol of Crea in wk 3 ($P = 0.013$) and decreased again till 0.117 ± 0.017 mmol/mmol of Crea in wk 11 ($P = 0.005$). Consequently, goat kid Ca/Crea content in urine was higher in wk 0 and 3 and lower in wk 7 compared with lambs ($P < 0.001$, $P = 0.001$, and $P = 0.001$, respectively). The content of P/Crea in urine did not vary significantly over time in any of the groups (from 0.097 ± 0.004 mmol/mmol of Crea in wk 0 to 0.111 ± 0.015 mmol/mmol of Crea in wk 11), but in wk 11 we found lower levels in goat kids than in lambs ($P = 0.011$, 0.071 ± 0.008 , and 0.144 ± 0.024 mmol/mmol of Crea, respectively).

Figure 2. Results of Ussing chamber technique: net Ca ion fluxes (J_{net}) through rumenal and duodenal mucosa of goat kids and lambs. Calculated from the mean detected unidirectional fluxes (J_{ms} fluxes from the mucosal to serosal side of the epithelium, and J_{sm} fluxes from serosal to mucosal) with the formula: $J_{net} = J_{ms} - J_{sm}$ (nmol/h per cm^2). LUV = UVB-exposed lambs; LC = lambs control; GUV = UVB-exposed goat kids; GC = goat kids control. Groups within 1 location with different letters differ significantly ($P < 0.050$). Mean $\pm$ SE.

Ussing Chamber

We observed a net active Ca absorption in rumen and a net active Ca secretion in duodenum (Figure 2). In goat kids, a significant irradiation-dependent difference was found: the absorption in rumen as well as the secretion in duodenum ($P = 0.009$ and $P = 0.001$, respectively) was lower in GUV than in GC. A species-specific effect was detected in both localizations, in rumen with GUV lower than LUV ($P = 0.004$) and in duodenum with GC higher ($P = 0.004$) than LC.

Feces

In feces samples, we did not observe either an irradiation-dependent effect or a time effect for AD of Ca and P (Table 5). The species difference showed higher AD of Ca in goat kids than in lambs in wk 3 ($P = 0.006$) and 7 ($P < 0.001$), and a higher AD of P in lambs than in goat kids at those time points ($P < 0.001$ and $P = 0.001$, respectively).

PCR/WB

In the kidneys, expression of VDR, Calb-D28k, 1α -OHase, and 24 -OHase on RNA level was compared within 1 species in relation to the respective control group. No irradiation-dependent effect was found (Table 6). Expression on protein level of VDR in duodenum within 1 species was not influenced by irradiation (Table 6).

Table 5. Effect of irradiation on mean ($\pm$ SE) apparent digestibility of Ca and P calculated with ADL used as an indicator, in lambs and goat kids

Item	Group ¹				<i>P</i> -value group ²
	LUV	LC	GUV	GC	
Ca (%)					
wk 3	18.4 $\pm$ 2.53 ^m	25.7 $\pm$ 3.21	29.9 $\pm$ 2.39 ⁿ	35.1 $\pm$ 2.98	0.002
wk 7	20.6 $\pm$ 3.5 ^m	23.3 $\pm$ 4.34 ^m	38 $\pm$ 2.62 ⁿ	37.1 $\pm$ 3.41 ⁿ	0.002
wk 11	24.4 $\pm$ 2.7	26.1 $\pm$ 6.78	32.6 $\pm$ 3.46	37.6 $\pm$ 3.2	NS ³
<i>P</i> -value, time ⁴	NS	NS	NS	NS	
P (%)					
wk 3	9.76 $\pm$ 3.73 ^m	10.67 $\pm$ 3.47 ^m	12.76 $\pm$ 6.46 ⁿ	-4.58 $\pm$ 4.68 ⁿ	0.003
wk 7	9.61 $\pm$ 3.02 ^m	14.69 $\pm$ 5.04 ^m	-4.9 $\pm$ 5.27 ⁿ	-3.83 $\pm$ 4.44 ⁿ	0.009
wk 11	13.94 $\pm$ 3.19	11.15 $\pm$ 5.97	5.44 $\pm$ 7.17	-0.04 $\pm$ 6.17	NS
<i>P</i> -value, time	NS	NS	NS	NS	

^{m,n}Different superscripts within a row indicate a significant influence by species ($P < 0.05$).

¹LUV = UVB-exposed lambs; LC = lambs control; GUV = UVB-exposed goat kids; GC = goat kids control.

²Differences in groups reflect both differences between species as well as irradiation treatment.

³NS: $P > 0.05$.

⁴Differences in time points reflect the differences between measurements in wk 3, 7, and 11.

pQCT

At that the end of the experiment, we found no irradiation-dependent effect on any of parameters measured by pQCT (Table 7). Total BMD (diaphysis and metaphysis) as well as cortical BMD and CBT increased significantly over time in all groups ($P < 0.014$) except in GC where the increase of total BMD and CBT in diaphysis did not reach significance. In the trabecular BMD, the time-dependent effect was not significant.

In the diaphysis we found significantly higher values of cortical and total BMD in lambs compared with goat kids at the end of the experiment ($P < 0.001$). A species-specific difference in the metaphysis was found in wk 0, where the total and trabecular BMD was significantly lower in lambs compared with goat kids ($P < 0.022$). Also, LUV had lower values of total and trabecular BMD in wk 0 compared with LC. These differences in the metaphysis disappeared until the end of the trial.

Table 6. Effect of irradiation on mean ($\pm$ SE) RNA expression of vitamin D receptor (VDR), calbindin D_{28k} (Calb-D28k), 1 α -hydroxylase (CYP27B1), 24-hydroxylase (CYP24A1) normalized to either β -actin (VDR) or GAPDH (Calb-D28k, CYP27B1, and CYP24A1) in kidneys and on protein expression of VDR normalized to GAPDH in duodenum determined with Western blot in lambs and goat kids

Item	Group ¹				<i>P</i> -value, group ²
	LUV	LC	GUV	GC	
Lambs					
Kidney					
VDR	0.64 $\pm$ 0.17	1 $\pm$ 0.18			NS ³
Calb-D28k	1.45 $\pm$ 0.77	1 $\pm$ 0.25			NS
CYP27B1	0.87 $\pm$ 0.42	1 $\pm$ 0.43			NS
CYP24A1	1.85 $\pm$ 0.42	1 $\pm$ 0.16			NS
Duodenum					
VDR	1.27 $\pm$ 0.13	1 $\pm$ 0.2			NS
Goat kids					
Kidney					
VDR			1.37 $\pm$ 0.23	1 $\pm$ 0.25	NS
Calb-D28k			1.64 $\pm$ 0.45	1 $\pm$ 0.21	NS
CYP27B1			0.76 $\pm$ 0.34	1 $\pm$ 0.34	NS
CYP24A1			0.43 $\pm$ 0.15	1 $\pm$ 0.38	NS
Duodenum					
VDR			1.22 $\pm$ 0.14	1 $\pm$ 0.1	NS

¹LUV = UVB-exposed lambs; LC = lambs control; GUV = UVB-exposed goat kids; GC = goat kids control.

²Reflects differences between irradiation treatment within 1 species.

³NS: $P > 0.05$.

Table 7. Effect of irradiation on mean ($\pm$ SE) metatarsal bone mineral density (BMD) and cortical bone thickness (CBT) in lambs and goat kids

Item	Group ¹				<i>P</i> -value, group ²
	LUV	LC	GUV	GC	
Total BMD diaphysis ³ (mg/cm ³)					
wk 0	593 $\pm$ 21.8	609 $\pm$ 18.7	572 $\pm$ 29.4	605 $\pm$ 26.9	NS ⁴
wk 13	801 $\pm$ 11.3 ^m	791 $\pm$ 20 ^m	659 $\pm$ 15 ⁿ	639 $\pm$ 18.2 ⁿ	<0.001
<i>P</i> -value, time ⁵	<0.001	<0.001	0.014	NS	
Total BMD metaphysis ⁶ (mg/cm ³)					
wk 0	321 $\pm$ 8.03 ^{a,m}	361 $\pm$ 7.06 ^{b,m}	440 $\pm$ 23.6 ⁿ	419 $\pm$ 15.6 ⁿ	<0.001
wk 13	465 $\pm$ 14.8	451 $\pm$ 9.03	479 $\pm$ 16.7	475 $\pm$ 12.9	NS
<i>P</i> -value, time	<0.001	<0.001	0.014	0.002	
Trabecular BMD metaphysis (mg/cm ³)					
wk 0	313 $\pm$ 12.1 ^{a,m}	369 $\pm$ 8.86 ^b	402 $\pm$ 37.7 ⁿ	387 $\pm$ 18.9	0.032
wk 13	354 $\pm$ 18.6	327 $\pm$ 15.9	379 $\pm$ 27.7	372 $\pm$ 18.9	NS
<i>P</i> -value, time	NS	0.044	NS	NS	
Cortical BMD diaphysis (mg/cm ³)					
wk 0	913 $\pm$ 15.2	928 $\pm$ 8.97	868 $\pm$ 24	904 $\pm$ 15.3	NS
wk 13	1,078 $\pm$ 6.35 ^m	1,070 $\pm$ 6.95 ^m	996 $\pm$ 5.48 ⁿ	979 $\pm$ 13.2 ⁿ	<0.001
<i>P</i> -value, time	<0.001	<0.001	0.001	<0.001	
CBT diaphysis (mm)					
wk 0	2.44 $\pm$ 0.07 ^m	2.46 $\pm$ 0.04 ^m	2.76 $\pm$ 0.08 ⁿ	2.84 $\pm$ 0.18 ⁿ	0.018
wk 13	3.34 $\pm$ 0.07 ^m	3.27 $\pm$ 0.08 ^m	2.94 $\pm$ 0.07 ⁿ	2.99 $\pm$ 0.1 ⁿ	0.004
<i>P</i> -value, time	<0.001	<0.001	0.026	NS	

^{a,b}Different superscripts within a row and the same species indicate a significant influence by irradiation treatment ($P < 0.05$).

^{m,n}Different superscripts within a row indicate a significant influence by species ($P < 0.05$).

¹LUV = UVB-exposed lambs; LC = lambs control; GUV = UVB-exposed goat kids; GC = goat kids control.

²Differences in groups reflect both differences between species as well as irradiation treatment.

³Measuring point at 50% of metatarsus length.

⁴NS: $P > 0.05$.

⁵Differences in time points reflect the differences between measurements in wk 0 and 13.

⁶Measuring point at 10% of metatarsus length.

The CBT was significantly higher in wk 0 in goat kids compared with lambs ($P = 0.001$), whereas at the end of the experiment, in wk 13, the situation was the inverse ($P < 0.001$).

DISCUSSION

Vitamin D Status and Synthesis in the Skin

In this study, we investigated the hypothesis that growing lambs and goat kids of dairy breeds are able to fully compensate for a reduced vitD content in food by producing vitD within their skin. Level of 25vitD in serum is recognized to be the most reliable indicator for vitD status (Adams et al., 1982). In the test kit used to determine 25vitD level in serum, no differentiation is present between 25-dihydroxyvitamin D₂ (coming from the vitD₂ acquired through nutrition), 25-dihydroxyvitamin D₃ (coming from endogenous production), and 24,25-dihydroxyvitamin D, and therefore all of these storage forms are included independent of their origin (Horst and Reinhardt, 1983). Both irradiated groups (LUV and GUV) had a better vitD status with higher 25vitD serum levels compared with the control groups

(LC and GC). In the experiment of Kovács et al. (2015), lambs and goat kids were irradiated in the same way as in the present study, but had a high vitD supply in the diet. The values of 25vitD at the end of that trial were in a similar range as the values of our irradiated groups (LUV and GUV), whereas the levels of 25vitD of our control groups (LC and GC) without any vitD source were clearly lower. This shows that the irradiation was sufficient to compensate for the lack of vitD and maintain the serum 25vitD levels. Additionally, the drop of the initially high serum 25vitD levels in our control groups confirms that the natural sources of vitD in the feedstuffs in this study were not sufficient to cover the vitD requirements of the animals.

The natural content of vitD in hay depends on composition, season, and climatic conditions of the region as well as drying method. Therefore, there is a high variation in the vitD contents described in the literature. Jäpelt et al. (2011), for example, found levels between 2.8 and 255.6 IU of vitD₂/kg of OM (analyzed by liquid chromatography tandem mass spectrometry) in different ryegrass over the seasons in Denmark, whereas Kohler et al. (2013) found a higher level of 2,508 IU of vitD₂/kg of DM in lowland hay in Switzerland (ana-

lyzed by HPLC). Considering that the recommended vitD supply, to reach a normal vitD status, is between 120 and 500 IU/d for sheep, and between 250 and 1500 IU/d for goats (Agroscope, 2013), the mean content of vitD in the hay used must have been under 153 IU of vitD/kg of DM. Therefore, the absorbed vitD naturally present in the hay did not cover the vitD requirement of the animals (which explains the dropped vitD levels in the control groups), but still provided a significant vitD amount, which prevented the drop of the vitD levels in a vitD-deficient range.

In most species (including humans), the normal range of 25vitD in serum is considered to be between 75 and 150 nmol/L, whereas levels between 25 and 75 nmol/L are defined as vitD insufficiency (meaning that endocrine function such as immune regulation and cell differentiation are compromised), and levels <25 nmol/L are defined as actual vitD deficiency with negative effects on the classical vitD function (such as bone metabolism and calcium homeostasis investigated in this study; Norman, 2011; Weber et al., 2014; Nelson et al., 2016). Different authors also described lower ranges of 25vitD in lambs and calves (compared with adult animals) with good health and no signs of classical vitD deficiency (Horst and Littledike, 1982; Handel et al., 2016; Nelson et al., 2016).

The animals in this study had a normal initial vitD status thanks to the high vitD intake due to the vitD content in the normal concentrate fed before starting the experiment and the vitD intake via milk before weaning. Indeed, for newborn ruminants, in contrast to other species, milk can be an adequate source of vitD under the condition of a high vitD status in combination with a moderate milk yield of the dam (Kohler et al., 2013; Weiss et al., 2015; Hymøller et al., 2017). The 25vitD can then be stored in the body tissue (primary fat, muscle, and liver) to help prevent a fast drop of vitD status (Hidioglou, 1987; Hidioglou and Karpinski, 1989). Therefore, the short duration of the experiment and the healthy initial vitD status also helped prevent a real vitD deficiency in our control groups.

The levels of the active metabolite 1,25vitD in serum, on the other hand, were neither influenced by irradiation nor by species difference. After 3 mo of observation, only a decrease of 1,25 levels in control groups could be observed. Therefore, the inexplicably higher serum 1,25vitD levels in irradiated goat kids found by Kovács et al. (2015) could not be confirmed. The production of 1,25vitD from 25vitD by the enzyme 1α -OHase is inhibited by the final product 1,25vitD, as well as by Ca and P, and stimulated by calcitonin, IGF-I, and parathyroid hormone (Nesbitt and Drezner, 1993; Holick, 2008; Dittmer and Thompson, 2011; Jones, 2013). The levels of

Ca and P in serum remained stable in a normal range (Pugh, 2002) during the whole experiment and IGF-I increased in a similar way in all the groups due to the physiological growing process (Zofková, 2003). Consequently, neither the amount of the enzyme 1α -OHase nor 24-OHase in the kidney was altered. In future studies, it would be interesting to analyze the reaction in an additional situation of reduced Ca supply.

Considering therefore that the difference in 25vitD status between the groups was not caused by a different degradation and the husbandry conditions and the feeding were identical for all groups, the difference must have been caused by the production with irradiation of the skin. These findings are in line with previous studies that showed a better vitD status in sheep exposed to UVB irradiation (Quarterman et al., 1964; Smith and Wright, 1980; Hidioglou and Karpinski, 1989; Kovács et al., 2015) and show evidence of the parallelism to the endogenous vitD production in dairy cows (Hymøller et al., 2009; Hymøller and Jensen, 2010, 2012; Hymøller et al., 2017). Also, the lower content of 7DHC in the skin of the irradiated animals compared with the control group supports our hypothesis. This goes along with the findings described by Kovács et al. (2015) and is caused by the transformation of 7DHC into pre-D3, as it has been proven *in vitro* for human skin (Holick et al., 1981). On the other hand, we could not find an irradiation-dependent increase in the concentration of the photoproducts (pre-D3, lumisterol, tachysterol, vitD3) in skin samples. Kühn et al. (2015) investigated the effect of UVB irradiation in skin of chicks and found no significant effect on 7DHC concentrations (but a slight irradiation-dependent decrease), and an irradiation-dependent increase on vitD3 concentrations. An explanation for the lack of accumulation of the produced vitD3 in the analyzed skin samples is that it has been released to circulation (Holick et al., 1980). In fact, the amount of 7DHC in the skin parts most exposed to UVB irradiation (back and neck) was negatively correlated with the 25vitD serum level. The lack of an increase of lumisterol and tachysterol remains unclear, but it could be hypothesized that the amount of UVB reaching the skin layers, which can be influenced by factors like skin pigmentation or thickness as well as hair coat density, was not high enough for their production or that other storage forms such as suprasterol I, suprasterol II, or 5,6-transvitamin D3 are involved (Forrest and Fleet, 1985; Webb and Holick, 1988; Handel et al., 2016). To clarify interspecies differences in those storage forms, further studies are needed. So far, we could show that lambs have a higher concentration of tachysterol and goat kids have a higher concentration of lumisterol within their skin. Also, the influence of the amount of

7DHC in the skin, as part of the cholesterogenic pathway, needs to be further investigated in both species. In fact, we could not confirm that sheep have 10 times lower concentration of 7DHC in their skin compared with goats as shown by Kohler et al. (2013) in adult animals, and for unclear reasons our 7DHC levels were higher compared with those measured in the study of Kovács et al. (2015).

Effect of Vitamin D Status on Bone Metabolism, Ca Absorption, and Ca Homeostasis

A sufficient vitD status, combined with a normal Ca availability, leads to the embedment of the minerals in the bone and these lead to an increase of BMD. In all groups BMD increased over the trial time, which is part of a normal physiological aging process (Liesegang et al., 2013), although the increase was higher in lambs than in goat kids. This agrees with the results of Kovács et al. (2015) and supports the suggestion of Liesegang and Risteli (2005) that growing sheep react faster in embedding minerals in the bone compared with growing goats. No difference between irradiated and control groups was seen in the BMD at the end of the experiment and the animals of the control groups did not show any signs of rickets or poor bone mineralization. This means that all the animals continued their normal bone growth and that the reduced vitD status in the control groups was not yet too low to influence bone formation (Van Saun, 2004; Dittmer et al., 2011; Dittmer and Thompson, 2011).

The concentrations of bone formation marker (OC) as well as bone resorption marker (SCL) in serum were higher in goat kids than in lambs, which leads to a higher bone turnover and conforms with reduced mineralization of the bones (Lips and van Schoor, 2011). Also, IGF-I and GH influence bone remodeling during the growth process. The pituitary gland produces and releases GH that stimulates the production of IGF-I in the liver and other tissues, which then increases osteoclastic and osteoblastic activity. It has been demonstrated in young men that IGF-I correlates well with bone turnover (Fatayerji and Eastell, 1999); therefore, it was not surprising that, as in Kovács et al. (2015), goat kids also had higher GH and IGF-I levels compared with lambs. However, this needs to be carefully interpreted because 2 different biases could be responsible for that significant interspecies difference: the pulsatile excretion of the 2 hormones during the day (which can also be affected by interspecies differences) and the high difference in sensitivity of the methods used for the different species.

In the gastrointestinal tract, we investigated the active Ca absorption with the background of a sufficient

Ca supply. Therefore, the paracellular absorption was probably sufficient to cover the Ca demand. Accordingly, even if goat kids had higher AD of Ca during the whole experiment, they did not have higher active Ca net fluxes compared with lambs. We detected an active Ca net absorption in rumen and an active Ca net secretion in duodenum, which goes along with previous studies (Schröder et al., 1999; Sidler-Lauff et al., 2010; Kovács et al., 2015). Ruminant Ca absorption plays an important role in Ca homeostasis in ruminants, but the underlying mechanisms are still not fully understood. It was previously demonstrated that, as in our study, the active trans-epithelial Ca absorption is not influenced by 1,25vitD (Schröder et al., 1999; Sidler-Lauff et al., 2010; Wilkens et al., 2011, 2012), but can be increased for example by alimentary administration of anionic salts, short-chain fatty acids, or Cl (Leonhard-Marek et al., 2007; Wilkens et al., 2016). As all the groups received the same diet, the reason why the control group (GC) had an unexpected higher active ruminal Ca absorption compared with GUV cannot be explained. However, the higher active net Ca secretion in duodenum in GC conforms with the results of Kovács et al. (2015) and could be a mechanism to compensate for the excess of Ca absorbed in the rumen to keep the Ca blood level constant. This supports the theory of Schröder and Breves (2006) who showed that the net absorption in the forestomachs and abomasum is negatively correlated with the intestinal net Ca absorption in cows, indicating a kind of compensatory mechanism that still needs to be investigated. The differences between the groups that we found in goat kids but not in sheep also indicate that goats probably adapt faster through the intestine in a situation of challenged Ca homeostasis and profit more from intestinal Ca absorption compared with sheep (Wilkens et al., 2012; Kovács et al., 2015).

In the duodenum of monogastric animals, the effect of 1,25vitD on the active Ca absorption is mediated by a VDR-dependent genomic action (Bronner, 2003), and in vitD deficient rats a reduction of VDR in duodenum was found (Zineb et al., 1998). In contrast, even if VDR was detected in the duodenum of ruminants (Boos et al., 2007; Riner et al., 2008), the transcellular Ca transport that is influenced by 1,25vitD is less important (Schröder et al., 1997; Sidler-Lauff et al., 2010; Wilkens et al., 2011). Indeed, we found no difference between the groups in VDR expression on protein level, probably also because the vitD status of the control group was not as low as in a situation of vitD deficiency.

Considering the AD of P, we only found lower AD in goat kids compared with lambs. This agrees with previous studies that showed that in goats fecal P losses might be so high that apparent digestibility results are

negative, but this does not reflect a negative true P digestibility. The amount of AD of P is linearly correlated with the dietary P intake, and the fecal P losses are also influenced by the dietary P intake, the salivary excretion, as well as the DMI and crude fiber content in feed (Bravo et al., 2003; Tayo et al., 2009).

The excretion of Ca and P through the kidneys was also not influenced by irradiation or vitD status. As already shown in earlier studies, in ruminants in contrast to monogastric animals, the kidneys are not a main site for the regulation of Ca homeostasis (Hoenderop et al., 2005; Herm et al., 2015).

In this study healthy animals with a good initial vitD status were used to demonstrate that the irradiation can prevent the drop of the vitD status even if the nutritional vitD supply is missing and therefore keep the healthy growing going, instead of curing an initial vitD deficiency, which is rare considering the currently used diets highly supplemented with vitD. In summary, during these experiment the animals were in a situation of challenged Ca homeostasis because of the growing process, but the AD of Ca and P as well as the Ca and P content in serum remained constant in both species over the whole time, and therefore the 25vit D levels and the absorbed Ca were sufficient to permit an embedment in the bones and a normal growth with increasing of BMD. Even if the vitD status in the control groups was declining, the period of the experiment was not sufficient to really achieve a vitD deficiency and therefore no negative effects on Ca homeostasis were detected. However, effects of the declined vitD status on other physiological processes, for example, on the immune system or on cell differentiation (Holick, 2004; Dittmer and Thompson, 2011), which were not analyzed in this study, cannot be excluded. In ruminants, it was demonstrated that vitD status interferes with reproductive performance in an observational study done in a wild Soay sheep population (Handel et al., 2016), and that vitD improves the immune function in cows (Nelson et al., 2010; Vieira-Neto et al., 2017). Therefore, future studies should include effects on fertility and the immune system.

CONCLUSIONS

In conclusion, we could demonstrate that, like cows, growing dairy goats and sheep are able to compensate a vitD-reduced diet by producing vitD within their skin while exposed to UVB irradiation and therefore to keep a high vitD status and a balanced Ca homeostasis. In contrast, the vitD status of the control groups with no UVB exposure dropped, but not enough to induce an actual vitD deficiency and therefore no effects on Ca homeostasis were yet detected. In future studies, with

a prolonged observation time, it should be clarified which is actually the ideal vitD status in young sheep and goats to ensure a healthy growing process and a balanced Ca homeostasis and how much UVB irradiation is needed to achieve that goal with the practical consequences in livestock farming.

ACKNOWLEDGMENTS

The authors thank Ines Mittner, Susanne von Waldow, and Reto Mühlemann for their technical assistance. The laboratory work was partly performed using the logistics of the Centre for Clinical Studies at the Vetsuisse Faculty of the University of Zurich.

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