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Influence of altitude on vitamin D and bone metabolism of lactating sheep and goats¹

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ABSTRACT: This study investigated the influence of alpine grazing on vitamin D (vitD) and bone metabolism in sheep and goats. Two groups of five adult lactating East Friesian milk sheep and Saanen dairy goats were kept on pastures at 2,000 to 2,600 m a.s.l. (SA: sheep alpine; GA: goats alpine) and 400 m a.s.l. (SL: sheep lowland; GL: goats lowland). The animals were milked twice daily and the milk yield was measured. Blood, milk, skin, and forage samples were collected and the left metatarsi were measured with peripheral quantitative computed tomography. The relative humidity and air temperature were recorded and the ultraviolet B (UVB) radiation was measured with a solar meter at both research stations. In addition, animals from the alpine group were equipped with a global positioning system receiver. The UVB radiation was higher at the alpine station ($P < 0.05$) compared to the lowland station. In contrast, both the relative humidity and the air temperature were higher at the lowland station ($P < 0.04$). The group GA produced more milk than GL ($P < 0.043$). No differences in milk production between SA and SL were detected. Only minor differences between the alpine and lowland species groups were found in the total 25-hydroxyvitamin D (25(OH)D)

and 1,25-dihydroxyvitamin D serum concentration and in the 25(OH)D milk concentration. 25-hydroxyvitamin D₂ concentration in serum was higher in sheep compared to goats and the 25(OH)D₃ concentration in serum increased in all four groups but was higher in the alpine groups during the experiment. In addition, no differences in 7-dehydrocholesterol (7-DHC) concentrations in the skin at high altitude and lowland groups were detectable. However the 7-DHC concentrations in the skin of sheep were less than a tenth of the concentrations in the skin of goats and were nearly not detectable. In both groups SA and SL bone strength index increased during the trial ($P = 0.043$). Bone strength index was lower in GA compared to GL at wk 12 ($P = 0.047$). Mean serum Ca concentrations were higher and P concentrations were lower in the alpine groups than in the lowland groups ($P = 0.047$). In both groups SA and GA the distance travelled increased during the trial. In conclusion, no effect of altitude on vitD status, vitD milk concentration and bone strength could be detected. Both sheep and goats are able to produce vitD in the skin, but sheep depend more on vitD intake with feedstuff, whereas goats rely more on cutaneous vitD production.

Key words: bone, lactation, mountain pasture, small ruminant, ultraviolet radiation, vitamin D

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INTRODUCTION

In Switzerland, ruminants often spend the summer grazing on alpine pastures located at high altitudes. This is an old tradition that also serves as landscape management. The ultraviolet B (UVB) radiation is especially high at these high-altitude pastures, which is considered to have a positive effect on vitamin D (vitD) metabo-

lism. The typical topography of these alpine pastures leads to stress on the locomotor system, resulting in an adaption of the bone, in particular bone strength. Other factors that are typical of the alpine pastures in Switzerland, like the low feed quality, which is due to the short vegetation period, fast growing of the feed and the extensive management (Brühlmann and Thomet, 1991), and the high calcium and low phosphorus content of the forage, which is due to low fertilization levels in connection with the limestone-based soil (Berry et al., 2002; Soder and Stout, 2003), also affect vitD and bone metabolism. Previous studies using lactating cows, growing lambs, and adult sheep and goats

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were performed to demonstrate the effect of high-altitude grazing on vitD and bone metabolism (Leiber et al., 2005; Kaulfers, 2009; Liesegang et al., 2013; Willems et al., 2013). However, to date, no studies with lactating sheep and goats were performed. The focus of this study was therefore to demonstrate to what extent a stay at the alpine pastures in Switzerland affect the vitD and bone metabolism in lactating dairy sheep and goats. By determining vitD metabolites in blood, skin, grass and milk samples, bone markers, and bone strength, the following hypothesis was tested: the higher UVB radiation at the alpine station leads to a better vitD status and higher vitD content of the milk, and the more intense exercise of lactating sheep and goats grazing at the hilly alpine landscape increases bone strength compared to animals grazing on flat terrain in the lowlands.

MATERIALS AND METHODS

The procedures in this research were approved by the Cantonal Veterinary Office of Zug and were based on the animal welfare law of Switzerland (approval ZG 106/2009).

Animals

Ten lactating East Friesian milk sheep and ten lactating Saanen dairy goats, which were housed in the same lowland barn prior to the experiment, were assigned (according to age) to two different groups: alpine and lowland group. The alpine group consisted of five sheep (sheep alpine = **SA**; aged 4.4 ± 1.2 yr; weighed 63.7 ± 4.0 kg) and five goats (goats alpine = **GA**; aged 3.4 ± 0.8 yr; weighed 58.8 ± 3.9 kg). Alpine sheep were in lactation for 98.4 ± 2 d whereas alpine goats lactated for 72.4 ± 6.7 d. The lowland group consisted of five sheep (sheep lowland = **SL**; aged 4.6 ± 1 yr; weighed 72.4 ± 5.2 kg) and five goats (goats lowland = **GL**; aged 4.2 ± 1.3 yr, weighed 61.9 ± 3.2 kg). Each group was in lactation for 94.4 ± 5.4 d (SL) and 93.4 ± 2.7 d (GL), respectively. All sheep were shorn before the experiment. The alpine group was kept at the high-altitude ETH research station "Alp Weissenstein" (Albula, Grisons, 2,000 to 2,600 m a.s.l). The lowland group stayed at the ETH research Station "Chamau" (Central Switzerland, Zug, 400 m a.s.l). All animals were kept in these locations for 12 wk and remained outside on pasture for 10 h during the day. At night, they either stayed in the barn or on pasture, depending on weather conditions. However, both groups were always treated the same way. They had access to a NaCl licking stone (38.5% Natrium; UFA AG, Herzogenbuchsee, Switzerland) and were fed 60 g concentrate (VitD₃: 5,000 I.U./kg DM; Ca: 9.5 g/kg DM; Haefliger AG, Herzogenbuchsee, Switzerland) a day per animal. In the barn, they had ad libitum access to straw

and on the pasture to fresh grass and were further excluded from other mineral or vitamin supplements. Last but not least, the animals were milked twice per day followed by measuring the respective yields.

Climate Data

On both research stations, the intensities of the UVB radiation were measured with a solar meter (Model 6.2 UVB; Solartech, Harrison Township, MI) every 2 wk during the experiment. Furthermore, we could access data from climate stations which were in use at both sites. The respective UVB radiation values were calculated for the entire study period by correlating the directly measured UVB radiation data from the solar meter with an additional sensor installed at the climate station (similar spectral range, CNR1 net radiometer; Kipp & Zonen, Delft, the Netherlands) measuring every 30 min. In addition to the radiation data, relative humidity and air temperature were also recorded in 30 min intervals at both sites.

Feed Analysis

Sward samples were taken twice from both research sites, at the beginning and at the end of the trial, respectively. The samples were dried for 48 h at 60°C. All grass samples and a hay sample from an average hay (second cut) fed at the home barn (for comparative purposes) were sent for analysis of vitD₂ by HPLC (DIN EN 12821: 2009) to the company Eurofins Scientifica AG (Schönenwerd, Switzerland). The feed samples were ashed at 550°C and then analyzed with an autoanalyzer (Cobas mira roche-Autoanalyzer; F. Hoffmann-La Roche Ltd., Basel, Switzerland) for Ca and P.

Measurement of the Daily Movement Patterns

Two randomly assigned sheep and goats from the alpine group were equipped with a global positioning system (GPS) receiver (G-Rays WBT-201; Wintec, New Taipei City, Taiwan) to determine the daily movement patterns of each group.

Collection and Analysis of Skin Samples

Skin biopsies were taken at wk 0, 7, and 12 from a shaved area on the neck of the animals under local anaesthesia (3ml lidocain 2%; G. Streuli & Co. AG, Uznach, Switzerland). The sampling was performed in the early morning before the animals were turned out on pasture. After collecting the biopsies of the skin, the wound was closed with staples. Animals received tetanus serum (MSD Animal Health GmbH, Luzern, Switzerland) and

the staples were taken out 10 d after the sampling. The skin samples were analyzed for 7-dehydrocholesterol (7-DHC) as described in Morris (1999).

Collection and Analysis of Milk Samples

Milk samples were obtained every second wk. The samples were taken in the morning (0530 h) and the evening (1700 h) milking and stored at -20°C until analysis.

Analysis of 25-hydroxyvitamin D in Milk. For the determination of 25-hydroxyvitamin D (25(OH)D), a commercial RIA was used (25-hydroxy vitamin D RIA, sheep-anti 25(OH)D antibody; Immunodiagnostic Systems GmbH, Frankfurt am Main, Germany). The intra- and interassay CVs were 5.0% to 6.1% and 7.3% to 8.2%. The sensitivity was < 3 nmol/L.

Collection of Blood Samples

Blood samples (10 mL) were collected at wk 0, 3, 7, 9, and 12. The samples were taken in the morning before the animals returned to pasture from the jugular vein using a Vacutainer (5 mL, without additives; Vacuette Greiner Bio-One, St. Gallen, Switzerland). The blood was centrifuged ($3,000 \times g$, 10 min, 4°C) within 30 min after collection and the serum was stored at -20°C until analysis.

Analysis of Serum Samples

Analysis of Ca and P. Ca and P were determined with commercial test kits (Ca: test kit DIA00460B, Cresolphthalein complexone; P: test kit DIA00620C, ammonium molybdate; diatools ag; Villmergen, Switzerland) by colorimetry with an autoanalyzer (Cobas mira Roche-autoanalyzer; F. Hoffman-La Roche Ltd., Basel, Switzerland). The intra- and interassay CVs were 0.62% to 1.45% and 1.66% to 2.7% for Ca, and 0.86% to 1.61% and 1.07% to 2.22% for P, respectively. The sensitivities were 0.05 mmol/L (Ca) and 0.065 mmol/L (P).

Analysis of 25(OH)D and 1,25-dihydroxyvitamin D in Serum. For the determination of 25(OH)D in serum, the same commercial RIA as for the determination of 25(OH)D in milk was used. For the determination of 1,25-dihydroxyvitamin D (1,25(OH)₂D), a commercial RIA was used (1,25-dihydroxy vitamin D RIA, sheep-anti-1,25(OH)₂D antibody; Immunodiagnostic Systems GmbH, Frankfurt am Main, Germany). The intra- and interassay CVs were 9.1% to 10.8% and 9.6% to 13.6%. The sensitivity was < 8 pmol/L.

Analysis of 25(OH)D₂ and 25(OH)D₃ in Serum. Selected serum samples from all four groups were analyzed for 25(OH)D₂ and 25(OH)D₃ by LC-MSMS (Labor Dr. Limbach und Kollegen, Heidelberg, Germany).

Analysis of Osteocalcin (OC). A commercial EIA test kit (Metra Osteocalcin; Quidel Corporation, San Diego, CA) with a murine monoclonal anti-OC antibody was used for the analysis of OC in serum. The intra- and interassay CVs were 4.8% to 10% and 4.8% to 9.8%. The sensitivity was 0.45 ng/mL.

Analysis of Carboxy-Terminal Cross-Linking Telo-peptide and Type I Collagen (CTX). A commercial ELISA (Serum CrossLaps ELISA; Immunodiagnostic Systems GmbH, Frankfurt am Main, Germany) was used for the determination of CTX. The intra- and interassay CVs were 1.7% to 3.0% and 2.5% to 10.9%. The sensitivity was 0.020 ng/mL.

Peripheral Quantitative Computed Tomography

At wk 0 and 12, the left metatarsus of each animal was measured with peripheral Quantitative Computed Tomography (pQCT, Stratec XCT 960A; Stratec Medizintechnik GmbH, Pforzheim, Germany). The measurements were done in the middle of the bone (diaphysis, 50% of length). Cortical area and total bone mineral density were calculated by the attenuation of the X-rays from the computer program. Bone strength index (BSI) was calculated as the product of cortical bone mineral density and cortical area (Siu et al., 2003).

Statistical Analysis

All data were expressed as means \pm standard errors (SE). To test the differences of the time-dependent patterns in the alpine and lowland groups, a multivariate analysis of variance for repeated measurements (MANOVA) was performed with group 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 nonparametric Mann-Whitney U-test. 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. The correlations between the factors age and 7-DHC concentration in the skin were calculated using SYSTAT.

RESULTS

Climate Data

The mean UVB dose was greater at the alpine station compared to the lowland station ($P < 0.05$) during the whole experiment (Table 1). In contrast, the relative humidity was lower at the alpine station than at the

Table 1. General climate variables (mean \pm SE) at the alpine and lowland station

Week	UVB dose, J cm ⁻² d ⁻¹		Relative humidity, %	
	Alpine	Lowland	Alpine	Lowland
1	12.2 \pm 0.9* ^{ac}	1.4 \pm 0.1* ^a	61.0 \pm 2.4* ^{ac}	70.8 \pm 2.9* ^{ab}
2	12.5 \pm 1.0* ^a	1.3 \pm 0.1* ^{ac}	58.8 \pm 5.5 ^{ad}	70.6 \pm 2.1 ^a
3	11.4 \pm 1.4* ^{ad}	1.3 \pm 0.1* ^{ad}	65.7 \pm 4.1	75.7 \pm 2.6 ^{abc}
4	8.0 \pm 2.4* ^a	1.0 \pm 0.2* ^a	70.4 \pm 6.7	80.1 \pm 5.5
5	7.2 \pm 2.0* ^a	0.9 \pm 0.2* ^a	77.3 \pm 4.8 ^{bc}	83.7 \pm 3.3 ^{bcd}
6	6.2 \pm 1.8* ^{bcd}	0.7 \pm 0.2* ^{bd}	82.9 \pm 3.2 ^b	87.2 \pm 2.7 ^d
7	5.3 \pm 1.5* ^b	0.8 \pm 0.2* ^{bc}	78.0 \pm 2.5 ^{bdc}	83.9 \pm 3.1 ^{de}
8	7.9 \pm 1.3* ^{bcd}	0.7 \pm 0.2* ^{bd}	74.1 \pm 5.0* ^{bc}	87.0 \pm 1.8* ^d
9	7.2 \pm 1.5* ^{bc}	0.7 \pm 0.2* ^b	73.4 \pm 5.2	80.8 \pm 2.7 ^{bcd}
10	8.0 \pm 0.8* ^b	0.9 \pm 0.1* ^{bd}	72.2 \pm 4.1 ^{be}	78.7 \pm 1.3 ^{ce}
11	5.3 \pm 1.4* ^b	0.7 \pm 0.1* ^{bd}	75.8 \pm 3.2* ^c	86.6 \pm 2.7* ^d

^{a-d}Within a column, means without a common superscript differ ($P < 0.05$).

*Indicates significant differences within groups ($P < 0.05$).

lowland ($P = 0.04$; Table 1). The mean air temperature was $10.5 \pm 1.0^\circ\text{C}$ and $18.0 \pm 0.9^\circ\text{C}$ at the alpine and lowland stations, respectively (data not shown).

Vitamin D₂ in Forage Samples

The vitD₂ content of both the alpine and lowland pasture grass was lower in the first compared to the second samples. The vitD₂ content was greater in the lowland pasture grass compared to the alpine pasture grass (Table 2). The Ca content was greater and the P content was lower in the alpine compared to the lowland forage (Table 2).

Movement Patterns

The average distance covered was 4.7 ± 0.2 km/day (SA) and 3.6 ± 0.2 km/day (GA). The mean altitude covered was 216 ± 14 m/day (SA) and 95 ± 9 m/day (GA).

Milk Yield

The average daily milk yield during the trial was 0.7 ± 0.06 kg (SA), 0.9 ± 0.06 kg (SL), 2.5 ± 0.16 kg (GA), and 1.9 ± 0.17 kg (GL). No differences within groups of sheep were detected. The group GA produced more milk than the group GL at wk 4 to 6 ($P = 0.021$) and wk 10 ($P = 0.043$; data not shown).

Vitamin D Metabolites in Serum and Milk

Mean serum 25(OH)D concentrations were higher in SA compared to SL at wk 0 ($P = 0.047$) and wk 12 ($P = 0.028$; Table 3). No differences between GA and GL in mean serum 25 vitD concentrations were detected.

In contrast, mean serum 1,25(OH)₂D concentrations were higher in SL compared to SA at wk 12

Table 2. Mineral concentration and vitamin D₂ content in forage samples

Item	Vitamin D ₂ content, $\mu\text{g}/\text{kg DM}$	Calcium, $\text{g}/\text{kg DM}$	Phosphorus, $\text{g}/\text{kg DM}$
Alpine pasture grass, early summer	20.6	13.06	1.18
Alpine pasture grass, late summer	32.0	12.35	1.13
Lowland pasture grass, early summer	52.4	5.87	3.28
Lowland pasture grass, late summer	73.6	5.23	3.66
Average lowland hay, second cut	62.7	4.3	3.1

($P = 0.009$; Table 3) and in GA compared to GL at wk 9 ($P = 0.016$; Table 3).

Between the 1,25(OH)₂D and P concentrations in serum, a negative correlation with coefficients of $R^2 = 0.29$ (SA), $R^2 = 0.17$ (SL), $R^2 = 0.49$ (GA), and $R^2 = 0.88$ (GL) was shown.

Serum 25(OH)D₂ concentrations of selected samples were higher in sheep compared to goats, and serum 25(OH)D₃ concentration of the same samples were higher in the alpine groups compared to the lowland groups (Table 3), where the GA group showed the highest concentrations.

The mean 25(OH)D concentrations in milk were 1.45 ± 0.2 pmol/mL (SA), 1.65 ± 0.2 pmol/mL (SL), 2.42 ± 0.3 pmol/mL (GA), and 1.78 ± 0.2 pmol/mL (GL). The mean concentrations were higher in SL compared to SA at wk 1 ($P = 0.009$), but no further differences between species groups or time points in one group were detected.

7-Dehydrocholesterol in Skin

No differences in mean 7-DHC concentrations in the skin were observed between the groups within species (Table 4). Between the 7-DHC concentration in the skin and the age of the animals, a negative correlation coefficient of $R^2 = 0.16$ in sheep and $R^2 = 0.71$ in goats was detected.

Calcium and Phosphorus Concentrations in Serum

Mean serum Ca concentrations were higher in SA compared to SL from wk 0 to 12 ($P < 0.036$) and in GA compared to GL from wk 3 to 12 ($P < 0.028$; Table 5). Within the alpine and lowland group, the serum Ca concentration was higher in sheep compared to goats. Mean serum P concentrations were lower in SA compared to SL and in GA compared to GL at wk 3, 7, 9, and 12 ($P < 0.047$; Table 5).

Table 3. Concentration (mean \pm SE) of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D and concentration of 25-hydroxyvitamin D₂ and 25-hydroxyvitamin D₃ in serum of selected samples

Item	Week				
	0	3	7	9	12
25-hydroxyvitamin D, nmol/L					
SA ¹	153.6 \pm 7.6 ^{*a}	141.0 \pm 9.9	141.1 \pm 12.3	134.6 \pm 6.5 ^b	143.9 \pm 8.6 ^{*a}
SL ²	125.6 \pm 6.5 [*]	130.7 \pm 6.4	136.0 \pm 9.3 ^a	129.0 \pm 6.5	114.9 \pm 6.2 ^{*b}
GA ³	122.3 \pm 6.8 ^{ad}	128.5 \pm 9.4	122.4 \pm 15.1 ^{ac}	140.6 \pm 16.3 ^{bd}	149.7 \pm 11.8 ^{bc}
GL ⁴	136.6 \pm 10.8	144.8 \pm 14.8	147.9 \pm 12.8	146.0 \pm 15.5	162.2 \pm 23.0
1,25-dihydroxyvitamin D, pmol/L					
SA ¹	43.4 \pm 5.5 ^a	63.0 \pm 10.6 ^{bd}	70.7 \pm 5.2 ^b	79.9 \pm 5.0 ^{cd}	60.9 \pm 5.5 ^{**b}
SL ²	45.8 \pm 4.1 ^a	74.4 \pm 10.7 ^{ac}	81.1 \pm 10.0 ^{bc}	84.6 \pm 11.4 ^{bc}	109.1 \pm 9.9 ^{**b}
GA ³	124.8 \pm 17.2 ^{ac}	116.4 \pm 20.1 ^a	197.6 \pm 22.1 ^{bc}	268.1 \pm 8.1 ^{*b}	250.7 \pm 29.7 ^{bc}
GL ⁴	106.6 \pm 7.3 ^a	164.4 \pm 24.4	185.9 \pm 18.6 ^b	201.5 \pm 14.4 ^{*b}	206.6 \pm 22.3 ^b
25-hydroxyvitamin D ₂ , nmol/L					
SA ¹	55.7	–	27.5	–	36.7
SL ²	44.2	–	28.7	–	26.0
GA ³	45.6	–	21.1	–	21.7
GL ⁴	41.4	–	19.2	–	17.3
25-hydroxyvitamin D ₃ , nmol/L					
SA ¹	140.0	–	192.7	–	157.5
SL ²	92.4	–	129.3	–	104.8
GA ³	107.1	–	202.3	–	215.5
GL ⁴	99.8	–	141.9	–	173.5

^{a-d}Within a row, means without a common superscript differ ($P < 0.05$).

¹SA = sheep alpine.

²SL = sheep lowland.

³GA = goats alpine.

⁴GL = goats lowland.

*Indicates significant differences within species groups ($P < 0.05$).

**Indicates significant differences within species groups ($P < 0.01$).

Bone Strength Index

No differences in BSI were observed between the species groups except for a greater BSI in GL compared to GA at wk 12 ($P = 0.047$; Table 6).

Bone Markers in Serum

No significant differences were observed between species groups in mean serum OC and CTX concentrations except for higher CTX concentrations in GA compared to GL at wk 7 ($P = 0.009$; data not shown). The bone remodeling rate (ratio OC/CTX) did not show differences between groups within one species (Table 6).

DISCUSSION

The UVB radiation was higher at the alpine site as previously shown by various studies. Through this higher UVB radiation at high altitude, the cutaneous vitD₃ production in the alpine groups and the vitD₂ content of the alpine forage was expected to be larger and to result in a better vitD status and higher vitD content of the milk.

Most of the literature on vitD₂ content in grass and hay is at least 50 yr old (Newlander and Riddell, 1952; Keener, 1954; Henry et al., 1958; Wallis et al., 1958). All studies used biological assays, which are based on the curative effect of the test material when fed to vitamin D-deficient rats (Parrish and Richter, 1979). However, this method is known to be imprecise and unable to distinguish between different vitD compounds (Jäpelt et al., 2011). To the authors' knowledge, only three recently published papers exist that used an HPLC method for vitD₂ determination (Horst et al., 1984; Magalhães et al., 2007; Jäpelt et al., 2011). The vitD₂ contents found in these studies range from 10.5 μ g to 1950 μ g vitD₂/kg DM. In the study of Jäpelt et al. (2011), the vitD₂ content changed by more than a factor of ten during the season. Jäpelt et al. (2011) also found an effect of forage maturity on the vitD₂ content, and further described an effect of humidity, precipitation, and UVB radiation on the vitD₂ content of forage. Humidity and precipitation contribute to the development of mold and therefore ergosterol formation. Ergosterol is a cell membrane component specific to fungi (Jäpelt et al., 2011). UVB radiation is required for the conversion of ergosterol to vitD₂. The

Table 4. Concentration (mean \pm SE) of 7-dehydrocholesterol in the skin

Item	Week		
	0	7	12
7-dehydrocholesterol, $\mu\text{mol/g}$			
SA ¹	13.8 \pm 2.9 ^a	7.0 \pm 2.7 ^b	8.1 \pm 2.2 ^b
SL ²	18.6 \pm 3.3 ^a	12.6 \pm 2.6 ^b	9.8 \pm 2.8 ^c
GA ³	170.6 \pm 24.5	140.8 \pm 20.6	106.8 \pm 21.8
GL ⁴	190.1 \pm 60.3 ^a	117.5 \pm 47.9 ^b	122.0 \pm 32.8 ^b

^{a-c}Within a row, means without a common superscript differ ($P < 0.05$).

¹SA = sheep alpine.

²SL = sheep lowland.

³GA = goats alpine.

⁴GL = goats lowland.

higher vitD₂ content of the samples collected at the end of the trial in the present study corresponds well to previously found results (Keener, 1954), which describe a lower vitD₂ content in first cutting forages compared to subsequent cuttings as in the presented study. In contrast to our expectations and hypothesis, the vitD₂ content was higher in the lowland forage. These differences in vitD₂ content of the alpine and lowland station may be explained by the phenomena described above, namely, because of the higher values in relative humidity at the lowland site. Jäpelt et al. (2011) stated that the vitD₂ content was maximum 2‰ of the ergosterol content, which might indicate that not UVB, but rather ergosterol concentration is the limiting factor in vitD₂ production in plants. This thesis would also correspond to our results, as we see that plants do not benefit from higher UVB radiation in terms of vitD₂ content. Another aspect is the higher plant diversity at the alpine station (Leiber et al., 2005). The animals on the alpine station had many more

plant species available in contrast to the animals grazing in the valley. In the present study, with data acquired from the GPS receiver, movement tracks were generated using a GPS visualizer. The tracks in combination with vegetation maps (Keller, 2006; Schmid, 2007) of the “Alp Weissenstein” were used to determine the most frequented habitats and the preferred vegetation. The group SA grazed on habitats dominated by *Poaceae* (*Sesleria caerulea*, *Festuca violacea*), *Cyperaceae* (*Carex sempervirens*), *Ericaceae* (*Erica carnea*), *Fabaceae* (*Trifolium thalii*), *Rosaceae* (*Potentilla caulescens*), *Asteraceae* (*Hieracium humile*, *Petasites paradoxus*), *Primulaceae* (*Androsace helvetica*), *Brassicaceae* (*Draba tomentosa*), *Caprifoliaceae* (*Valeriana montana*), *Caryophyllaceae* (*Moehringia ciliate*), and *Saxifragaceae* (*Saxifrage aphylla*). The group GA stayed in plant communities dominated by *Poaceae* (*Poa alpine*), *Apiaceae* (*Ligusticum mutellina*), *Asteraceae* (*Leontodon hispidus*, *Crepis aurea*), *Fabaceae* (*Trifolium* spp.), *Cupressaceae* (*Juniperus communis*), *Ericaceae* (*Arctostaphylos uva-ursi*, *Calluna vulgaris*, *Vaccinium* spp.; Keller, 2006; Schmid, 2007). The vegetation available to the SL and GL was *Poaceae* (*Lolium* spp.) and *Fabaceae* (*Trifolium repens* and *Trifolium pratense*). However, as the animals' feed intake of grass was not monitored, it is not known if they ate from all available plants or which plants they favored, as different plant species have different vitD₂ contents. The plants analyzed in the studies mentioned above were *Fabaceae* (*Medicago sativa*; Horst et al., 1984), *Cannabaceae* (*Humulus lupulus*; Magalhães et al., 2007), and *Poaceae* (*Lolium perenne*; Jäpelt et al., 2011). The vitD₂ content found in the studies of Horst et al. (1984) and Jäpelt et al. (2011) correspond to our results. The high vitD₂ contents in the forage analyzed by Magalhães et al.

Table 5. Concentration (mean \pm SE) of calcium and phosphorus in serum

Item	Week				
	0	3	7	9	12
Calcium, mmol/L					
SA ¹	2.68 \pm 0.04 ^{*a}	2.56 \pm 0.04 ^{**b}	2.57 \pm 0.05 [*]	2.64 \pm 0.06 ^{**}	2.66 \pm 0.04 [*]
SL ²	2.44 \pm 0.07 ^{*a}	2.22 \pm 0.04 ^{**b}	2.18 \pm 0.12 ^{*b}	2.24 \pm 0.04 ^{**b}	2.24 \pm 0.10 ^{*b}
GA ³	2.21 \pm 0.04 ^a	2.35 \pm 0.04 ^{*ac}	2.38 \pm 0.06 [*]	2.45 \pm 0.03 ^{**bc}	2.55 \pm 0.06 ^{*b}
GL ⁴	2.01 \pm 0.12	1.99 \pm 0.09 [*]	2.05 \pm 0.11 [*]	2.05 \pm 0.06 ^{**}	2.10 \pm 0.12 [*]
Phosphorus, mmol/L					
SA ¹	1.37 \pm 0.09 ^a	1.13 \pm 0.08 ^{*ab}	0.90 \pm 0.12 ^{*b}	0.99 \pm 0.08 ^{**bc}	0.67 \pm 0.04 ^{**c}
SL ²	1.61 \pm 0.08	1.49 \pm 0.10 ^{*a}	1.32 \pm 0.13 ^{*a}	1.72 \pm 0.05 ^{**b}	1.38 \pm 0.10 ^{**a}
GA ³	1.91 \pm 0.12 ^a	0.76 \pm 0.12 ^{**bc}	0.60 \pm 0.08 ^{**b}	0.58 \pm 0.12 ^{**bc}	0.46 \pm 0.09 ^{*c}
GL ⁴	2.60 \pm 0.35 ^a	1.81 \pm 0.08 ^{**b}	1.61 \pm 0.20 ^{**b}	1.76 \pm 0.17 ^{**}	1.27 \pm 0.23 ^{*b}

^{a-c}Within a row, means without a common superscript differ ($P < 0.05$).

¹SA = sheep alpine.

²SL = sheep lowland.

³GA = goats alpine.

⁴GL = goats lowland.

*Indicates significant differences within species groups ($P < 0.05$).

**Indicates significant differences within species groups ($P < 0.01$).

(2007) may be the result of the different plant species analyzed and difference in UVB exposure because the forage was dried in the summer sun in Portugal.

All animals were fed hay before the experiment. This hay contained more vitD₂ than the first samples of the alpine and lowland pasture grass. Therefore, the vitD₂ intake with forage decreased at the onset of the experiment, also 25(OH)D₂ concentration in serum decreased in all four groups. However, even though the alpine and lowland grass samples differed greatly in vitD₂ concentrations, no corresponding differences between the alpine and lowland groups were detectable in 25(OH)D₂ serum concentrations. Because the grass was the only source of vitD₂, it is possible that the animals from the alpine groups selected the forage containing the most vitD₂. It was also observed that 25(OH)D₂ only represents a small proportion of the total 25(OH)D. According to Horst et al. (1981), vitD₂ is the major vitD form in sheep. The present study shows that exactly the opposite is the case, i.e., 25(OH)D₃ is the major form in sheep and in goats. However, as the 25(OH)D₂ concentrations are higher in sheep compared to goats, vitD intake with feedstuff seems to be more important for sheep than for goats.

The 25(OH)D₃ concentrations in serum represent the intake of vitD₃ with concentrate and the cutaneous vitD₃ production. The amount of vitD₃ intake with concentrate was 300 IU/d, which only represents a small part (6–16%) of the total vitD intake with feedstuff. As the alpine groups were exposed to a higher amount of UVB radiation than the lowland groups, they were expected to reach higher 25(OH)D₃ concentrations in serum (Armas et al., 2007). As expected, the 25(OH)D₃ concentrations were higher in the alpine groups compared to the lowland groups. But, as the difference in SA and SL was in the same order of magnitude before and during the experiment, it is unlikely to be related to the UV radiation. In goats, there seems to be a clear positive effect of high-altitude grazing on 25(OH)D₃ concentration in serum, as the 25(OH)D₃ concentrations were the same in GA and GL at the beginning of the experiment and were clearly higher in GA than GL during the experiment. However, in all four groups, the concentrations of 25(OH)D₃ increased at the onset of the experiment. This indicates that even the lower UVB radiation at the lowland station was sufficient to increase cutaneous vitD₃ synthesis, but not to the same extent as in GA. But only goats benefitted from the higher UVB radiation at the alpine station, since they showed higher 25(OH)D₃ concentrations. It is also noteworthy that in both sheep groups the 25(OH)D₃ concentration decreased in the second part of the experiment. The sheep were shorn before the experiment and the growing wool might have prevented the UVB radiation from penetrating through the skin. From the lower 25(OH)D₂ and the higher 25(OH)D₃ concentrations in serum of goats compared

Table 6. Bone remodeling rate and bone strength index (mean ± SE)

Item	Week				
	0	3	7	9	12
Bone remodeling rate					
SA ¹	48.5 ± 12.8 ^a	26.8 ± 6.7 ^b	15.7 ± 2.9 ^{bc}	14.4 ± 4.4 ^c	13.0 ± 2.7 ^{bc}
SL ²	32.6 ± 6.7 ^a	12.8 ± 2.2	17.3 ± 3.6 ^{bc}	20.3 ± 3.0 ^{ac}	14.5 ± 2.3 ^b
GA ³	55.5 ± 11.0 ^a	17.0 ± 4.2 ^{bc}	17.3 ± 1.5 ^{bc}	14.4 ± 3.1 ^c	18.2 ± 2.3 ^b
GL ⁴	83.3 ± 25.3 ^a	24.9 ± 4.0 ^b	34.9 ± 8.6 ^b	27.9 ± 5.8 ^b	24.6 ± 7.6
Bone strength index					
SA ¹	179 ± 6.5 ^a	–	–	–	205 ± 3.3 ^b
SL ²	179 ± 8.9 ^a	–	–	–	205 ± 10.5 ^b
GA ³	152 ± 7.0	–	–	–	145 ± 6.1*
GL ⁴	164 ± 3.5	–	–	–	165 ± 3.7*

^{a-c}Within a row, means without a common superscript differ ($P < 0.05$).

¹SA = sheep alpine.

²SL = sheep lowland.

³GA = goats alpine.

⁴GL = goats lowland.

*Indicates significant differences within species groups ($P < 0.05$).

to sheep, we assume that goats depend more on cutaneous vitD₃ production than sheep.

The total 25(OH)D concentrations measured revealed only numerical, but no significant differences, whereas the addition of the 25(OH)D₂ and 25(OH)D₃ concentrations, measured in selected samples, clearly showed a difference between GA and GL and SA and SL, respectively. This data has to be interpreted with caution since not all samples were measured. However, this information gives an evidence toward increased vitD production in the skin on the high-altitude groups.

The animals in our study were assigned to groups according to age. Age has, at least in humans, a negative impact on the capacity of skin to produce vitD₃ due to a reduced skin thickness and therefore decreased 7-DHC concentrations with advancing age (MacLaughlin and Holick, 1985; Need et al., 1993). However, in our study we could only detect a weak negative correlation between age and 7-DHC concentrations in the skin of sheep but also a stronger and positive correlation in goats, which is in contrast to aforementioned studies conducted in humans (MacLaughlin and Holick, 1985; Need et al., 1993). Thus, additional research on the results found in previous studies but also in this trial is needed.

While the concentrations of 25(OH)D in serum are comparable in sheep and goats, the 7-DHC concentrations in the skin of the sheep are strikingly low compared to the concentrations found in goats. Morris (1999) also describes a low 7-DHC concentration in the skin of sheep, although not as low as in cats, which are completely dependent on vitD in their diet. Due to this fact, sheep most likely also depend more on vitD in their diet than goats due to low 7-DHC in the skin. This thesis

is also supported by the 25(OH)D₂ concentrations in serum, as previously discussed.

The hypothesis also suggests an increase in vitD metabolites in milk with higher altitudes. The 25(OH)D concentration in milk is correlated to the corresponding concentration in plasma (Hollis et al., 1981; McDermott et al., 1985) and rises with increasing exposure to UVB radiation (Weckel, 1941; Greer et al., 1984) and with increasing amount of dietary vitD (McDermott et al., 1985; Hollis and Wagner, 2004). Therefore, we expected an increase in 25(OH)D concentration in milk in the high-altitude groups. As 25(OH)D in serum was not different between the alpine and lowland groups, the lack of differences in 25(OH)D concentrations in milk was not surprising.

The alpine sward shows a very high Ca:P ratio of 11, in contrast to the low ratio in the lowland sward (1.4 to 1.8). These differences in Ca and P contents were also described in Leiber et al. (2009), Liesegang et al. (2013), and Willems et al. (2013). Reasons for these differences in mineral composition are a result of different factors playing together. As mentioned above, the plant diversity is higher at the alpine station. The lowland pastures contain mostly grass species, whereas the alpine station contained grass, legumes, and herbs in a balance (Leiber et al., 2005). In legumes, Ca content is higher and P content lower (Suttle 2010). Another factor leading to the high Ca content of the alpine forage is the limestone-based soil (Berry et al., 2002). The low fertilization levels at the alpine site explain the low P content (Soder and Stout 2003). In view of these differences, the diverging serum concentrations of Ca and P in the lowland and alpine groups are not surprising. In a study also conducted in Switzerland by Tschuor et al. (2008), the reference range for serum Ca are 2.3 to 2.8 mmol/L in sheep and 2.2 to 2.7 mmol/L in goats. Lactating meat sheep and dairy goats were used to generate the reference ranges. It is well known that serum Ca concentrations depend on the physiological stage (lactating versus dry) and breed (milk versus meat) of animals. Peterson and Waldern (1981) described a higher serum Ca concentration in dry compared to lactating cows and Smardzija et al. (2011) also detected a higher serum Ca concentration in meat compared to dairy goats. Therefore, the above mentioned reference ranges must be interpreted with caution. According to these reference ranges, the group GL was hypocalcaemic during the whole trial. However, no clinical signs of hypocalcaemia like ataxia, dullness, and tremors (Yamagishi et al., 1999) were evident in this group. If Ca concentration is low in blood, parathormone secretion is enhanced and consequently, 1,25(OH)₂D production in the kidney is increased (Holick 2004). Increases in 1,25(OH)₂D concentrations were detectable in all four groups, independent of the serum Ca concentra-

tions. Therefore we conclude that the group GL was low in calcium, but was not hypocalcaemic. Even though an increase in 1,25(OH)₂D in all four groups was detected, the group GA showed the most prominent increase with a very high peak value. The active vitD metabolite 1,25(OH)₂D results from hydroxylation of 25(OH)D and only increases when enough 25(OH)D is available. Therefore, it is possible that 25(OH)D serum concentration was originally higher in GA, but through the transformation into 1,25(OH)₂D, it stayed on the lower level.

Besides Ca, low P also stimulates the conversion of 25(OH)D to 1,25(OH)₂D. In all animals, negative correlations between 1,25(OH)₂D and P were detected. However, the correlation was weaker in sheep and stronger in goats.

The hypothesis also suggests an increase in bone strength due to more intense exercise from the hilly alpine landscape. As described in Haapasalo et al. (2000) and Firth et al. (2011), mechanical strains occurring during activity lead to an increase in BSI. An increase in stress-bearing characteristics of bone, such as bone mineral content and bone mineral density, were also described by Haapasalo et al. (2000) and Umemura et al. (2002). However, BSI serves as a better indicator of bone strength according to Siu et al. (2003). As shown in the collected GPS data, SA walked on average longer distances and covered larger altitudinal differences than GA. The reason for the differences in activity is most likely due to the different diet preferences between sheep and goats. As young grass preferred by sheep grew at higher altitudes, they had to walk longer distances and cover larger altitudinal differences than goats. The shrubs favored by goats grew closer to the barn at lower altitudes; therefore, they did not have to cover long distances (Kaulfers 2009). Besides the differences between species, both groups increased their physical activity during the trial. No movement patterns, and therefore extent of exercise, were monitored in SL and GL, but as the lowland pasture was clearly smaller than the alpine pasture area and the landscape is completely flat, the lowland groups exhibited lower walking activity than the alpine groups. As described in Bass et al. (2005), exercise only has the potential to increase bone strength when the nutritional conditions are adequate, which was not the case in the present study. According to the Ca and P concentrations in serum and forage, the alpine sward was inadequate in P. The low P supply at the alpine station prevented the bone to respond properly on the more intense exercise with an increase in bone strength. In SA and SL, a slight increase in BSI is detectable independent of the extent of exercise. We assume that the changes in BSI were not caused by the exercise, but rather by regeneration of bone after lactation-induced bone loss. As described in Liesegang et al. (2007), lactating milk sheep lose skeletal tissue during late

pregnancy and early lactation. The lost minerals are restored in the bone again during late lactation. The higher BSI of the GL compared to the GA at the end of the trial is most likely due to the slightly higher milk yield in the group GA. Cows kept at a high altitude exhibit an energy deficit which leads to a reduction in milk yield (Leiber et al., 2005). In our study, exactly the opposite was the case: the milk yield in GA was higher compared to GL. Goats are particularly sensitive to high air temperatures; feed intake decreases and leads to an associated decrease in milk yield (Di Grigoli et al., 2009). As the air temperature was higher at the lowland station, this might be a possible explanation for the differences in milk yield in goats. However, it is remarkable that despite the very low P in the alpine groups, they were able to maintain bone strength at the end of the experiment. As the P decreased continuously in the alpine groups and the lowest values had been reached toward the end of the experiment, it is possible that a decrease in bone strength would have happened in the following weeks. Another explanation may be that through the high $1,25(\text{OH})\text{D}_2$ concentrations, the absorption was high enough to keep bones healthy, although the serum content was low.

The bone formation and resorption markers OC and CTX were evaluated for sheep and goats (Liesegang et al., 2003) and have been used in these species in previous studies (Liesegang and Risteli, 2005; Liesegang et al., 2006, 2007). Bone remodeling rate is defined as the ratio of OC (bone formation) and CTX (bone resorption). If bone resorption outweighs bone formation, the bone remodeling rate decreases, as was initially the case in the present study. This initial modification in bone remodeling rate was only visible in bone markers in serum and not in pQCT because the markers in serum respond quickly to changes in bone metabolism (Allen, 2003), whereas the pQCT measurements provide a long-term overview (Christenson, 1997). This change in the alpine groups might have been caused by a low-P diet. Scott et al. (1994) found a low OC in growing lambs fed low-P diet. The low-P diet also led to a fall in plasma P concentrations. In the present study, P concentrations in serum correlated with OC concentration in serum in SA and GA ($R^2 \geq 0.85$). However, the lowland groups also exhibited an initial change in bone remodeling, even though the diet was not low in P. Another explanation for this variation might be the change from being housed indoors to be turned out to pasture, as described in Hüttenmoser (2007).

Contrary to our expectations, lactating dairy sheep and milk goats do not profit from a stay at high-altitude alpine pastures in Switzerland in terms of vitD status or vitD concentration in milk or bone strength. Our hypothesis therefore has to be rejected. However, other interesting facts have been discovered in this study. We were able to demonstrate that in sheep and in goats the

main vitD metabolite is $25(\text{OH})\text{D}_3$ and not $25(\text{OH})\text{D}_2$ as stated in previous studies. However, pronounced differences between species, i.e., sheep and goats, were illustrated. Sheep, who have very low 7-DHC concentration in their skin and higher $25(\text{OH})\text{D}_2$ concentrations in serum, seem to depend more on vitD intake with feedstuff. Goats on the other hand rely more on cutaneous vitD production. We also illustrated that the limiting factor for vitD₂ production in plants is ergosterol and not UVB radiation, and we confirmed that nutrition affects bone metabolism more than exercise.

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