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ORIGINAL ARTICLE

Influence of high-altitude grazing on bone metabolism of growing sheep

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Summary

The objective of this study was to identify the effect of high alpine grazing, associated with varying pasture grass qualities and more pronounced exercise on typically steep slopes, on bone metabolism by improving bone density and enhancing bone turnover in growing sheep. Twenty-four 5-month-old sheep were randomly assigned to two groups. One group was kept at high altitude (HA; 2000–2200 m a.s.l.) for 3 months, and the other group (C; control) remained in the lowlands (400 m a.s.l.). Both groups were kept in grazing pastures with access to good-quality swards. Before the start of the experiment, blood samples were taken, the sheep were weighed, and the left metatarsus of each animal was analysed by quantitative computer tomography. After 1 month, blood samples were taken and body weight was measured, followed by biweekly sampling. Finally, the animals were slaughtered, and the bones were collected for analysis of various bone parameters. Body weight development did not differ between the groups. Concentrations of 25-OH-Vitamin D, carboxy-terminal telopeptide of type I collagen and activities of bone-specific alkaline phosphatase were always higher in the HA group than in the C group, except on the last two sampling dates. Bone mineral content and density increased in both groups during the experiment, but more intensively in the HA group. In addition, the cortical thickness of the HA group increased. The present study demonstrates an increase in bone turnover and mineral content of the bones of the growing sheep grazing in high alpine pastures. The factors associated with HA grazing, therefore, clearly seem to improve bone composition.

Introduction

During the summer, it is common to graze ruminant species on mountainous pastures, where a number of factors influence nutritional status and metabolism (Christen et al., 1996; Leiber et al., 2004, 2006). The steep slopes and the low feed density require and promote considerable movement activity (Liesegang et al., 2010), and bone changes occur in the skeletal structure and metabolism during intensive straining movement (Bass et al., 2005). Bone tissue adapts to higher levels of physical activity (Carter, 1987; Beaupre et al., 1990; Turner and Pavalko, 1998), and about 15% of bone mass is turned over each year (Swaminathan, 2001). The physiological control of Ca metabolism and skeletal growth and remodelling is mainly regulated by the active form of vitamin D (VitD), 1,25-dihydroxyvitamin D (1,25VitD). Vitamin D may be either present in or added to feed, or it has to be

formed from 7-dehydrocholesterole in the epidermis under the influence of ultraviolet radiation (Holick et al., 1980; Bikle et al., 1993). A linear (but solar elevation dependent) increase with altitude of the global and direct components in both the ultraviolet radiation type A (UV-A) and ultraviolet radiation type B (UV-B) ranges has been observed for altitudes up to 5500 m above mean sea level (Piazena, 1996). 1,25VitD controls the absorption of Ca in the small intestines, as well as its reabsorption in the kidneys (Nemere and Norman, 1988). Another target tissue of 1,25VitD is bone, where it enhances the formation of osteoclasts and decreases collagen synthesis in the osteoblasts. Bone metabolism in ruminants can be monitored by measuring bone markers released during bone formation and resorption (Liesegang et al., 2000). In addition, peripheral quantitative computer tomography (pQCT) can be used for the determination of bone mineral density (BMD) and content (BMC) (Gasser, 1998; Liesegang and Risteli, 2005).

In the present study, we tested the hypothesis that high-altitude (HA) grazing of ruminants enhances bone formation and turnover in response to a presumed higher calcium supply by the pasture grass, an increased strain and movement as well as the more intense UV-B radiation. This could have implications for the utility of this farming practice, because these ruminants would develop a denser skeleton compared with their companions staying in the lowlands, which would have an impact on bone health and strength and therefore maybe also better daily gain. In addition, this study could have model character for other species as well as humans.

Materials and methods

Animals and measurements

Twenty-four Jura sheep (six males and 18 females), which were 4.4 ± 0.6 months old and weighed 28.5 ± 1.5 kg, were randomly assigned to two groups after balancing for gender (three males and nine females in each group): the HA group (mean 29.9 kg) and the lowland control group (C, mean 27.0 kg). The HA group was kept for 3 months at the ETH research station Alp Weissenstein on the northern alpine slope in the district of Grisons, Switzerland, at 2000–2200 m a.s.l. (average inclination of the pastures >20%). The animals grazed a natural pasture with *Crepido aureae–Festucetum rubrae* and *Deschampsio cespitosae–Poetum alpinae* as the main vegetation types, and they were observed daily for distance and steepness of movement. Concurrently, Group C remained in the lowlands at the ETH research station Chamau in the district of Zug, Switzerland, at 400 m a.s.l., where they grazed on flat pasture areas artificially sown with *Trifolium repens* and *Lolium multiflorum* as the dominating species. All the sheep were permanently kept outside in their groups, grazing one large pasture area; they were rotated among pastures from time to time. The animals had free access to NaCl in the form of a licking bowl in the pastures, but they received no other mineral or vitamin supplement. Before the start of the experiment, the animals were kept in a barn in the lowlands without access to pasture, and they were dewormed.

Before transport to the alpine pastures or turnout to the lowland pasture, blood and faeces samples were taken from all the sheep. Blood was collected from the jugular vein (Vacutainer®; 10 ml, without additives; Aichele Medico AG, Basel, Switzerland). Blood was centrifuged (1580 *g* for 10 min, 4 °C) within 30 min after sampling, and the resulting serum was stored at −20 °C. In addition, the sheep were weighed. Faeces were taken directly from the anus in plastic mugs with a lid. After 1 month of adaptation to the pasture, blood and faeces sampling and body weight measurements were performed every 2 weeks during the period the animals were on pasture. The last samples were taken as soon as all the sheep were back in the lowland barn. In addition, sward samples were taken at the time of each blood sampling at the corresponding pasture site. At the end of the experiment, the sheep were slaughtered and the left metatarsus was collected.

The left metatarsus of each animal was subjected to measurement of BMC and BMD using pQCT (XCT 960; Stratec, Pforzheim, Germany) once before the start of the experiment (*in vivo*) and once at the end of the experiment (after slaughtering). This included the measurement of the length of the metatarsus. With this information, records were obtained from the middle of the diaphysis (50% of length), as well as distal in the metaphysis (10% of length). The following parameters were calculated by automated computation: total BMC and total and cortical BMD, as well as cortical thickness (cortical mode 2; threshold for cortical bone >640 mg/cm^3) (Liesegang and Risteli, 2005). Total BMD was used as an indicator of bone stability.

The experiment was approved by the respective authority for animal welfare under the approval number ZG 28/03.

Laboratory analyses

Samples of grass, faeces and bones (left lateral phalanx proximalis) were analysed (in duplicate) for Ca and P contents after drying at 105 °C for 48 h and ashing in a muffle furnace at 600 °C for 96 h. The ash was dissolved in 80 g/g HCl solution. These solutions, as well as blood serum samples, were subjected to colorimetry with an autoanalyzer (COBAS MIRA® Roche-autoanalyzer; F. Hoffmann-La Roche, Basel, Switzerland), using commercial kits (Nos. A11A00112 and A11A00098, respectively—methylthymol blue method for Ca, phosphomolybdate without precipitation of proteins for P; ABX Diagnostics, Villmergen, Switzerland).

For the determination of cross-linked–carboxy-terminal telopeptide of type I collagen (ICTP), an immunoassay was used as previously described by Liesegang et al. (1998). The activity of bone-specific alkaline phosphatase (bAP) was determined using an immunoassay utilizing a monoclonal anti-bAP antibody and was detected using a para-nitrophenylphosphate substrate (Metra® BAP; Quidel, San Diego, CA, USA). Although this test was developed to measure bAP in humans, it has also been validated for use in sheep and goats (Liesegang and Risteli, 2005). The intra- and inter-assay coefficients of variation were 5.8% (n = 12) and 5.2% (n = 10) respectively. The remodelling rate was calculated using the ratio of bAP to ICTP.

Serum concentrations of 1,25VitD and 25VitD were measured with commercially available radioimmunoassays (IDS, Fountain Hills, AZ, USA). The intra- and inter-assay coefficients of variation were 11.3% (n = 20) and 11.4% (n = 20) respectively.

Statistical analysis

The results are presented as means ± SE. A multivariate analysis of variance for repeated measurements (MANOVA) was performed with group as a cofactor included in the model to test differences of the time-dependent patterns in the high- and the low-altitude groups. To avoid false conclusions owing to violation of the assumption of compound symmetry, a Huynh–Feldt correction was performed. The statistical differences between the sampling days within each group were analysed with a Wilcoxon signed-rank test for paired samples. Furthermore, the difference between groups was tested with the Mann–Whitney U-test (non-parametric) to limit the influence of extreme values. The level of significance was set at $p < 0.05$ for all tests. All statistical analyses were performed by using SYSTAT® for Windows® (Version 7.0; SPSS, Chicago, IL, USA).

Results

The analysed average Ca concentrations over the whole time-period in the lowland and highland sward samples were 3.2 ± 0.4 (n = 6) and 4.4 ± 0.7 g/kg dry matter (DM) (n = 5), respectively; the corresponding P concentrations were 2.4 ± 0.2 and 1.3 ± 0.2 g/kg DM respectively. Over time, the content of Ca and P in the sward samples changed (Table 1). Following the different mineral contents of the sward, the mineral contents in the faeces varied significantly between the groups and over time

Table 1 Means of calcium and phosphorus content in sward samples (mean of double determination) and faeces samples (in faeces with SE)

Treatment	Control	High altitude	p-value group	p-value time*
Swards (*g/kg DM*)				
Calcium content				
Week 0	4.1	4.1		
Week 4	2.3	5.9		
Week 6	4.6	5.3		
Week 8	1.8	2.9		
Week 10	3.2	†		
Week 12	2.7	3.3		
Phosphorus content				
Week 0	1.5	1.5		
Week 4	2.4	2.0		
Week 6	2.6	1.1		
Week 8	2.9	1.0		
Week 10	2.0	†		
Week 12	2.7	1.1		
Faeces (*g/kg DM*)				
Calcium content				
Week 0	15.5 ± 0.9	15.5 ± 0.7	0.485	
Week 4	15.0 ± 0.8	39.7 ± 0.2	0.001	0.003 (HA)
Week 6	26.5 ± 0.7	38.2 ± 0.3	0.027	0.002 (LA)
Week 8	31.9 ± 0.9	40.2 ± 0.1	0.017	0.006 (HA)
Week 10	21.9 ± 0.7	29.4 ± 0.8	0.001	0.015
Week 12	34.2 ± 0.1	33.6 ± 0.1	0.433	0.002
Phosphorus content				
Week 0	9.4 ± 0.3	9.7 ± 0.3	0.207	
Week 4	9.3 ± 0.1	4.2 ± 0.3	0.0001	0.002
Week 6	7.9 ± 0.2	4.0 ± 0.5	0.0001	0.002
Week 8	6.3 ± 0.6	2.7 ± 0.1	0.0001	0.003
Week 10	8.9 ± 0.1	5.0 ± 0.6	0.007	0.0026
Week 12	8.0 ± 0.3	4.3 ± 0.2	0.0001	0.018

DM, dry matter.
*The differences in time-points always show the difference of the week before to the week after; e.g., p = 0.003 in Ca content means the difference between week 0 (before experiment) and week 4.
†No sample available.

(Table 1). Concentrations of Ca and P in the faeces as mean of all samples over time in DM were 25 ± 0.9 g Ca/kg DM and 8 ± 1 g P/kg DM in group C, and 31 ± 1 g Ca/kg DM and 5 ± 0.1 g P/kg DM in group HA respectively. The sheep gained weight from 28.5 ± 1.5 kg at the beginning of the experiment to 49.5 ± 1.5 kg at the end of experiment (mean of all animals in both groups). No significant differences in average daily gains (231 ± 7 vs. 219 ± 7 g/day for control and HA sheep respectively) occurred between the groups. In both groups, the mean serum Ca and P concentrations remained in the reference range during the whole experimental period of 3 months. Significant differences between the groups were only observed for mean serum P concentrations at week 4 ($p = 0.001$; higher in HA compared with C), week 8 ($p = 0.007$; lower in HA compared with C) and week 12 ($p = 0.001$; higher in HA compared with C), although the concentrations were still in the normal range for sheep (reference concentrations: 1.8–2.8 mM; Fig. 1a,b).

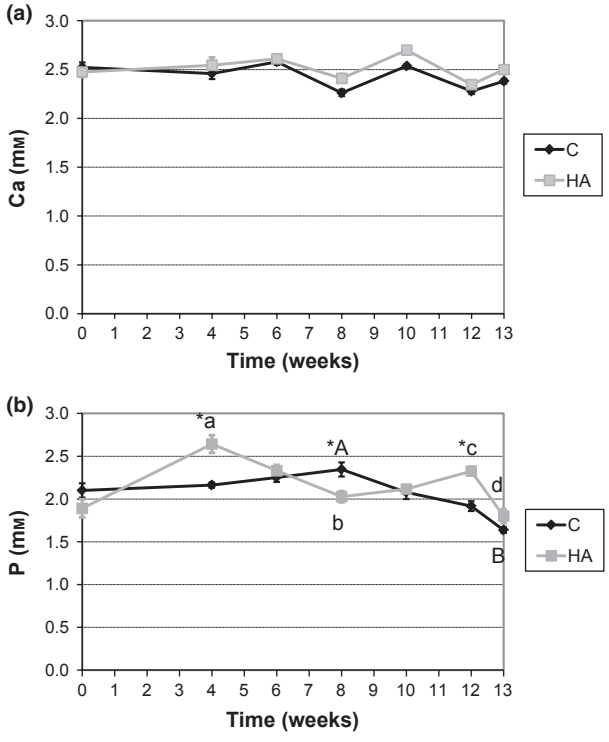

Fig. 1 Serum concentrations (mean ± SE) of calcium (a) and phosphorus (b) before (0), during (1–12), and just after (13) high-altitude (HA) grazing. Values marked with an asterisk differ significantly ($p < 0.05$) between groups (group effect at different time-points). Different letters (upper case and lower case) indicate differences between the time-point before and the time-point after ($p < 0.05$) within one group. ◆ = lowland control group (upper case for significant time effect in group C), ■ = HA group (lower case for significant time effect in group HA).

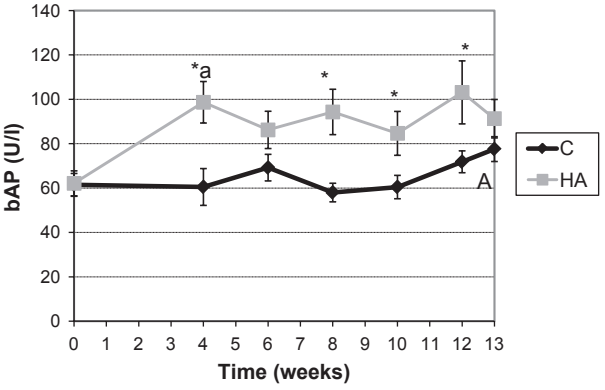

Fig. 2 Serum activities (mean ± SE) of bone-specific alkaline phosphatase before (0), during (1–12), and just after (13) high-altitude (HA) grazing. Values marked with an asterisk differ significantly ($p < 0.05$) between groups (group effect at different time-points). Different letters (upper case and lower case) indicate differences between the time-point before and the time-point after ($p < 0.05$) within one group. ◆ = lowland control group (upper case for significant time effect in group C), ■ = HA group (lower case for significant time effect in group HA).

At the beginning of the experiment, bAP activity was at a similar level (Fig. 2). It increased in the HA group from week 0 to week 4 ($p = 0.02$) but remained at the same level in group C (Fig. 2). Thereafter, bAP activity in group HA remained at this high level (different at $p < 0.05$ from group C until week 12). In addition, the ICTP concentrations were higher ($p < 0.05$) in the HA groups compared with the C group during the entire experiment, except for the last sampling date (Fig. 3). The ICTP

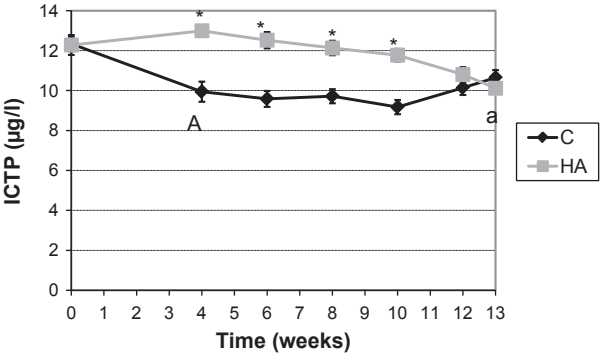

Fig. 3 Serum concentrations (mean ± SE) of cross-linked carboxy-terminal telopeptide of type I collagen before (0), during (1–12), and just after (13) high-altitude (HA) grazing. Values marked with an asterisk differ significantly ($p < 0.05$) between groups (group effect at different time-points). Different letters (upper case and lower case) indicate differences between the time-point before and the time-point after ($p < 0.05$) within one group. ◆ = lowland control group (upper case for significant time effect in group C), ■ = HA group (lower case for significant time effect in group HA).

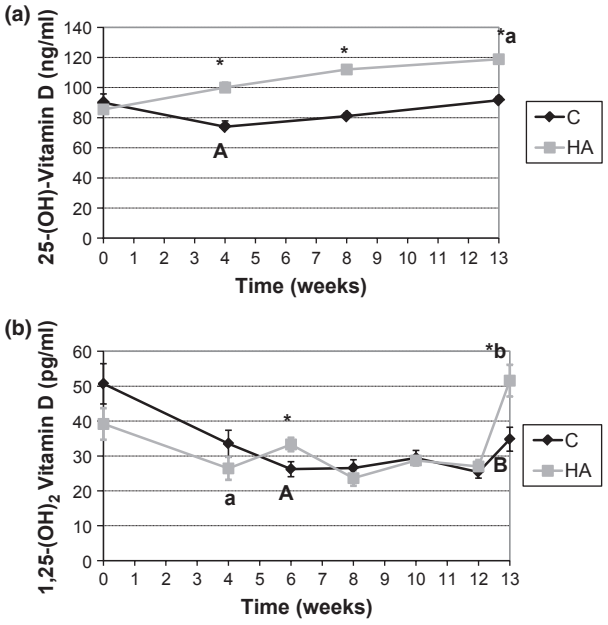

Fig. 4 Serum concentrations (mean ± SE) of 25-OH-Vitamin D (a) and 1,25-$(OH)_2$-Vitamin D (b) before (0), during (1–12), and just after (13) high-altitude (HA) grazing. Values marked with an asterisk differ significantly ($p < 0.05$) between groups (group effect at different time-points). Different letters (upper case and lower case) indicate differences between the time-point before and the time-point after ($p < 0.05$) within one group. ◆ = lowland control group (upper case for significant time effect in group C), ▫ = HA group (lower case for significant time effect in group HA).

concentrations in group C decreased ($p = 0.005$) until week 4 and remained at the same level until the end of the experiment, when concentrations increased again. The remodelling rate (Ratio bAP/ICTP) was similar in both groups at the beginning (HA: 4.4; C: 4.6). During the stay at HA, the remodelling rate increased to almost 7, while in the lowlands, the rate of the ration increased to only 5.8.

The 25VitD concentrations continuously increased ($p = 0.002$) with time in group HA, whereas in group C, the concentration decreased ($p = 0.012$) from week 0 to week 4, followed by an increase ($p < 0.05$) until the end of the experiment (Fig. 4a). In addition, the animals in group HA had higher ($p < 0.05$) serum concentrations of 1,25VitD in weeks 6 and 13 of the experiment compared with group C (Fig. 4b). In both groups, the 1,25VitD concentrations decreased ($p = 0.002$ for C; $p = 0.041$ for HA) with time and increased at the end of the experiment.

Total mean BMC in the metatarsus of both groups increased ($p = 0.0002$) during the experimental period but was higher ($p = 0.048$) at the end of the experiment in group HA (Table 2). The total mean BMD showed an increase with time ($p < 0.001$) in both groups. The cortical thickness also increased ($p = 0.00001$) in both groups, but it was higher ($p = 0.043$) in the HA group after the experiment. Cortical density increased in both groups with time ($p < 0.001$), but no significant difference between the groups was observed ($p = 0.051$). In the left *phalanx proximalis*, concentrations of Ca in the bone ash were 178 ± 4 and 174 ± 4 g/kg DM for groups C and HA, respectively; the corresponding P contents were 74 ± 2 and 73 ± 2 g/kg respectively.

Table 2 Means of different bone quality variables with SE (Week 0 = before experiment)

Treatment	Control	High altitude	p-value group	p-value time
Total bone minerals				
Content (mg/cm)				
Week 0	132 ± 5	139 ± 3	0.95	<0.0001
Week 13	154 ± 4	161 ± 4	0.048	
Density (mg/cm^3)				
Week 0	730 ± 11	741 ± 15	0.67	<0.0001
Week 13	819 ± 11	876 ± 16	0.026	
Cortical density (mg/cm^3)				
Week 0	1155 ± 35	1165 ± 19	0.75	<0.0001
Week 13	1214 ± 13	1235 ± 15	0.051	
Cortical thickness (mm)				
Week 0	9.41 ± 0.32	9.73 ± 0.21	0.22	<0.0001
Week 13	11.14 ± 0.15	12.10 ± 0.28	0.043	

Discussion

Effects of high-altitude grazing on bone quality and metabolism

The present study was conducted to test the hypothesis that HA grazing changes bone metabolism, improves bone density and enhances bone turnover in ruminants. For that purpose, biochemical bone markers and two different forms of VitD in serum were determined, as well as computer tomographical bone properties, comparing lowland and HA groups of growing sheep.

The lambs in the present study showed the expected weight gain, which was similar at both sites, suggesting that the higher energy requirements at HA (low oxygen pressure, physical activity, conditions when traversing steep slopes; Christen et al., 1996; Lachica et al., 1997; Leiber et al., 2006) were compensated for by a higher feed and energy intake. As the weight gain was not different between both groups, the first hypothesis on better weight gain on HA pastures had to be dismissed, although we

cannot rule out whether the meat composition of the animals on HA had a lower fat and higher lean body mass content compared with the other group. Unfortunately, this was not verified.

Serum Ca and P concentrations are regulated homoeostatically to remain within small ranges to achieve a well-balanced, steady state between requirements and intake. Mobilization of Ca from bone and increased absorption from the gastrointestinal tract are the main means of maintaining homoeostasis during times of low Ca supply or high Ca needs. The opposite is the case if there are excessive Ca supplies or low Ca needs. During the sampling period of the present study, serum Ca and P concentrations were always within the normal range (Tschuor et al., 2008). Thus, the differences in Ca and P in the forages obviously were counteracted in absorption or metabolism with the help of various hormones. The known antagonism of Ca and P in absorption apparently also had no effect and probably was balanced through increased secretion via the faeces over time. Unfortunately, no urine samples were collected. As the kidneys also play an important role in the regulation of Ca and/or P homoeostasis, this would have provided more information regarding the regulation. If Ca is low in the plasma pool, parathyroid hormone is secreted in the parathyroid gland. Parathyroid hormone acts directly on the kidneys, where it stimulates the conversion of 25-OH-VitD to 1,25-$(OH)_2$-VitD, as well as the excretion of P via the urine. If Ca levels in the plasma pool are high because of high absorption rates of Ca, less P usually is excreted via urine. In our study, Ca and P content in urine were not measured, so the influence of the kidneys was not described.

Upregulation of serum activity of the bone formation marker bAP observed in the HA group is consistent with the findings in other bone-specific traits. Similar to bAP (bone formation), bone remodelling (measured as quotient of bone formation marker and bone resorption marker) increased in the HA group, which was evident from changes in BMC, BMD, cortical density and cortical thickness. Therefore, bone resorption and formation were coupled and remained in balance, meaning that both formation and resorption processes were upregulated in the HA group, although bone formation was higher. An increase in the circumference of the bone may be reflected in a higher BMD. In interpreting the results, it is important to consider that peak bone mass had not been reached in the animals when they were slaughtered. In humans, peak bone mass occurs by the end of the second decade and does not reach its nadir before the fourth decade (Walsh et al., 2010), which corresponds to about 2–4 years of age in sheep (Pearce et al., 2007); the sheep in our study were still growing. Because two groups of the same age and balanced sex were used, the differences between the groups in BMC, BMD, cortical density and cortical thickness likely were due to the HA, whereas the increases in the bone parameters found within both groups were age related. Because peak bone mass was not reached in the animals investigated, only a transient difference between the groups may have been observed. As because of the experimental setup the animals could not be investigated at a higher age, it can only be assumed that having higher BMD during growth leads to better bone health in older sheep. In case of females, this would likely be advantageous at the onset of the lactation, as the reserves are better filled and the mechanisms causing bone turnover are already better established. Interestingly, the bone ash contents revealed no significant differences for either Ca or P content. However, the bone ash was analysed in the phalanx, a bone with lower turnover than the metatarsus where BMD and BMC had been determined. The best sites to measure bone density for humans are the ankle, hip or spine, depending on the tomography available and the method. Additionally, Calcium and phosphorus are not the only minerals retained in the bone; other minerals are dominantly found in bone, but they were not measured in this study.

Factors responsible for the effect of high altitude on bone properties

Three major factors typical of HA sojourn may have contributed to the differences in bone properties found between the two experimental groups: motion strain (largely elevated at the HA site with its steep slopes); mineral intake (the grass at the alpine site had higher Ca and lower P concentrations compared with the lowland site); and UV-B, which facilitates the formation of VitD in the skin.

Mechanical strain has a large influence on the structure and thickness of bone (Frost, 1997; Turner, 2000; Schönau et al., 2002; Bass et al., 2005). Therefore, it was assumed that motion on the steep pastures with more than 20% inclination was the main reason for the changes in bone characteristics found with HA grazing. By contrast, walking in horizontal plane areas is defined as low-peak strain and thus does not specifically support bone formation.

Accordingly, Skerry and Lanyon (1995) observed that constant slow movement of sheep on a treadmill did not stimulate bone formation. The increased bone remodelling found in the lambs at the beginning of the alpine period suggests that this was caused by the suddenly higher straining motion or by a sudden decrease in P content in the grass at HA. Because high-peak strains mainly cause formation of the final bone architecture (Rubin and Lanyon, 1985; Skerry and Lanyon, 1995), it could have been expected that animals that have to move under difficult topographical conditions would show larger changes in bone characteristics than those kept in the lowlands on flat pastures. Although the growth rate of the animals and the accompanying bone length did not differ between groups in the present study, cortical thickness of the bone increased in the HA group. This is also the reason why bone resorption was increased. Bone formation and resorption are usually coupled and in balance. If bone formation is increased, bone resorption is also increased. As the remodelling rate was increased in the HA group, both parameters were increased. This is consistent with two other studies using pQCT (Haapasalo et al., 2000; Joo et al., 2003). Although humans and rats were used in these studies, the increased exercise (tennis player; rats on treadmill) with high-peak strains led to increased diameter and cortical thickness of the long bones. Hiney et al. (2004) showed that calves that were able to exercise daily had higher total BMD and higher cortical thickness. The present study supports the observation of others (Eriksen, 1986; Mori et al., 2003; Hagihara et al., 2005; Rakovac et al., 2007; Tobias et al., 2007) that moderate exercise with high-peak strains may lead to higher bone mass or BMC. After the first few weeks of grazing the HA pasture, the lambs seemed to adapt to the specific topographical conditions, as the bone marker concentrations stayed at the same levels from then on. Higher physical activity is likely to lead to higher adaptation of the tissue (Carter, 1987; Beaupre et al., 1990; Turner and Pavalko, 1998). Lower physical activity leads to lower bone remodelling, which was shown by the starting point where the animals were kept under lowland indoor conditions. It is known that the influence of motion on bone is much higher in younger animals than in older animals (Notomi et al., 2002), which is why the effect may have been quite pronounced in the present study.

It is known that a higher Ca supply, likely to have occurred in the animals grazing the Ca-rich high alpine pastures, would affect mineral deposition in the bone (Lambert et al., 2008). However, this also requires extra VitD, as providing extra Ca or VitD alone does not change bone quality (Rauch and Schönau, 2001; Bass et al., 2005). In the HA lambs, the higher Ca content of the grass was accompanied by higher serum VitD (25 and 1,25) concentrations, suggesting that the Ca-VitD axis also contributed to the changes found in the bone properties. However, as mentioned before, the Ca content in the bone tested did not increase compared with the C group. In addition, the Ca content was higher in the high alpine grass, but also the excretion of Ca in faeces was higher. The higher UV-B radiation at HAs (Blumthaler et al., 1992; no measurements in this experiment) would be the most likely reason for the higher 25VitD concentrations found in the serum of these animals. However, more exercise and motion were also found to lead to higher 1,25VitD concentrations in rats (Iwamoto et al., 2004) as a result of the upregulated Ca metabolism. In another study in cows kept at the same HA pasture area, increased 25VitD concentration in blood plasma was observed as well (Leiber et al., 2005). In the present study, only 25VitD concentration increased in the HA group, but 1,25VitD concentration increased at only one time-point. Serum 25VitD level is the best indicator for VitD supply (Quarterman et al., 1964).

The grass on the alpine site was low in P [considered to be deficient even for ruminants by Berry et al. (2001) at the same site]. The bone resorption marker ICTP was higher in the HA group than in the control group during the entire experiment. In addition, the bone formation marker bAP was also higher, suggesting that low P concentrations in the grass is not a likely explanation for the shifts found in ICTP, as only bone resorption would then have increased. In the HA group, the increase in bone remodelling indicates a fast adaptation of the skeleton to the different influencing factors. Movement may be a possible cause, which is explained by the fact that the HA animals had to move up the hills to follow the vegetation, whereas the animals in the lowlands only had to change pastures if the feed was short.

The present study demonstrates that growing sheep kept at HA express a higher bone turnover, bone formation and quality. Possible reasons for this effect include more intensive motion strain, higher dietary Ca supply and increased UV-B radiation, leading to an increased formation of VitD in the skin. It is not possible to identify unambiguously the major factor of influence from the results or to determine whether all factors contributed equally.

However, the current practice of summer grazing of ruminants on HA pastures seems an efficient means to improve bone health.

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