INTRODUCTION: Numerous large animal models for postmenopausal osteoporosis currently exist including the mature, OVX ewe. One limitation of this model is that the amount of bone mineral density lost post-ovariectomy has been approximately 5-10% after 12 months (6) as measured by DEXA. A more desirable model would have greater BMD losses in a shorter period of time.

Previous studies in other species have utilized calcium restricted diets to induce osteoporosis in animal models. However, calcium restriction in the mature, OVX ewe is difficult to achieve as their basal requirements are low. In addition, humans may develop osteoporosis despite adequate calcium intake. One possible explanation has been proposed in recent studies that have implicated diets that induce metabolic acidosis (1-5). Such diets in may be referred to as having a low cation-anion difference. It is unknown whether estrogen depletion and acidifying diets induce osteoporosis in a similar manner and are therefore additive in effect or exert their effect through similar albeit different pathways and therefore may be synergistic in causing bone mineral density loss. The purpose of the studies reported here was to determine the effect of diet on bone mineral density in mature OVX ewes and in non-OVX ewes.

METHODS: Study 1: 52 skeletally mature (4-7 year old) Columbia-Rambouillet cross ewes were used. 38 sheep underwent OVX and 14 sheep underwent sham surgery (ShOVX). Following surgery sheep consumed a low DCAD diet (n = 24, OVX), or a normal DCAD diet (n = 14 ShOVX and n = 14 OVX). All sheep underwent dual energy X-ray absorptiometry (DEXA) of the lumbar vertebrae on day 0. All 24 low DCAD sheep had DEXA at 90 days and 14 normal DCAD diet sheep (7 OVX and 7 ShOVX) were euthanized and DEXA scanned at 90 days. The balance of the sheep are being held for future study. DCAD was calculated as (Na + K) – (S + Cl) = DCAD. The normal DCAD diet consisted of free choice grass hay (DCAD 300 mEq/kg dry matter (DM)). The low DCAD diet consisted of grass hay and a low DCAD pellet, such that the total DCAD consumed per sheep per day was approximately –350 mEq/kg DM. Day 0 and 90 DEXA scans were compared and data was analyzed using an ANOVA. Percent change in BMD (%BMD) was compared using a standard of significance of p < 0.05.

RESULTS: There was no statistical difference in %BMD within the normal DCAD group between OVX and ShOVX, therefore these sheep were grouped together for comparison with the low DCAD group. Mean %BMD for the low DCAD diet group was significantly greater than for the normal DCAD diet group. Sheep fed the low DCAD diet lost 10.19% ± 1.27% of their BMD compared to 1.95% ± 0.78% BMD loss in the sheep fed the Hay diet (p = 0.00004).

Study 2: 24 skeletally mature (4-7 year old) Columbia-Rambouillet cross ewes were used. 12 sheep underwent OVX and 12 sheep did not (non-OVX). 12 sheep (6 OVX and 6 non-OVX) consumed a normal DCAD diet and 12 sheep (6 OVX and 6 non-OVX) consumed a low DCAD diet (diets as described above). Sheep received a DEXA scan of the lumbar vertebrae at 0 and at 6 months. Monthly serum and urine samples were collected and were analyzed for serum bone alkaline phosphatase (sBAP), urine DPD (deoxypyridinoline) and fractional elimination (FE) of calcium and phosphorus. Arterial blood gas measurements were taken at 6 months. Significance was set at p ≤ 0.05.

RESULTS: Body weights did not change significantly within or between groups through the course of the study. Arterial pH taken at 6 months demonstrated no significant differences between groups. Mean %BMD was -1.4% (+/- 3.3) non-OVX NDCAD, -5.3% (+/- 2.0) OVX NDCAD, -11.5% (+/- 6.9) non-OVX LCDAD, 14.4% (+/- 5.5). A two-way ANOVA demonstrated a significant difference between groups. One way ANOVA (OVX status and diet variables combined as categorical data) between groups revealed the following significant differences (p < 0.05): between NDCAD OVX and non-OVX, NDCAD non-OVX and LCDAD non-OVX and LCDAD OVX, and NDCAD OVX and LCDAD OVX. FE of calcium and phosphorus showed increases in calcium and phosphorus in urine throughout the study. sBAP was significantly greater in all groups compared to controls in months 3, 4, 5 and 6, whereas urine DPD was not different.

DISCUSSION: These two studies demonstrate that a low DCAD diet and a low DCAD diet with OVX can induce a greater decrease in bone mineral density over a shorter period of time when compared to the conventional model of a normal DCAD diet in combination with OVX. Urinary losses of phosphorus and calcium support the calcium wasting effect of the dietary change without causing a change in arterial pH that is outside the normal physiologic range. Rises in bone alkaline phosphatase support that bone turnover is increased. Decreased bone mineral density appears greatest when dietary changes and ovariectomy are combined. However, they were not significantly different within the time frame of these studies. Additional studies are necessary to determine the implications of acidifying diets on the development of osteoporosis. However, this model shows considerable promise for use in the study of human osteoporosis and the combined effect of dietary lifestyle and estrogen depletion. The model also shows promise in the study of surgical implants into osteoporotic/osteopenic bone.

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