Introduction. Recombinant or purified transforming growth factor-beta (TGF-ß) has been shown by our group and others to stimulate bone ingrowth into porous coated implants and bone regeneration adjacent to the implants in young, adult animals. One report with negative findings suggested that TGF-ß might not be an effective means of enhancing bone regeneration in the aged skeleton. Although little attention has been paid to the inherent reparative capacity of the osteoporotic or aged skeleton, most clinicians would agree that, even in the presence of excellent mechanical stability, fracture healing is slower in aged or osteoporotic individuals. Consistent with these clinical impressions is a recent report of reduced numbers of osteoprogenitor cells as a function of aging in humans and suppressed osteoblastic gene expression following femoral marrow aspiration in aged rats. The purpose of the present study was to determine if stimulation of intramembranous bone regeneration with TGF-ß2 was equivalent in skeletally mature young and old animals.

Methods. Porous metallic implants were made from titanium fiber metal and were placed bilaterally in the presence of a 3 mm gap in the proximal humeri of skeletally mature young adult (age 1-2 years) and old adult (age 10-12 years) beagles. The study protocol was approved by our institution’s IACUC. The test implant was treated with hydroxyapatite/tricalcium phosphate (HA/TCP) and recombinant human TGF-ß2 in buffer at a dose of 3.2 g or 35 g. In these same animals, an internal control implant treated only with HA/TCP and buffer was placed in the contralateral humerus. Two additional groups were studied in old beagles. In one of these groups, 0.32 g of TGF-ß2 was tested. In the second group, the effect of the HA/TCP coating was assessed by comparing implants treated with HA/TCP to implants not treated with the ceramic (these animals, therefore, received no TGF-ß). All groups had a sample size of 6 and were studied at 4 weeks. We determined the volume fraction and characterized the architecture of the newly formed bone within the 3 mm gap adjacent to the implant using previously described methods.

Results. The HA/TCP coating had no significant effect in the old animals (as with our previous studies in young adult mongrel dogs, also in the presence of a 3 mm gap). There was no stimulation of bone regeneration with the 32 g dose in the old animals. In both the young and old animals, there was a relatively small (~2.5-fold) stimulation of bone regeneration with the 3.2 g dose and a much larger stimulation (3-fold) with the 35 g dose (paired t-tests to compare the treated and internal control sides) (Fig. 1). An analysis of variance for repeated measures with age and dose as the between-subjects factors showed that implant treatment with the growth factor was a significant within-subjects main effect (p < 0.001). There was a significant interaction between treatment and dose (p < 0.001) because of the greater degree of enhancement in the 35 g dose group compared to the 3.2 g dose group. In addition, the analysis of variance showed that dose was a significant main effect (p = 0.003). Interestingly the analysis showed that age was not a significant main effect, although there was a trend in this direction (p = 0.089). There was no significant interaction between age and dose.

At the tissue level, the mechanisms underlying the growth factor stimulated increase in the volume fraction of bone in the gap surrounding the implant were similar to those we have observed previously in mongrels. Namely, we found an increase in the number of trabeculae per mm and an increase in the average trabecular width (p < 0.05).

Discussion. The present study demonstrates that TGF-ß stimulation of bone regeneration is as effective in aged beagles as it is in young, adult beagles. From a practical point of view, our finding that the stimulatory effect of TGF-ß on bone regeneration was similar in young and old adult beagles is important because it suggests that clinically it would not be necessary to adjust dosage based on the patient’s age.

Although aging did not influence the effect of TGF-ß, aging did have a statistically marginal effect on bone regeneration. With a larger sample size, this 10 to 20% age-related reduction in bone regeneration might become statistically significant. Whether or not a suppression of intramembranous bone formation of this magnitude would have later clinical implications cannot be answered by the present study.

Figure 1. Bone volume fraction in the 3 mm gap surrounding the implants plotted for the untreated internal control implants and the TGF-ß2 treated implants as a function of animal age and test dose. Difference from the paired internal controls: * p < .05, ** p < .01, *** p < .001.

Acknowledgements: Funding provided by NIH Grant AR42862. Zimmer-USA donated the implants.

References
4. Lane JM et al. Instructional Course Lectures 36:71, 1987

**Genzyme Tissue Repair, Framingham, MA.