INTRODUCTION: Growth factors such as TGF-β and VEGF are found in significant amounts in bone extracellular matrix, and can be mobilized by mechanical loading from devitalized bone matrix [1]. In vitro, mechanical stimulation of osteoblasts will increase the production of TGF-β mRNA [2]. Presence of TGF-β or VEGF can induce osteoblast differentiation and/or proliferation [3,4], and inhibit the formation and function of osteoclasts [5].

It is possible that these growth factors, deposited in the matrix at the time of apposition in proportion to current load, may provide a long-term matrix memory. Released at the time of matrix damage, they could serve as a signal to direct the repair and remodeling process. Here we used a model of new bone formation resulting from fracture repair. We hypothesized that such new bone that is not loaded would have lower matrix concentrations of these growth factors than bone that is loaded.

METHODS: The study combined a rat tibial-fracture model [5] with a tail-suspension model of weightlessness [6]. Under local IACUC approval, 32x400gm male SD rats were anesthetized and the right tibia pinned through a percutaneous approach. Half of the operated tibiae were fractured at the distal tibial-fibular junction. The animals were then divided into tail-suspension or free (loaded) groups. Four weeks after surgery, the animals were euthanized and the right and left tibiae harvested. The portion of the tibia and fibula that was within 5mm of the distal tibial-fibular junction was cleaned of soft tissues, power under liquid N2, and growth factors extracted with 4M guanidine HCl, 0.05M EDTA, bovine serum albumin (1mg/ml) 0.03 M Tris pH 7.4, with proteinase inhibitors (2mM PMSF, 2ug/ml leupeptin, 5 mM benzamidine hydrochloride, 0.1 M 6-aminohexanoic acid). After dialysis against PBS, extracts were analyzed for TGF-β1, TGF-β2, and VEGF by ELISA (R&D Minneapolis MN). Growth factor concentrations were expressed as ng/g of powdered bone tissue. For each leg, the effect of fracture and loading status on the matrix concentration of each growth factor was evaluated using a 2-way ANOVA, with posthoc SNK tests.

RESULTS: Both local and systemic effects on matrix concentrations of these growth factors were noted (Fig.2):

VEGF: There was no significant effect of loading (p>.260) or fracture (p>.440) on the matrix concentration of VEGF, on either the operated or contralateral legs.

TGF-β1: Operated leg: The combination of fracture and loading was sufficient to significantly decrease the matrix concentration of TGF-β1 (interaction p=.004). Contralateral leg: There was no significant effect of loading (p=.394) or fracture (p=.709), interaction p=.502.

TGF-β2: Operated leg: Loading (p=.011) and fracture (p=.007) were both associated with a significant decrease in the matrix concentration of TGF-β2 (interaction p=.444). Contralateral leg: The contralateral tibia of fractured animals had significantly lower matrix concentrations of TGF-β2 (p=.003) than those of non-fractured animals, regardless of loading status (p=.194, interaction p=.779).

DISCUSSION: These data do not support an hypothesis of decreased matrix deposition of these growth factors with decreased loading. When a significant effect of loading was found, it was in the direction of increased matrix concentrations of growth factors with decreased loading. Specifically, matrix VEGF levels were not demonstrably affected by either fracture or loading. Matrix levels of TGF-β1 were affected locally, with loading and fracture affecting the matrix concentration synergistically (both in the opposite direction from that expected), there was an additional, systemic effect of fracture, in that the matrix concentrations of this growth factor on the opposite, unfractured tibia was also decreased, albeit to a lesser extent. The mechanisms behind these systemic changes are unclear from these data.

The differences between the effects seen for TGF-β1 and TGF-β2 may reflect different anatomic localizations for the two growth factors (e.g., matrix proper vs canalicular and lacunar surface), which could result in differential mobilization of the two factors on matrix damage and subsequent repair.