Final Report for NASA Contract NCC 2-1201

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Title: Do Integrins Mediate the Skeletal Response to Altered Loading? (Subproject B)

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Summary of Research NCC 2-1201:

Our major findings have been published in two peer-reviewed publications (Globus et al. 2005, Iwaniec et al. 2005). These papers report our phenotype characterization and short term unloading data. Several additional manuscripts are in preparation for peer-review publication, and will include the results presented here. These data have been presented at several international conferences (Globus et al. 2002, 2003; van der Meulen et al. 2004, Dovi et al. 2004). Drs. Globus and van der Meulen are also preparing a review article for *Current Opinion in Orthopaedics* on the subject of weightlessness, bone disuse, and the hindlimb suspension model.

In vivo experiments were performed to examine the role of ß1 integrin in skeletal adaptation to reduced and increased loading. Transgenic mice were generated with a dominant negative form of the ß1 integrin cytoplasmic domain with expression driven by the osteocalcin promoter (pOCb1DN). This fragment consists of the transmembrane and intracellular domains and interferes with endogenous integrin signalling *in vitro*. This promoter targets expression of the transgene to mature bone cells. Expression of the transgene was confirmed by immunoprecipitation and western blotting. Reduced loading was generated by hindlimb suspension and increased loading the resumption of normal loading following hindlimb suspension. Two groups of female 35-day old mice were examined: pOCb1DN transgenic mice (TG) and wild-type littermate controls (WT). Animals were hindlimb suspended for 1 week (HU, n=10/gp) or 4 weeks (HU, n=4-7/gp) or suspended for 4 weeks followed by reloading by normal ambulation for 4 weeks (RL, n=10/gp). Age-matched controls (CT) were pairfed based on the HU food intake. The protocols were approved by the NASA Ames Research Center IACUC.

Upon completion of the experimental protocol, body mass was recorded and tissues of interest removed and analyzed following standard procedures. Femoral whole bone structural behavior was measured in torsion to failure to obtain whole bone strength (failure torque) and torsional rigidity. Ash content (ash) and fraction (%ash) were determined for the tibia. Total ash is indicative of bone size whereas %ash is a material property. Tibial curvature was measured from microradiographs. For each experiment, the effects of genotype (TG, WT) and treatment (CT, HU/RL) were assessed by two-factor ANOVA followed by the Tukey-Kramer posthoc to identify significant differences at an alpha level of 0.05. Our goal was to understand differences resulting from altered integrin function in the adaptation to altered loading.

Hindlimb unloading of growing mice for 1 week significantly reduced their body mass, but did not impact whole bone strength, torsional rigidity or energy to failure (Figure 1). TG mice were significantly lighter than WT mice. When normalized by body mass, femora of HU mice were significantly stronger per unit body mass after short-term unloading. Energy to failure was not different across the four groups. Tibial ash was not altered by HU, but ash fraction was reduced after 1 week HU. Tibial curvature was reduced by HU. TG femoral failure torque and stiffness, ash, ash% and curvature were significantly lower than WT values.

Hindlimb unloading of growing mice for 4 weeks decreased all parameters examined in TG and WT relative to normally loaded controls. Body mass was reduced significantly in HU mice, and this effect was smaller in TG than WT mice. Failure torque was significantly reduced with hindlimb unloading in both WT and TG relative to CT, and this effect was less in TG than WT femora ($p=0.009$, Figure 1). The difference in whole bone strength in TG compared to WT with HU disappeared when normalized by body mass ($p=0.19$). Torsional rigidity was significantly reduced due to unloading, and this effect was similar in TG and WT mice. Energy absorbed to failure was only significantly lower after 4 weeks of unloading. Tibial ash was significantly reduced with unloading, and significantly less so in TG than WT; %ash was reduced with unloading but not different between TG and WT. Tibial curvature was not affected by HU, but lower in TG than WT.

An equal period of reloading was not sufficient to completely recover all skeletal properties following hindlimb unloading. Body mass recovered to control values, but was significantly lower in TG than WT. Whole bone torsional strength and stiffness remained significantly decreased following reloading and were similarly affected in TG and WT mice. Energy to failure was not different across the four groups. Both tibial ash and ash fraction were still suppressed following reloading. Tibial curvature was also still reduced after reloading compared to normally loaded controls. Torsional strength and stiffness, ash, %ash and curvature were significantly lower in TG than WT mice.

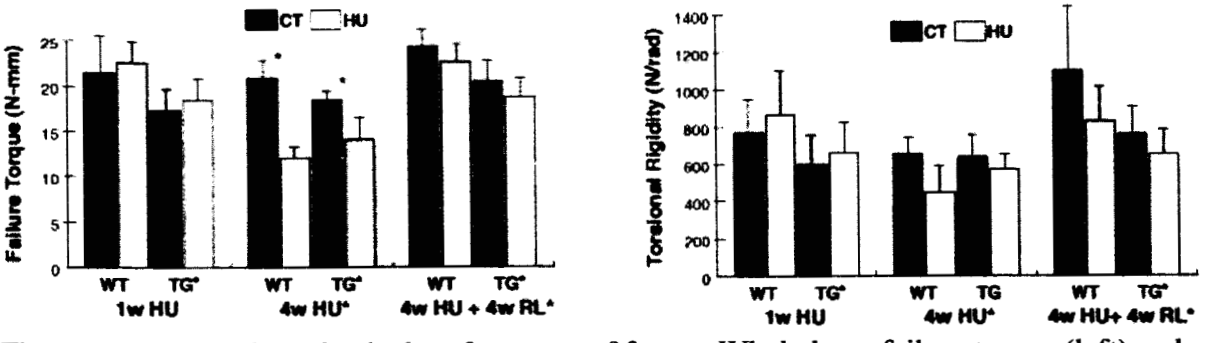

Figure 1: Torsional mechanical performance of femur: Whole bone failure torque (left) and torsional rigidity (right). See text for description of experimental groups. *$p<0.05$ for HU vs CT or TG vs WT

In conclusion, adaptation of whole bone strength was reduced in mice with osteoblasts expressing a function disrupting ß1 integrin fragment. After 4 weeks of hindlimb unloading both TG and WT femora were weaker than controls, but, more importantly, this effect was less in TG than WT. Our results also show that unloading-related bone changes included geometric and

material properties. The difference between TG and WT adaptation appeared to be size (total ash), not material (%ash) differences. Cross-sectional geometry of the femur is needed to confirm this observation. When body mass differences were accounted for, the adaptation to hindlimb unloading was similar across genotypes, suggesting femoral strength differences may result from systemic influences reflected in body mass. Unfortunately our statistical power was the least for the 4w unloading experiment among the three studies performed. However, combined with the reloading results, the 4w unloading and reloading data show similar, slower structural responses in TG than WT mice when adapting to changes in mechanical stimuli. These data support our hypothesis and suggest an *in vivo* role for integrin receptors in mechanotransduction and functional adaptation of bone, particularly bone strength, an important functional measure. Our findings are critical for understanding skeletal adaptation to reduced loading during spaceflight and will help in the development of countermeasures to inhibit microgravity-induced bone mass and strength losses.

Peer-review papers:

Globus RK, Nishimura Y, Amblard D, Iszwaniec U, Kim J-B, Almeida EAC, Damsky CD, Wronski TJ, van der Meulen MCH (2004) Skeletal phenotype of growing transgenic mice that express a function-perturbing form of ß1 integrin in osteoblasts. *Calcif Tissue Int* 76: 39-49

Iwaniec UT, Wronski TJ, Amblard D, Nishimura Y, van der Meulen MCH, Wade CE, Bourgeouis MA, Damsky CD, Globus RK (2005) Effects of disrupted ß1 integrin function on the skeletal response to short-term hindlimb unloading in mice. *J Appl Physiol* 98: 690-696

Peer-review conference proceedings:

Dovi JV, van der Meulen MCH, Kim JB, Damsky CD, Almeida EAC, Globus RK (2004) In vitro functions of beta1 integrins in mature cells of the osteoblast lineage. *Amer Soc Bone Miner Res*, Seattle, WA, SU150

Globus RK, Amblard D, Nishimura Y, Litzenberger J, Corcoran JL, Almeida EAD, Wade C, Morey-Holton E, Damsky CD, Kim J-B, Wronski TJ, Iwaniec UT, van der Meulen MCH (2003) Altered loading of growing transgenic mice that express a function-perturbing form of beta1 integrin in osteoblasts. *Amer Soc Bone Miner Res,* Minneapolis MN, SA176

Globus RK, van der Meulen MCH, Damsky C, Kim J-B, Amblard D, Nishimura Y, Iwaniec UT, Wronski TJ (2002) Skeletal phenotype of transgenic mice expressing the ß1 integrin cytoplasmic tail in osteoblasts. *Amer Soc Bone Miner Res*, San Antonio TX, M096

van der Meulen MCH, Nishimura Y, Netravali NA, Amblard D, Litzenberger J, Damsky CH, Globus RK (2004) Role of osteoblast's ß1 integrin in skeletal structural adaptation during growth. *Trans Orthop Res* Soc 29: 396