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**A. Summary of Proposed Research**

CASI

Disuse causes osteolysis of the skeleton, with attendant hypercalcemia, hypercalciuria and osteoporosis. Immobilization with a cast and stress shielding by an orthopedic implant result in localized bone loss. Although these problems have been recognized for decades, there has been limited progress towards identifying the underlying cellular and biochemical changes. Consequently, at present, there is no effective treatment to prevent bone wasting associated with disuse. Previous studies in man and animals have emphasized the role of increased bone resorption in development of disuse osteopenia. However, the results of studies that we carried out in animal models indicate that decreased bone formation may account for most of the deficit. These findings suggest to us that osteoblast activity is modulated by gravitational loading and muscle loading. The hypothesis of this proposal that osteoblasts are under direct regulation of mechanical loading at the level of gene expression will be tested in bone cells subjected to the near weightlessness of orbital spaceflight. We postulate that gravitational loading modulates the elaboration of specific signaling molecules (growth factors) by osteoblasts and, in turn, these signaling molecules promote normal coupling of bone formation and bone resorption. Normal coupling is essential to maintenance of bone volume; it is the pronounced uncoupling that occurs during disuse which leads to potentially catastrophic bone loss in humans. Although several signaling molecules may be involved, the weight of evidence favors transforming growth factor-beta (TGF- $\beta$ ) as having an important role in this process. TGF- $\beta$  is produced by rat and human osteoblasts, acts on both osteoblasts and osteoclasts of rats and humans, is incorporated in relatively large amounts into bone matrix, and its expression is altered in rats by weight bearing.

The purpose of the proposed study is to establish whether changes in gravitational loading have a direct effect on osteoblasts to regulate TGF- $\beta$  expression. The effects of spaceflight and reloading on TGF- $\beta$  mRNA and peptide levels will be studied in a newly developed line of immortalized human fetal osteoblasts (hFOB) transfected with an SV-40 temperature dependent mutant to generate proliferating, undifferentiated hFOB cells at 33-34°C and a non-proliferating, differentiated hFOB cells at 37-39°C. Unlike previous cell culture models, hFOB cells have unlimited proliferative capacity yet can be precisely regulated to differentiate into mature cells which express mature osteoblast function. If isolated osteoblasts respond to changes in mechanical loading in a manner similar to their response in animals, the cell system could provide a powerful model to investigate the signal transduction pathway for gravitational loading.

**B. Specific Aims**

The specific aims of the proposed research are to:

1. Determine the effects of spaceflight and reloading on expression of TGF- $\beta$  mRNA and protein by hFOB cells.
2. Determine the effects of spaceflight and reloading on expression of osteoblast marker mRNAs and proteins, e.g. osteonectin, osteocalcin, and type 1 collagen.

**C. Studies Performed**

Year 1: These studies were performed to demonstrate that hFOB cells could grow on cytodex 3 beads in the cell culture model. It was also essential to establish that the cells would remain viable during flight conditions for at least 3 weeks. These goals were accomplished during the first year and the results reported.

Years 1-2: A pilot spaceflight experiment was performed in which we were assigned 4 cell cartridges. The results of the pilot study, which were previously reported, were used to make the final experimental design decisions for the main study.

Years 2-3: The main experiment in which we were assigned 8 cartridges was performed. This report will summarize the results of this study.

### Materials and Methods:

#### Cell Culture

The cell culture module (CCM) designed for maintenance of the flight and ground control samples contained the following devices: 1) heated metal sleeves for 37°C incubation, 2) temperature monitors, 3) CO<sub>2</sub> gas canister and infusion system calibrated to 5%, 4) peristaltic pumps for each pair of cartridges, 5) media bags containing 250ml 1:1 Delbecco's Modified Eagle's (DME)/ Ham's F12 (F12) medium containing 10% (v/v) FBS and 150 µg/ml geneticin (G418) medium for each cartridge, 6) hollow fiber cartridges for culturing cells, 7) fraction collection tubes and valves for in-flight media samples.

Pretreatment of Cytodex 3 beads consisted of hydration in phosphate-buffered saline (1X PBS), followed by rinses with serum-free medium, medium + 10% fetal bovine serum (FBS), and 24hr incubation with 100% FBS at 37°C. Similarly, the inter and extra-capillary spaces of the cartridges were rinsed with serum-free medium and medium + 10% FBS. The extracapillary space was incubated with 100% FBS for 24hr at 37°C, then rinsed with medium + 10% FBS. Media bags were filled with 250ml DME/F12+10% FBS+G418 medium, equilibrated at 37°C for 24hr, degassed, and stored at 4°C until needed.

The hFOB cell line was cultured under standard conditions as described previously (1), except that the culture temperature was 37°C, instead of 34°C. This temperature has been previously shown to be permissive for the temperature sensitive SV40 large T antigen in the hFOB cell line (unpublished data). Briefly, stock flasks of cells were cultured in DME/F12+10% FBS+G418. Seven days prior to launch, T-75 flasks of cells were passaged with 1ml trypsin-EDTA. The trypsin was inactivated with 9ml DME/F12 + 10% medium and the cells were counted with a hemacytometer. For each experimental cartridge,  $6 \times 10^5$  cells were mixed gently with 50mg of pretreated Cytodex beads and incubated at 37°C in 5ml of equilibrated DME/F12+10% FBS+G418 medium in 15ml conical tubes for 45 min, to allow for cell attachment. The cells on beads were then transferred to 100mm non-adherent petri plates containing equilibrated DME/F12 + 10% FBS+G418 medium. Culture medium was removed and replaced with fresh medium daily. Two days prior to launch, the cells on beads were removed from the petri plates with a pipette and transferred to a 15ml conical tube. The beads were allowed to settle (~2min) and excess media was removed. The cells on beads were injected with ~5ml of medium into the extracapillary space of each cartridge with a 10ml syringe. The cartridges containing the cells were installed into the CCM and incubated at 37°C until launch 2 days later. Baseline samples were prepared in identical fashion except that the media was pumped through the cartridges with a Cellmax quad peristaltic pump in a 37°C incubator rather than a CCM. For purposes of nomenclature, the launch was designated "day 0", the landing (R) and R+8hr timepoint were "day 18", and the R+24hr timepoint was "day 19".

Fractions (4ml) of conditioned media were collected on days 1, 4, 8, and 12 from both flight and ground control cultures. In all cases fractions were taken from 2 different cultures such that each flight or ground culture had only one fraction collected during the 17 day mission. The remainder of the conditioned media was collected after landing on days 18 and 19.

### RNA Isolation

At the appropriate time following recovery of the spacecraft, the cartridges were removed from the CCM and the ends of the cartridges were cut off to expose the hollow fibers. The hollow fiber bundle was removed from the cartridge and the cells+beads+collagenous matrix was gently scraped and rinsed off of the fibers with a spatula and pipette into 1X PBS. A small aliquot of beads with attached cells was transferred to buffered fixative for later examination by electron microscopy. The cells/beads were transferred to 15 ml conical polypropylene tubes and centrifuged at 500 x g for 5min. Excess PBS was removed leaving a cell/bead pellet in 1.5-2ml of PBS. RNA was isolated by the method of Chomczynski (2), using guanidine isothiocyanate-phenol lysis reagent (TRI reagent-Molecular Research Center, Cincinnati, OH). An additional extraction with a 1 ml volume of chloroform and a precipitation step in 1M LiCl were added for further RNA purification.

Following ethanol precipitation, RNA pellets were dissolved in molecular biology grade water and quantitated by UV spectrophotometry for Northern or RNase protection assays.

### Northern Analyses

Total RNA isolated from hFOB cells was denatured in glyoxal/DMSO buffer and separated by agarose gel electrophoresis (3), blotted to nylon filters by capillary diffusion in 20X SSC (3M NaCl, 0.3M NaCitrate pH 7.0), and bound to nylon filters by vacuum baking at 80°C for 2hr. Hybridization was performed in a hybridization incubator (model 400, Robbins Scientific, Sunnydale, CA) at 65°C for 2hr with 10ml of rapid hybridization buffer (Amersham, Arlington Heights, IL) containing 50 µg/ml salmon sperm DNA (Stratagene, La Jolla, CA). Blots were rinsed in 1X SSC + 0.1% w/v SDS at 50°C, then at 65°C. Each hybridization contained ~2 x 10<sup>7</sup> cpm (~10 ng) of <sup>32</sup>P-labeled cDNA probe. Labeling of cDNAs was performed with a random primer labeling kit (Amersham) as per the manufacturer's instructions using [<sup>32</sup>P]-dCTP (3000 Ci/mmol, Amersham). The labeled cDNAs were purified by gel filtration chromatography (Pharmacia nick columns). The membranes were hybridized with DNA probes for osteonectin, alkaline phosphatase, type I collagen, and 18S rRNA as a control for gel loading. Following hybridization, each blot was exposed to phosphorimager screens (Molecular Dynamics) and band intensities were quantitated with a Molecular Dynamics Phosphorimager.

### RNase Protection Assay

RNA isolated from hFOB cells was hybridized to antisense RNA probes and further treated as specified by the manufacturer (RPA II kit manual #1410, Ambion, Austin, TX). Briefly, antisense RNA probes were synthesized by *in vitro* transcription (23) with T3 or T7 polymerase in the presence of <sup>32</sup>P-UTP (800 Ci/mmol, New England Nuclear, Boston, MA), then 50 x 10<sup>3</sup> cpm of probe was hybridized 16-24hr at 43°C in 20 µl hybridization buffer (80% v/v deionized formamide, 100 mM sodium citrate pH 6.4, 300 mM sodium acetate pH 6.4, 1 mM EDTA) containing 10-20 µg of total RNA. Following hybridization, the solution was mixed with 0.5 units of RNase A and 20 units of RNase T1 and incubated for 30min at 37°C, then ethanol precipitated. The RNA pellet was dissolved in gel loading buffer (95% v/v formamide, 0.025% xylene cyanol, 0.025% bromophenol blue, 0.5 mM EDTA, 0.025% SDS) and the reaction products separated by urea-polyacrylamide (50% urea w/v, 7% w/v acrylamide 29:1 bis) gel electrophoresis in 1X Tris-borate EDTA (45mM Tris-borate, 1mM EDTA) buffer. The gel was then vacuum dried and placed on a phosphorimager screen for quantitation of band intensities.

### Procollagen Quantitation

The level of carboxyterminal propeptide of type I procollagen (PICP) in hFOB conditioned media was measured by radioimmunoassay as specified by the manufacturer ( PICP kit manual # 35100,

Incstar Corp., Stillwater, MN). Briefly, 3-100  $\mu$ l of conditioned media was incubated with dilute rabbit anti-PICP antiserum and a constant amount of  $^{125}$ I-PICP for 2hr at 37°C. The complexes were precipitated by incubation with a goat anti-rabbit antibody conjugated to solid particles, then centrifuged at 2000 x g for 15 min at 4°C. The amount of precipitated  $^{125}$ I-PICP was quantitated in a gamma counter and compared to a standard curve of known PICP standards, including a 0 standard. Non-specific background was determined with samples lacking PICP-antiserum, and media background was determined using base (non-conditioned) media.

#### PGE<sub>2</sub> Quantitation

The level of prostaglandin E<sub>2</sub> in hFOB conditioned media was measured by enzyme immunoassay as specified by the manufacturer (Amersham). Briefly, 5-50  $\mu$ l of hFOB conditioned media was incubated at 25°C for 60 min with a mouse anti-PGE<sub>2</sub> antibody and PGE<sub>2</sub>-horseradish peroxidase conjugate in a 96 well plate coated with goat anti-mouse IgG. The plate was washed 4 times with 10 mM phosphate buffer containing 0.05% (v/v) Tween 20, then substrate (3,3',5,5'-tetramethylbenzidine/hydrogen peroxide in 20% (v/v) dimethylformamide) was added and incubated for 30min at 25°C. The reactions were stopped with 1M sulphuric acid (0.25M final) and the absorbance at 450nm was measured with an ELISA plate reader. The amount of bound PGE<sub>2</sub> for each sample was compared to a standard curve of known standards, including a 0 standard. Non-specific 0 was determined with samples lacking anti-PGE<sub>2</sub> antibody, and media background was determined using base (non-conditioned) media.

#### Results

To determine whether the rate of hFOB glucose utilization is altered during spaceflight, conditioned media fractions were collected from flight and ground based control cells prior to lift-off, during orbit, and after landing and analyzed for glucose content. Both flight and ground controls metabolized glucose at a steady and nearly identical rate (fig.1) from the day of lift-off (day 0) until day 12 of orbit, after which glucose utilization appeared to have slowed to a similar extent in both groups until landing (day 18).

To assess the effect of spaceflight on matrix production in hFOB cells, the level of type I collagen accumulation in conditioned media was quantitated by radioimmunoassay. A high level of collagen accumulation in both flight and control media was detected by day 4 of orbit and continued to increase steadily to day 12 (fig.2). The levels of collagen after landing (days 18,19) were similar to or slightly lower than at day 12, suggesting that a decrease in the rate of collagen synthesis or an increase in collagen degradation occurred between day 12 and landing. There was an apparent reduction in collagen levels in flight cell media at day 18, but not at day 19. However, due to the loss of two day 18 ground control samples, it is not known if this is statistically significant. The overall rate of collagen accumulation in flight and control cells were indistinguishable, consistent with similar rates of collagenous matrix production.

To examine whether spaceflight affected the production prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) by hFOB cells, the accumulated level of PGE<sub>2</sub> by flight and control cells was measured by enzyme immunoassay (EIA). The level of PGE<sub>2</sub> measured in media from day 0-day 8 cells was below detection limits or very low, but was detectable (~1 ng/ml) in day 12 media, and high (~10-30 ng/ml) in day 18-19 media (fig.3). No significant difference in PGE<sub>2</sub> levels was detected in media from flight or control cells at days 12,18, or 19.

The effect of spaceflight on the gene expression of osteoblastic phenotypic markers was assessed by northern analyses of total RNA isolated from flight and control cells on day 18 and 19. The RNA in two of R + 8 hr (day 18) ground control samples was degraded. The remaining two samples did not differ from the R 24 hr ground controls for any of the measurements. These two ground control groups were subsequently combined for comparisons with the flight cultures.

These data indicate that the steady state level of osteonectin (ON) mRNA decreased dramatically, whereas the levels of alkaline phosphatase (AP) and collagen mRNA decreased slightly from day 0 (baseline) to day 18-19 (fig. 4), when normalized to 18S rRNA. Similar levels of collagen AP and ON were measured in flight and control cells at day 18-19. However, there was a trend towards lower levels of osteonectin ( $p < .06$ ) and collagen ( $p < .09$ ) mRNA levels in flight cells on day 19. Additionally, the steady state level of osteocalcin mRNA in flight and control cells was similar in day 18-19 cells as measured by RNase protection assay (fig. 4).

The effect of spaceflight on cytokine gene expression was measured by RNase protection assay. These data indicate that the steady state level of TGF- $\beta_2$  mRNA decreased dramatically from day 0 (baseline) to day 18-19 (fig. 5), whereas TGF- $\beta_1$  mRNA levels decreased slightly, when normalized to rpl32. The level of TGF- $\beta_2$  mRNA decreased slightly (~30%) in flight cells compared to ground controls (fig. 5), although this decrease was only statistically significant ( $p < .05$ ) in day 19 cells. Similarly, TGF- $\beta_1$  mRNA levels decreased slightly (~20%) in flight cells, but this decrease was only significant ( $p < .05$ ) in day 19 cells. The steady state levels of mRNA for several interleukins were also measured. These data indicate that there was a transient decrease in IL-1 $\alpha$ , (~70%) and IL-6 (~50%) mRNA levels in day 18 cells (fig. 6). Similarly, there was a trend towards a transient reduction in IL-12 ( $p < .07$ ) and IL-1 $\beta$  ( $p < .06$ ) mRNA levels. In all cases, IL mRNA levels returned to near normal levels in day 19 cells.

Scanning electron microscopy demonstrated no qualitative morphological difference between cells flown in space and those maintained on earth (fig. 7). Cellular ultrastructural details could not be examined at this time since it was not possible to employ transmission electron microscopy on these specimens. Figure 8 clearly demonstrates the extensive cell/bead clumping observed in both ground-based and spaceflight-flow cultures.

### Discussion

The deleterious effects of weightlessness on human and animal skeletal tissues are quite dramatic. However, the mechanisms which control the response of bone to mechanical loading or unloading are poorly understood. To determine whether isolated human osteoblastic cells are directly affected by weightlessness, several cellular parameters were measured in cultures subjected to a 17 day space flight. These data indicate that glucose utilization, type I collagen and PGE<sub>2</sub> accumulation, and gene expression for several bone matrix proteins were essentially unaffected by prolonged spaceflight. The lack of effect on glucose utilization suggests, albeit indirectly, that the rate of cell proliferation in flight and ground control cultures was similar. Histological studies in young rats suggest that the reduction in bone formation during spaceflight is not due to a decrease in osteoblast number (4,5). Therefore, it is possible that osteoblast activity is reduced during spaceflight rather than osteoblast number. To address this possibility, several parameters of osteoblastic cell activity were measured. Both type I collagen and PGE<sub>2</sub> accumulation were unaffected in hFOB cells during spaceflight. Similarly, the steady state mRNA levels of osteocalcin, alkaline phosphatase, and osteonectin were unchanged after spaceflight. Finally, there was no qualitative differences in the morphology of the cells and extracellular matrix between the flight and ground control cultures. These findings contrast with the decrease in osteocalcin and type I collagen mRNA levels in rat bone (6-9). Thus, it is possible that hFOB cells have a reduced ability to respond to weightlessness with regard to bone matrix protein synthesis.

The lack of a direct effect of weightlessness on osteoblast growth and matrix production is not totally unexpected. The force imparted by earth's gravity on an individual osteoblast is less than  $1 \times 10^{-10}$  of the force on an adult human. The direct effects of gravity on osteoblasts on a bone surface would result in strains of  $\sim 10^{-2}$  microstrain, a value which is  $\sim 10^{-5}$  of that imparted by the indirect actions of gravity. These indirect actions are primarily due to ground reaction forces and

muscle loading (10). Therefore, dynamic loading rather than static loading would be considered the predominant factor regulating of bone mass.

As discussed in the Introduction, other investigators have reported changes in osteoblast growth and/or differentiation following spaceflight (11-13). We cannot rule out the possibility that osteosarcoma (ROS 17/2.8 and MG63) and spontaneously transformed (MC3T3-E1) cells are more responsive to static gravitational loading than are fetal osteoblasts. Alternatively, there are many factors which could influence the growth and differentiation of cultured osteoblasts during spaceflight which are indirectly due to weightlessness. For example, buoyancy (resulting from the difference in density between the cells and the tissue culture media) and shear (due to circulation of media) forces depend upon the choice of media and culture system and are likely to be altered by spaceflight. Other factors which change during spaceflight, whose specific effects on cultured cells are difficult to evaluate, may include diffusion of gasses and nutrients and variations in temperature gradients. Thus, differences between the culture systems as well as between the cell lines could account for the reported differences in the response of bone cells to spaceflight.

As discussed above, the spaceflight-induced reductions in bone matrix synthesis and mRNA levels for bone matrix proteins in weight bearing bones of rats were not observed in cultured hFOB cells. Importantly, hindlimb unloading and disuse models for skeletal unloading, which are similar to ground based cell culture in that bone cells are subjected to static 1G load, result in bone changes similar to spaceflight (4,8,14-16). These differences indicate that gravitational loading per se is insufficient to maintain the skeleton; dynamic weight bearing is essential to maintain normal levels of bone formation.

Interestingly, steady-state mRNA levels for selected cytokines and growth factors believed to be important in bone remodeling differed between the flight and ground control cells following spaceflight. There was a small reduction in  $TGF\beta_1$  and  $TGF\beta_2$  mRNA levels in flight cells. These data are consistent with a previous report of decreased  $TGF\beta_1$  mRNA levels in rat bone (7). However, it is difficult to assess the physiological significance of such a small reduction in mRNA levels in hFOB cells. Also, the RNA was isolated 8-24hrs after reloading. Growth factor expression can change quickly in bone (17), so it is possible that the mRNA levels changed following spaceflight. Due to hardware limitations, in flight RNA isolation could not be performed in this study.

In addition to  $TGF\beta$  mRNA levels, the steady state mRNA levels of IL-1 $\alpha$  and IL-6 in hFOB cells were transiently, but significantly reduced following spaceflight. These findings contrast with Zhang and Turner (18) who found no effect of spaceflight on mRNA levels for IL-6 in proximal tibia of ovariectomized rats and an increase in mRNA levels for IL-1. Interestingly, Miller, et al. (19) reported that thymus cell, but not spleen cell production of IL-6, increased in rats subjected to a 7 day spaceflight. Since several interleukins are known regulators of bone remodeling, [for review see Horowitz (20)], it is possible that interleukin gene expression plays a role in mediating the response of osteoblastic cells to mechanical usage.

As discussed, the observed changes in gene expression are not necessarily due to changes in gravitational loading of the cells during spaceflight. On the other hand, as the osteoblast population increased and synthesized extracellular matrix, the Cytodex beads covered with osteoblasts began to adhere to one another, greatly increasing the effective mass and increasing the likelihood that changes in the net acceleration due to gravity would directly influence the cells. Furthermore, we cannot yet rule out the possibility that the changes in cytokine and growth factor expression may have been due to the brief transient increase in acceleration which occurred during reentry.

In summary, these studies suggest that response of hFOB cells *in vitro* to weightlessness during spaceflight is characterized by changes in cytokine gene expression rather than detectable changes in cell proliferation and function. Since there is scant data on the effects of loading or unloading on osteoblast cytokine gene expression *in vivo*, it is unclear whether these findings will extrapolate to intact bone. However, it is clear that cytokine production must be examined as a possible mechanism which mediates the effects of mechanical loading on bone.

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Figure Legends:

Figure 1. Glucose utilization. The mean level of glucose in hFOB conditioned media for each time point is shown. Launch was on day 0 and landing was on day 18. Error bars= SD , n=4 for day 0, day 18 flight, day 19 flight, day 19 ground, n=2 for all others.

Figure 2. Type I collagen accumulation. The mean level of type I collagen in hFOB conditioned media for each time point is shown. Error bars=SD, n=4 for day 0, day 18 flight, day 19 flight, day 19 ground, n=2 for all others.

Figure 3. PGE<sub>2</sub> accumulation. The mean level of PGE<sub>2</sub> in hFOB conditioned media for each time point is shown. Error bars=SD, n=4 for day 18 flight, day 19 flight, day 19 ground, n=2 for all others.

Figure 4. Bone matrix protein gene expression. A) The mean steady state level of alkaline phosphatase (AP), type I collagen (Coll), osteocalcin (OC), and osteonectin (ON) mRNA normalized to 18S rRNA is shown. Relative units are arbitrary based on phosphorimager analysis of northern blots and RNase protection gels with 100 equal to the mean level in ground control cells. Cont=ground control, FL+8=flight + 8hr (day 18), FL+24= flight + 24hr (day 19). Error bars=SEM, n=6 for ground control, n=4 for flight B) A representative northern blot is shown. RNA samples were hybridized with the indicated probes. The position of molecular weight size markers is denoted. BL= baseline, R+8= reentry + 8hrs (day 18), R+24= reentry + 24 hrs (day 19), GR= ground control, FL= flight.

Figure 5. TGF- $\beta$  gene expression. A) The mean steady state level of TGF $\beta$ 1 and TGF $\beta$ 2 mRNA normalized to L32 is shown. Relative units are arbitrary based on phosphorimager analysis of RNase protection gels with 100 equal to the mean level in ground control cells. Cont=ground control, FL+8=flight + 8hr (day 18), FL+24= flight + 24hr (day 19). Error bars=SEM, \* - p< .05, n=6 for ground control, n=4 for flight. B) A representative RNase protection analysis gel is shown. RNA samples were hybridized with the indicated TGF $\beta$  antisense RNA probes. GAPDH (glyceraldehyde 3-phosphate dehydrogenase) and rpL32 (ribosomal protein L32) probes were used as internal controls for sample loading. The position of molecular weight size markers are denoted. Probes were also hybridized to yeast RNA and incubated with or without RNase (No RNase) to show the position of digested and undigested probes respectively. BL= baseline, R+8= reentry + 8hrs (day 18), R+24= reentry + 24 hrs (day 19), GR= ground control, FL= flight.

Figure 6. Interleukin gene expression. A) The mean steady state level of IL-1 $\alpha$ , IL-1 $\beta$ , IL-6 and IL-12 mRNA normalized to L32 is shown. Relative units are arbitrary based on phosphorimager analysis of RNase protection gels with 100 equal to the mean level in ground control cells. Cont=ground control, FL+8=flight + 8hr (day 18), FL+24= flight + 24hr (day 19). Error bars=SEM, \* - p< .05, n=6 for ground control, n=4 for flight. B) A representative RNase protection analysis gel is shown. RNA samples were hybridized with the indicated interleukin antisense RNA probes. The position of molecular weight size markers are denoted. Probes were also hybridized to yeast RNA and incubated with or without RNase (No RNase) to show the position of digested and undigested probes respectively. R+8= reentry + 8hrs (day 18), R+24= reentry + 24 hrs (day 19), GR= ground control, FL= flight.

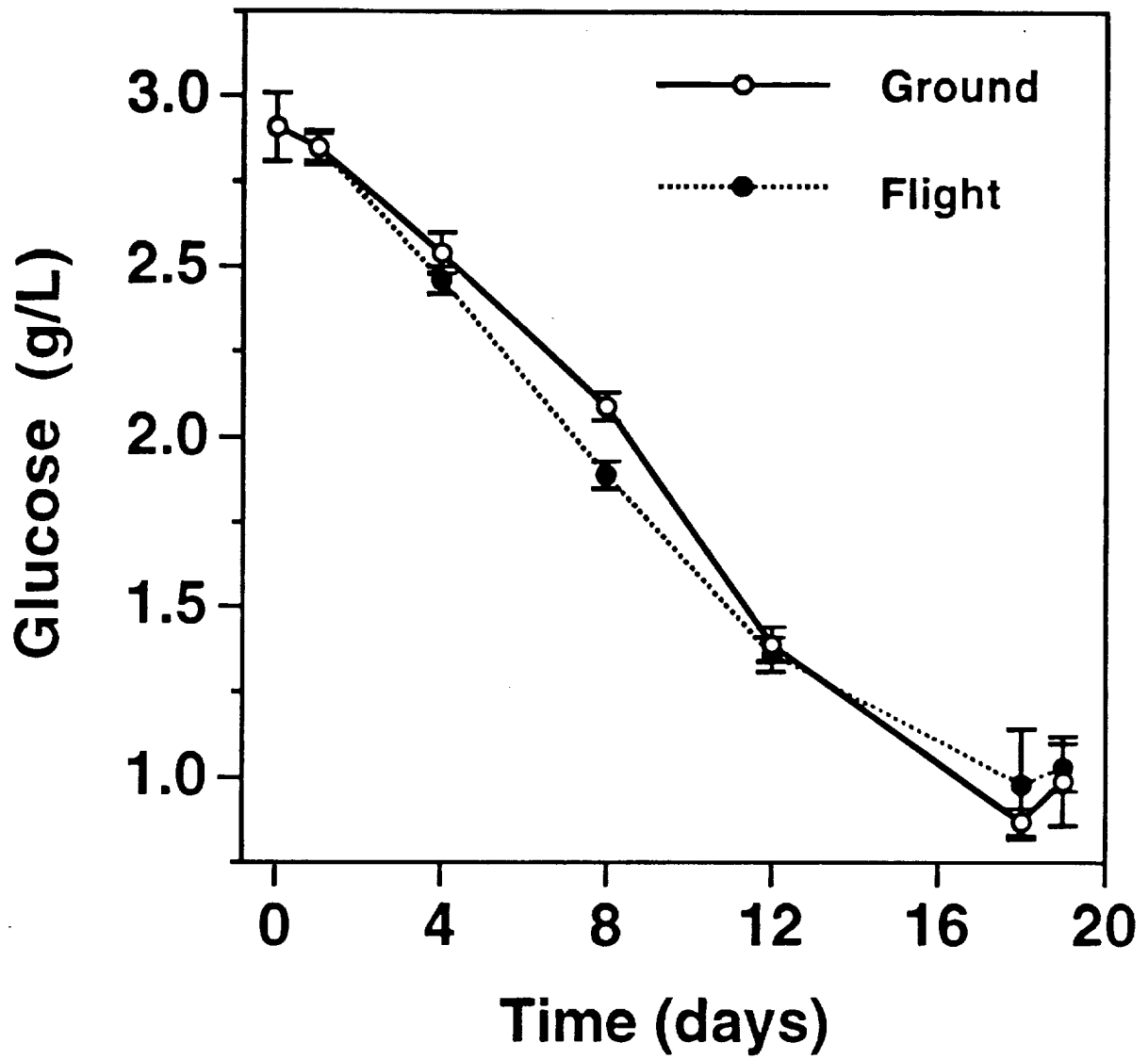
Figure 7. A representative scanning electron micrograph of hFOB cells adhering to a cytodex bead. Magnification is 2,300x.

Figure 8. A representative scanning electron micrograph of an aggregate of hFOB cells and cytodex beads. Magnification is 350x.

Publications:

1. Harris SA, Zhang M, Evans GL, Kidder LS, Spelsberg TC, Turner RT: The effects of orbital spaceflight on human osteoblastic cell physiology and gene expression. J Bone Miner Res, Submitted.

Figure 1



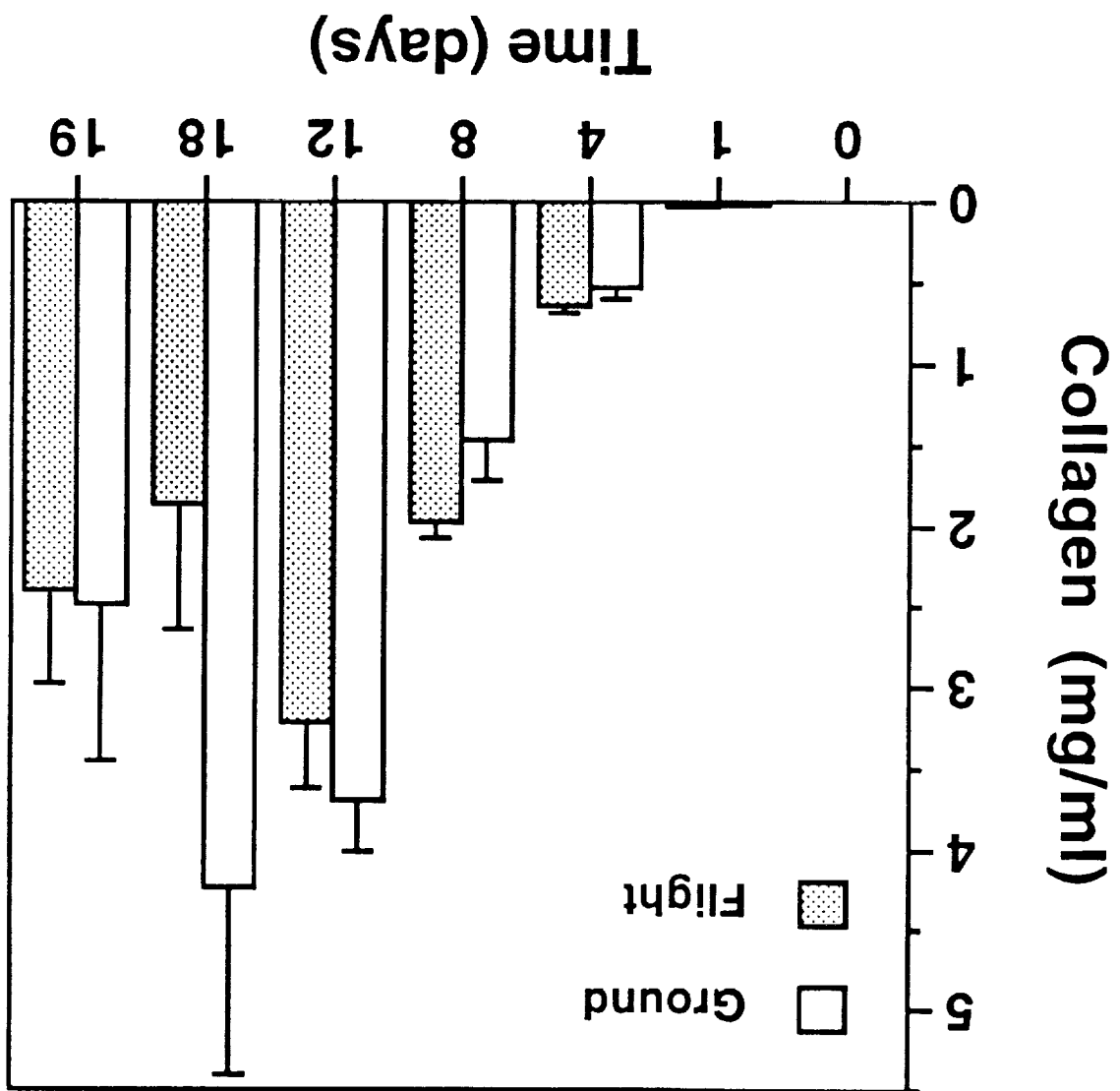


Figure 2

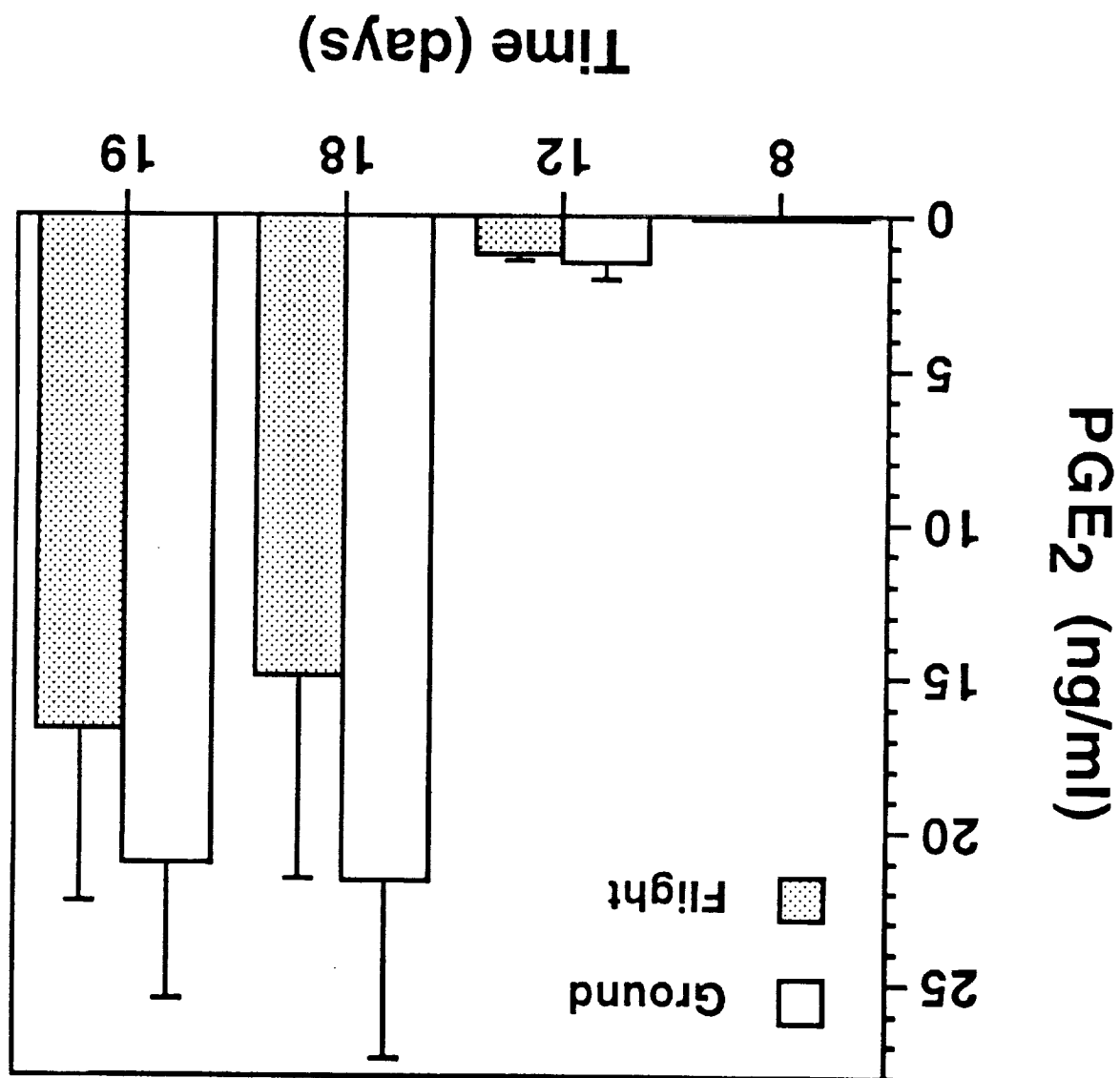


Figure 3

Figure 5

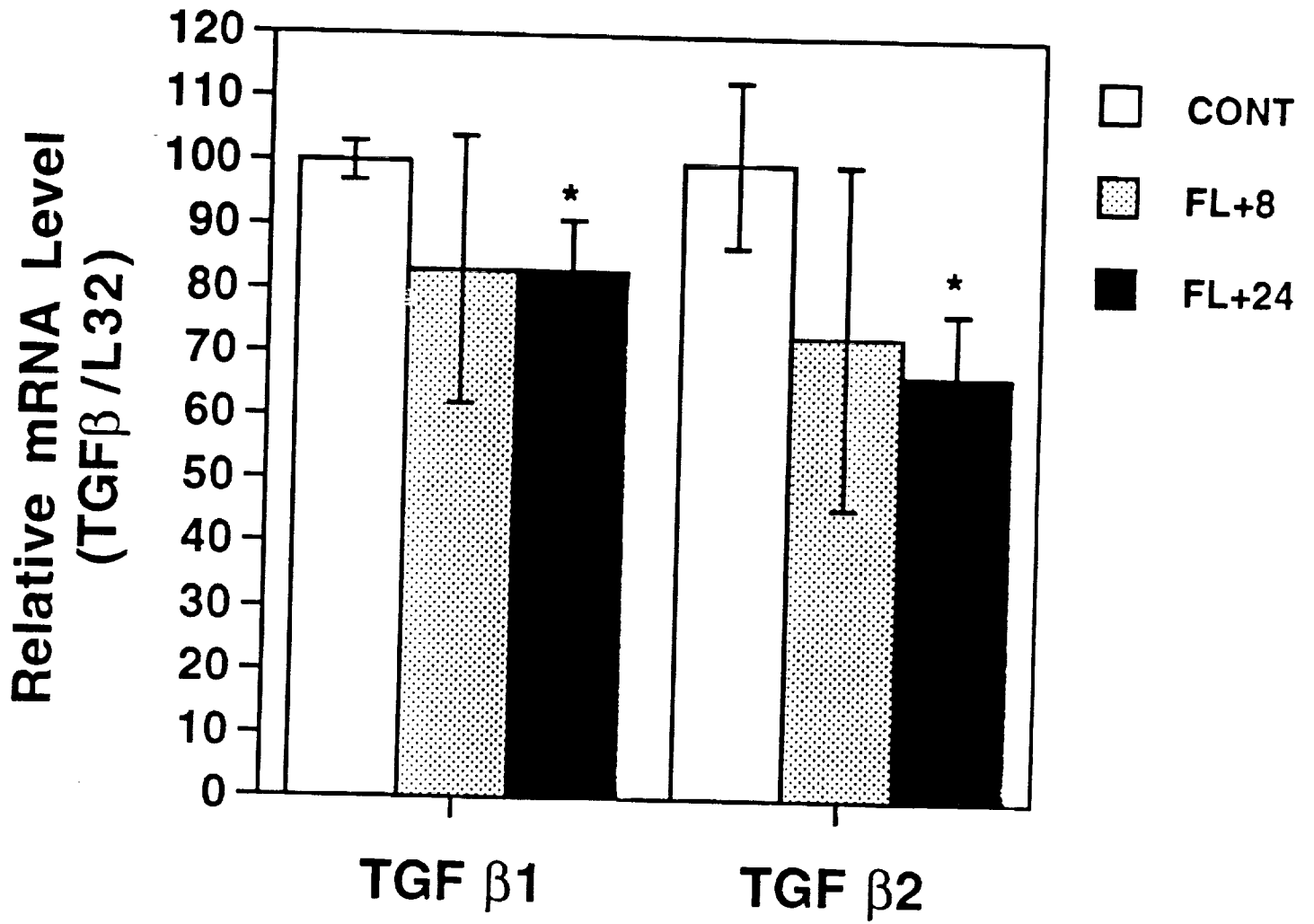


Figure 5B

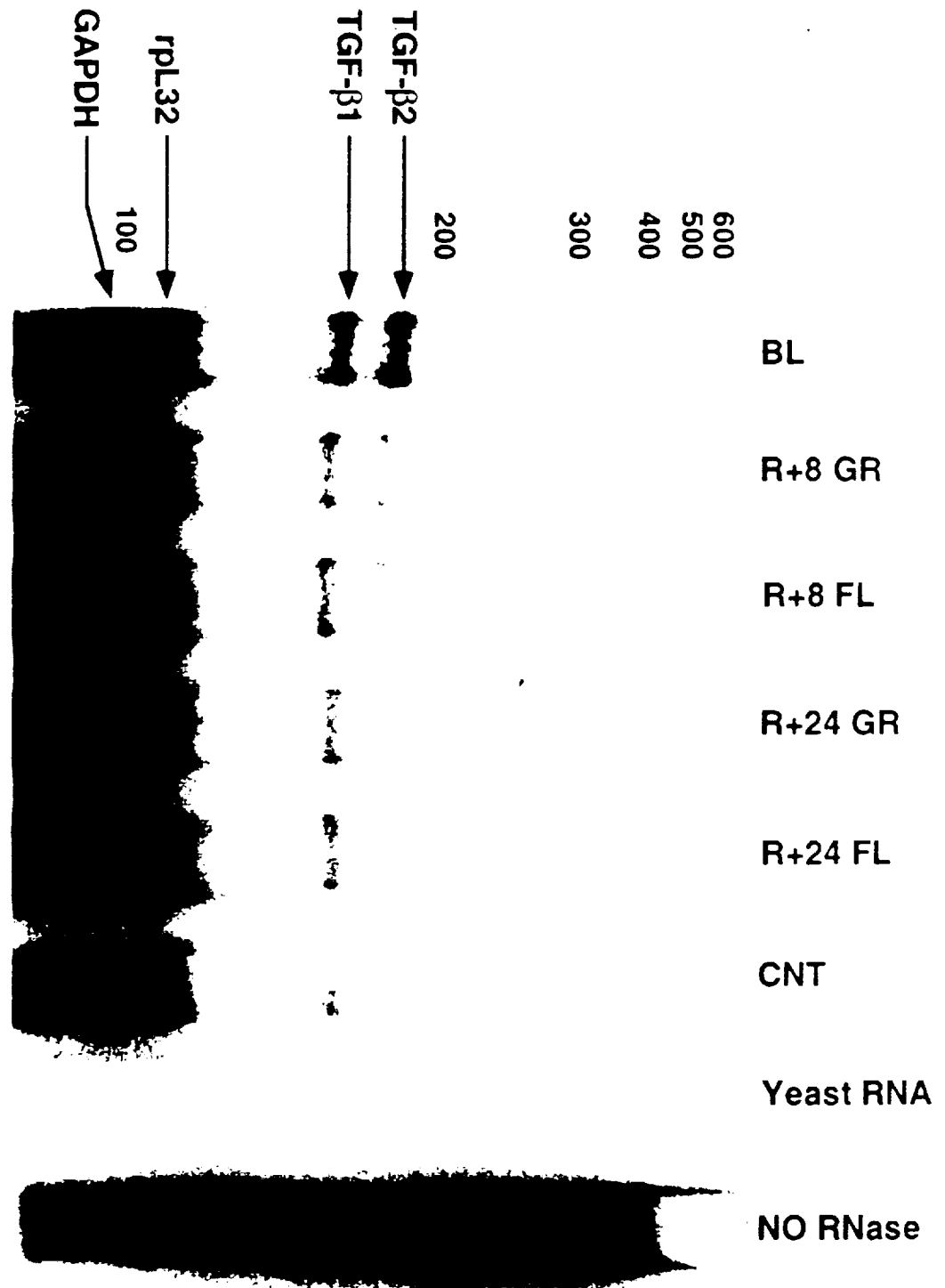


Figure 6

