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Mechanical stimulation and intermittent parathyroid hormone treatment induce disproportional osteogenic, geometric, and biomechanical effects in growing mouse bone

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Abstract

Mechanical loading and intermittent parathyroid (iPTH) treatment are both osteoanabolic stimuli, and are regulated by partially overlapping cellular signaling pathways. iPTH has been shown clinically to be effective in increasing bone mass and reducing fracture risk. Likewise, mechanical stimulation can significantly enhance bone apposition and prevent bone loss, but its clinical effects on fracture susceptibility are less certain. Many of the osteogenic effects of iPTH are localized to biomechanically suboptimal bone surfaces, whereas mechanical loading directs new bone formation to high-stress areas and not to strain-neutral areas. These differences in localization in new tissue, resulting from load-induced vs iPTH-induced bone accumulation, should affect the relation between bone mass and bone strength, or “tissue economy.” We investigated the changes in bone mass and strength induced by 6 wks mechanical loading, and compared them to changes induced by 6 wks iPTH treatment. Loading and iPTH both increased ulnar bone accrual, as measured by bone mineral density and content, and fluorochrome-derived bone formation. iPTH induced a significantly greater increase in bone mass than loading, but ulnar bone strength was increased approximately the same amount by both treatments. Mechanical loading during growth can spatially optimize new bone formation to improve structural integrity with a minimal increase in mass, thereby increasing tissue economy i.e., the amount of strength returned per unit bone mass added. Furthermore, exercise studies in which only small changes in bone mass are detected might be more beneficial to bone health and fracture resistance than has commonly been presumed.

INTRODUCTION

Osteoporosis is a bone disease characterized by low bone mass and deteriorated bone structure, resulting in increased susceptibility to fracture. Osteoporosis is a serious health threat for over 44 million people, 55% of whom are over 50 years of age.[1] While the severity and localization of the disease vary, most fractures resulting from osteoporosis occur in the spine, hip, and wrist. Osteoporosis increases mortality and morbidity; roughly 25% of individuals over the age of 50 die within one year of a hip fracture, and only 15% of

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patients can walk unaided 6 months after a hip fracture.[1] While most treatments for osteoporosis involve the use of anti-catabolic agents, including bisphosphonates and selective estrogen receptor modulators (SERMs), a truncated form of the human parathyroid hormone (PTH)—or teriparatide—is currently the only FDA-approved anabolic compound for the treatment of osteoporosis.

Intermittent PTH (iPTH) treatment has been shown clinically to be effective in increasing bone mass and reducing fracture risk.[2, 3] Although the molecular mechanisms of iPTH action on bone cells are incompletely understood, the tissue-level effects in patients are clear. iPTH increases bone formation on trabecular, endocortical, and periosteal surfaces.[4] Although some of the anabolic effects of iPTH on particular bone surfaces appear to be dependent upon prior resorption in the remodeling cycle,[5, 6] it is difficult to identify surfaces in the iPTH-treated skeleton that have not undergone enhanced bone formation. These observations are consistent with iPTH treatment having a global effect on the skeleton, i.e., PTH increases bone mass by adding bone to most, if not all, available surfaces of the skeleton. Functionally, this distribution pattern results in increased bone mass and strength in clinically relevant sites (e.g., hip, spine) but also in clinically irrelevant sites (e.g., mandible, skull, humerus) [7, 8]. Moreover, among the clinically relevant sites where iPTH generates new bone, it is unclear whether the bone is deposited along biomechanically advantageous (e.g., principal bending) axes, or whether it accrues only at currently modeling/remodeling sites, or perhaps even randomly.

Mechanical loading (exercise) is another osteo-anabolic strategy that can significantly enhance bone apposition, particularly during childhood and adolescence, and can also retard the loss of bone.[9] Much like iPTH signaling, the molecular mechanisms of mechanotransduction in bone cells are incompletely understood. Despite its well-documented osteogenic potential, exercise intervention studies in humans have yielded equivocal results in terms of improving bone mass, with the most efficacious studies reporting gains in aBMD of only a few percent at most.[10] Furthermore, a lack of prospective studies on exercise-induced fracture-risk reduction are lacking. Using the non-invasive rodent ulnar loading model, we previously reported that small gains in load-induced aBMD and BMC imparted very large increases in bone strength because the new bone formation was localized to the most biomechanically relevant sites.[11] Thus it might be possible to significantly enhance fracture resistance through mechanical loading (e.g., exercise), even if commonly used noninvasive measurements of bone mass or density (e.g., DXA) reveal only slight or negligible changes. This is perhaps an important difference between load-induced bone formation and pharmaceutically- (e.g., iPTH-) induced bone formation. Many of the osteogenic effects of anabolic compounds are localized to biomechanically suboptimal bone surfaces, whereas loading directs new bone formation to high-stress areas and not to strain-neutral areas. This potential difference in new bone distribution is postulated to change the “tissue economy,” i.e., the amount of strength returned per unit bone mass added.

We investigated the changes in bone mass and strength induced by 6 wks mechanical loading, and compared them to changes in bone mass and strength induced by 6 wks iPTH treatment, using low and high doses of each stimulus. We hypothesized that a mechanical stimulation would result in improved strength-to-mass ratio, as compared to iPTH treatment, largely as the result of favorable geometric changes. We found that loading and iPTH both increased bone accrual. iPTH induced a significantly greater (~3X) increase in bone mass than loading, but ulnar bone strength was increased approximately the same amount by both treatments, despite the much lower gains induced by loading. Our data suggest that mechanical loading can spatially optimize new bone formation to improve structural integrity with a minimal increase in mass, thereby increasing tissue economy.

MATERIALS AND METHODS

Animals

Fifty-six female C57BL/6J mice were purchased from Jackson Labs (Bar Harbor, ME) at 5 weeks of age. Twenty-four mice were chosen for the PTH experiment and 32 were chosen for the mechanical loading experiment. The mice were housed in cages of 3–5 and each mouse was given standard mouse chow (Harlan Teklad 2018SX; 1% Ca; 0.65% P; 2.1 iu/g vitamin D₃) and water *ad libitum* during the 5 week acclimation period and throughout the duration of the experiment. All procedures were performed in accordance with the Institutional Animal Care and Use Committee (IACUC) guidelines.

Intermittent parathyroid hormone treatment

Twenty-four mice were divided into 3 groups ($n=8$ /group) for daily human PTH 1–34 (Bachem, Torrance, CA) treatment. The treatment groups comprised vehicle treatment (0 μ g/kg/day), low dose intermittent PTH (10 μ g/kg/day) or high dose intermittent PTH (30 μ g/kg/day). Details on PTH preparation have been described previously.[12] PTH concentrations in the 10 and 30 μ g groups were adjusted weekly based on weekly body mass measurements. All PTH/vehicle injections were given subcutaneously once daily, 7 dy/wk for 6 wks, beginning when the mice reached 10 wks of age. Fluorochrome labels were injected intraperitoneally into all mice in the PTH study, in the following sequence: xylenol orange (90 mg/kg) on day 11 (11.6 wks of age) and alizarin complexone (20 mg/kg) on day 42 (16 wks of age; two days prior to sacrifice). Mice were sacrificed 44 days after start of PTH/vehicle injections. Post mortem, the left and right ulnae were dissected free of adhering soft tissues. The right ulna was processed for embedding in methylmethacrylate for histological purposes. The left ulna was wrapped in saline soaked gauze and saline and frozen at -20°C for radiography and mechanical testing (see below).

Mechanical loading *in vivo*

Thirty-two 10-week old mice were divided into high force and low force groups. Under isoflurane-induced anesthesia, the right forearm of each mouse was loaded 120 cycles/d using 2-Hz haversine waveform for 3 days with a day of rest in-between each loading day (Fig. A) as described previously.[13–15] The low force group began treatment at a peak force of 1.95 N; the high load force group began at 2.25N. Peak force was increased each week by 0.05 N to maintain consistent strain on the ulna during growth that occurs during 10–16 wks of age. At the conclusion of the experiment (week 6), the maximum force administered to the two load force groups was 2.2N for the low force group and 2.5N for the high force group. The forces used generated strains ($\sim 1800 \mu\epsilon$ and $\sim 2200 \mu\epsilon$) slightly greater than has been measured in human bone during vigorous activity [16]. The left arms of the animals were not loaded and served as an internal control for loading effects. All mice were allowed normal activity between loading bouts of treatment. Intraperitoneal fluorochrome injections were given in the following sequence: xylenol orange (90 mg/kg) on day 10 and alizarin complexone (20 mg/kg) on day 42. All animals were sacrificed 44 days after the first loading day. The right and left ulnas from half of the low force mice ($n=8$) and half of the high force mice ($n=8$) were dissected free of adhering soft tissues and processed for histology (see below). The left and right ulnas from the remaining mice ($n=8$ /group) were frozen in saline soaked gauze and at -20°C for radiography and mechanical testing (see below).

Ulnar areal bone mineral density (aBMD) and content (BMC)

Standard mouse DEXA instruments (e.g., pixiMUS, pDEXA sabre) for measuring bone mineral density and content in isolated mouse ulnae failed to provide sufficient resolution to

accurately detect the isolated ulnar bone profile, so a customized method for measuring aBMD and BMC was adopted using a high resolution digital x-ray cabinet (Faxtron MX-20; Faxitron, Inc., Lincolnshire, IL). Each ulna was positioned lateral side down and centered on the detector of the x-ray cabinet. A small custom-fabricated density standard was positioned next to each ulna while the radiograph was taken. The density standard comprised a disk with 5 hydroxyapatite cylinders, each of different density. Briefly, the hydroxyapatite (HA) cylinders were prepared using a polymer-ceramic slurry method. Sintered hydroxyapatite powder (Plasma Biotol Limited, North Derbyshire, England) was mixed with a 1:1 mass ratio of isobornyl acrylate esters (Sartomer Company, Exton, PA) and propoxylated neopentyl glycol diacrylate (Sartomer) to form slurries containing 1.35, 1.1, 0.85, 0.6, 0.35, and 0.1 g/cm³ hydroxyapatite ($\rho_{\text{hydroxyapatite}} = 3.14 \text{ g/cm}^3$; $\rho_{\text{monomer}} \approx 1 \text{ g/cm}^3$). Benzoyl peroxide initiator (Acros Organics, Geel, Belgium) and N,N – dimethyl – p – toluidine accelerant (ICN Biomedicals, Irvine, CA) were added to the slurry at 1% and 0.5% to the weight of the monomer, respectively. The slurry was then mixed and poured into 2 mm cylindrical teflon molds. The cylinders were allowed to cure for 10 min, embedded in methylmethacrylate, and sectioned using a diamond wafering saw. The finished density standard disk was laid flat on a pixiMUS densitometer, and the areal (2D) mineral density of each standard within the disk was measured on 3 separate scans.

Individual radiographs displaying the whole ulnae with adjacent mineral standard were imported into ImageJ, where each HA cylinder within the density standard disk was measured for pixel intensity and used to create an internal standard curve (pixel intensity vs. mineral content) for each radiograph. The pixel intensities within the ulnar profile were measured, as were the profile areas, and the pixel values were converted to mineral values using the internal standard curve (=BMC). aBMD was then calculated based on the calculated BMC and the measured bone profile area.

Bone formation measurements at the ulnar midshaft

Post dissection, the right and left ulnas from the loaded mice, and the right ulnas from the PTH-treated mice were cleaned of soft tissue, fixed in 10% neutral buffered formalin for 48 h, dehydrated in graded alcohols, cleared in xylene, and embedded in methyl methacrylate (Aldrich Chemical, Milwaukee, WI, USA). Using a diamond-embedded wire saw (Histo-saw; Delaware Diamond Knives, Wilmington, DE, USA), transverse thick sections (~70 μm) were removed from the ulnar midshaft. The wafers were ground to a final thickness of ~20 μm and were mounted unstained on standard microscope slides.

One section per bone was photographed digitally on a Nikon Optiphot fluorescence microscope (Nikon, Garden City, NJ, USA) equipped with a Spot RT fluorescence camera (Diagnostic Instruments, Inc., Sterling Heights, MI). The digitized fluorescent images were imported into ImagePro plus (Media Cybernetics, Inc., Gaithersburg, MD), in which the following primary data were measured manually on the periosteal surface: total perimeter; single label perimeter (sL.Pm); and total new bone area (area between the two labels).

Geometric properties at the ulnar midshaft

Prior to mechanical testing, the frozen ulnas removed from the both the PTH and loading study animals were scanned in the transverse plane through the midshaft on a desktop μCT (μCT -20; Scanco Medical, Bassersdorf, Switzerland) using 9 μm voxel size. The midshaft tomographs were imported into Scion Image 4.0.2 for Windows (Scion Corporation, Frederick, MD) equipped with a customized macro for calculating cortical area (Ct.Ar; mm²) and principal second moments of area (I_{MAX} and I_{MIN} ; mm⁴).

Axial compression tests of whole ulnas

Whole ulnas were brought from -20°C to room temperature slowly (~ 1.5 hr) in a saline bath. Subsequently, each bone was mounted, distal end down and posterior end up, between two opposing cup-shaped platens of a miniature materials testing machine (ElectroForce 3200; Bose Inc., Eden Prairie, MN), which has a force resolution of 0.001N. The bone was fixed in place using a ~ 0.2 N static preload and kept hydrated via a saline bath attached to the lower platen. The ulnas were loaded to failure in monotonic compression using a crosshead speed of 2 mm/s, during which force and displacement measurements were collected every 0.01 sec. From the force versus displacement curves, ultimate force (F_U ; in N) and energy to failure (U ; in mJ), were calculated using standard procedures.[17]

Statistical Methods

Effects of loading (loaded right ulna vs nonloaded left ulna) on mechanical properties, bone formation measurements, geometric properties, and bone mineral density/content were tested for significance using paired t-tests. Effects of iPTH treatment on the same outcome measurements were tested for significance using unpaired t-tests, where the treated groups (10 μg and 30 μg) were compared to the vehicle group (0 μg). The relation between bone mass measurements (aBMD, BMC) and mechanical properties (energy to failure) were tested for significance using reduced major axis regression (because of the error terms inherent to both axes). Analysis of covariance (ANCOVA) was used to test for differences among slope elevations when slopes were found to be statistically similar. For all tests, significance was taken at $p < 0.05$.

RESULTS

Intermittent PTH and mechanical loading are anabolic to bone, but not equally

To verify that both low and high doses of intermittent PTH (iPTH) and loading were anabolic to bone, we measured areal bone mineral density (aBMD) and content (BMC) in excised whole ulnae after 6 weeks of treatment. As expected, both low-dose and high-dose iPTH induced significant gains in whole-bone BMD and BMC, as compared to vehicle treated mice (Table 1). Mechanical loading of the right ulna at both low and high forces also induced significant gains in whole-bone BMD and BMC, as compared to the nonloaded left ulna. However, the load-induced gains in bone mass were significantly more modest than those incurred by iPTH treatment.

Similar to the gains reported for ulnar bone mass, both low-dose and high-dose iPTH induced significant increases in fluorochrome-derived bone formation parameters on the ulnar periosteal surface, as compared to vehicle-treated mice (Figure 1, left axis). *In vivo* mechanical loading of the right ulna at both low and high forces also resulted in a significant increase in ulnar bone formation parameters, but the magnitude of the increase was significantly less ($\sim 50\%$ less) than that observed for iPTH treatment.

Intermittent PTH and loading improve bone strength and geometry, but not equally

To explore the possibility that iPTH and mechanical loading, while both anabolic to bone, might have different effects on the structure and strength of the bone tissue, the iPTH-induced and load-induced changes in the second moments of area at the midshaft ulna and whole-bone mechanical properties were measured. Both low-dose and high-dose iPTH induced significant gains in the maximum second moment of area (I_{MAX}), reaching a 12–23% increase (Figure 2A). I_{MAX} was also improved significantly by mechanical loading at both forces, reaching 7–14% increase, but the load-induced changes were significantly less than those measured for iPTH. Conversely, iPTH-induced changes in the minimum second moment of area (I_{MIN}), were significant only for the high dose group ($\sim 24\%$ increase), while

among the loaded animals, I_{MIN} increased significantly for both low- (22% increase) and high- (35% increase) force groups (Figure 2B). Moreover, the I_{MIN} values measured for the loading groups were significantly greater than those measured for the iPTH-treated mice (compared across doses).

The anatomical basis for the discrepancies between greater improvement in I_{MAX} among the iPTH-treated mice, and greater improvement in I_{MIN} among the loaded mice, can be appreciated in Figure 2(C&D). Fluorochrome labeling reveals an even distribution of new bone formation around the cortex (or even a preference for formation along the major axis [along the dorsoventral plane]) among the iPTH treated mice, whereas loading preferentially added bone along the minor axis (in the mediolateral plane), with very little new bone added along the major axis.

In light of the stark contrast in geometric properties between iPTH and loading, and the effects they might have on bone mechanical properties, mechanical tests were performed on the whole ulnae. As expected, axial compression tests of the ulnae revealed significant gains in ultimate force and energy absorption among low- and high-dose iPTH-treated mice, reaching 12–26% improvement in those properties (Figure 1, right axis). Similarly, mechanical tests conducted on the ulnae from the loaded mice showed significant gains in ultimate force and energy absorption in both load groups, reaching 14–28% improvement in those properties. No significant difference in ultimate force or energy absorption was detected between loaded- and iPTH-treated ulnae (compared across doses).

Evaluation of tissue economy: the relation between gains in bone mass and gains in bone strength

Because it is difficult to compare the doses chosen for loading with the doses chosen for PTH, in terms of their equivalence in stimulatory effects on the anabolic processes, the amount of bone gain was compared to the degree of strength gain, for each of the groups evaluated. Regression of the increase in bone mass (BMD and BMC) against the increase in bone strength (e.g., energy absorption) revealed a significant relation for both iPTH ($r^2=0.63-0.65$) and loading ($r^2=0.46-0.58$). However, comparison of the intercepts *via* ANCOVA revealed that loading increased bone strength to a similar degree as iPTH, but did so at significantly lower gains in bone mass (significantly lower slope elevation). In other words, a 20% increase in bone strength was achieved via loading by increasing BMC by only ~4%, whereas the same 20% increase in bone strength induced by PTH required an 11% increase in BMC. Thus, loading appears to be more efficient at improving bone strength, using significantly less tissue, than iPTH.

DISCUSSION

We undertook an investigation into the differences in effectiveness of two anabolic treatments—mechanical loading and intermittent PTH (iPTH)—on bone formation and strength, with the goal of elucidating the functional consequences of site-targeted vs uniformly deposited bone formation. Numerous previous experiments have shown that both anabolic stimuli can increase bone formation and strength significantly, but the two treatments are known to add bone in different amounts and according to different distribution patterns. We sought to determine how these differences affected the relation between bone mass gain and bone strength gain. Our results indicate that while iPTH treatment induced significantly greater bone gain (e.g., BMC, BMD, newly labeled bone) than mechanical loading, the structural consequences were not different between the two stimuli. In other words, mechanical stimulation improved bone strength to the same degree as iPTH, but did so at less than half of the bone gain as that required by PTH.

The large gains in strength, despite relatively small gains in mass, observed among the loaded mice appears to be related to the optimization of the bone's geometry. We found that loading improved I_{MIN} to a significantly greater extent than did iPTH treatment. Because of the morphology of the ulnar shaft, the I_{MIN} plane of bending experiences the greatest strains during axial loading. This is a common phenomenon in vertebrate long bones, where bending is directed or channeled to a predictable orientation.[18] Consequently, the addition of bone along this axis would have disproportionately greater effects on resistance to bending, and ultimately, strength. iPTH treatment, on the other hand, appears to have added bone uniformly around the periosteal perimeter (Fig. 2C & 2D), which had the effect of increasing I_{MAX} to roughly the same extent as I_{MIN} . Because the addition of bone to the I_{MAX} plane contributes relatively little to bending resistance to axial loads, the iPTH-induced increases in I_{MAX} yielded little return in mechanical properties.

In a previously reported rat ulna loading experiment conducted over 16 weeks, we found a 7% load-induced increase in ulnar BMC, and a concomitant 64% and 94% load-induced increase in ultimate force and energy to failure, respectively.[11] Those large increases in bone strength despite meager increases in bone mass accrual are consistent with the mouse ulna data presented here, with a few exceptions. In the current (mouse) study, we found smaller load-increases in both DXA-derived bone accrual (1–4% increase) and mechanical properties (15–30% increase). The reduced overall response in the mouse study is probably the result of (1) the duration of the study; the rat study lasted 16 wks whereas the mouse study lasted only 6 wks, and (2) the daily loading bout duration, which was 66% less in the mouse study. Nevertheless, the data are proportionally consistent, in that both loading studies revealed a ~10-fold greater increase in strength compared to the increase in DXA-derived bone mass accrual. In the current study, we were able to gauge the impact of this result (i.e., mass-to-strength ratio induced by loading) by conducting a parallel experiment using another potent anabolic stimulus—iPTH. Mechanical loading induced similar gains in bone strength as iPTH, but did so using a fraction of the bone mass.

There are some data supporting a synergistic effect of PTH and mechanical stimulation,[19–21] and further, that loading can to some extent localize the anabolic effects of iPTH to biomechanically relevant sites.[22] However, it is unclear how these two anabolic stimuli, used in conjunction change the mechanical properties or tissue economy. Furthermore, the desensitization dynamics associated with these two anabolic treatments could have significant consequences on mechanical outcomes in the long-term. For example, we have found that bone cells desensitize to mechanical stimulation after several weeks of daily signaling.[23, 24] Likewise, iPTH treatment has been shown to induce tachyphylaxis on several time scales.[25, 26] It is not known whether these anabolic treatments will continue to yield the same disparities in mechanical properties when employed in the long-term. There is, however, data to suggest that loading effects on bone strength are maintained in the long term because of the positive geometric changes involving the periosteal surface.[27]

In conclusion, we found that intermittent PTH and mechanical loading, while both anabolic to growing bone, resulted in significantly different gains in bone mass and geometry, but statistically similar gains in bone strength. These observations suggest that mechanical loading induces a more favorable tissue economy (strength per unit mass) than iPTH. Furthermore, our data indicate that exercise studies in which only small changes in aBMD or BMC are detected might be more beneficial to bone health and fracture resistance than has commonly been presumed. We found that very modest changes in aBMD and BMC can translate into large changes in mechanical properties because mechanical loading tends to add bone to the most structurally relevant loci. If these observations can be translated to human bone, the benefits of exercise to bone health might have previously been vastly underappreciated. Harnessing the highly specific localization properties of mechanical

loading, in conjunction with the prolific anabolic effects of iPTH, might be a potentially high-yield approach to improving bone strength, regardless of changes in mass.

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Figure 1.

Six weeks of *in vivo* mechanical loading induced significant increases in bone formation (labeled surface and new bone area; left axis, solid bars) in a load-dose dependent manner (indicated by *). Treatment with intermittent PTH (iPTH) for the same period induced also induced significant dose-dependent increases in bone formation (indicated by *), but to a greater extent than induced by loading (indicated across dose groups by #). Among the loaded mice, the mechanical properties ultimate force and energy to failure (right axis, hatched bars) followed similar trends as the histomorphometry, but among the iPTH-treated mice, mechanical properties did not increase nearly as much as bone formation, reaching the statistically equivalent level as was found in the loaded mice. Means and standard errors are depicted.

Figure 2.

(A) The maximum second moment of area (I_{MAX}) at the midshaft ulna was enhanced significantly by mechanical loading and iPTH treatment (indicated by *), but iPTH induced significantly greater increases in I_{MAX} than loading (indicated across dose groups by #). (B) The minimum second moment of area (I_{MIN}) at the midshaft ulna was enhanced significantly, and to a much greater extent than I_{MAX} , by mechanical loading. iPTH treatment induced a significant increase in I_{MIN} only among the high-dose mice, which reached a lower magnitude than was found for the high force loaded mice (indicated across dose groups by #).

Figure 3.

Photomicrographs taken under filtered fluorescent light of the midshaft ulna from 16 wk old mice in the iPTH experiment (panels A and B) and the ulnar loading experiment (panels C and D). Panel A is from a mouse in the vehicle-treated group, and panel B is from a mouse in the high dose iPTH group. Panels C and D are from a single mouse in the high-force loading group (panel C is the left, nonloaded ulna, and panel D is the right, loaded ulna). Labeling in the control images for both treatments (left panels) illustrate the small amount of growth that occurred during the experimental period, which was confined mainly to the dorsal cortex. Note the large area between the labels in the I_{MIN} plane (mediolateral plane) in the loaded section, compared to the more even distribution of bone formation in the iPTH section (right panels) These differences in localization of stimulus-induced new bone formation (e.g., prolific bone formation along the mediolateral axis in the loaded mice) are likely responsible for the large increase in bone strength in spite of minimal changes in new bone formation. M=medial; L=lateral; D=dorsal, V=ventral.

Figure 4.

Both iPTH- and load-induced gains in bone mass (aBMD, panel A; BMC, panel B) were positively associated with increases in energy to failure. However, the elevation of the iPTH slope was significantly higher than that for loading, in both comparisons, indicating that loading required far less new bone mass to achieve the same strength benefit as did iPTH.

Table 1Summary of treatment-induced changes in ulnar areal bone mineral density (aBMD) content (BMC)^{a,b}

Treatment		
<i>Group</i>	<i>%Δ in aBMD</i>	<i>%Δ in BMC</i>
Loading		
<i>Low Force</i>	1.31 ± 0.68 *	2.73 ± 0.95 *
<i>High Force</i>	2.36 ± 0.56 *	4.16 ± 0.96 *
PTH		
<i>Low iPTH</i>	4.42 ± 0.53 *	9.75 ± 0.89 *
<i>High iPTH</i>	7.61 ± 0.81 *	14.90 ± 1.28 *

^a mean and standard error are reported^b* indicates significantly different from zero