INTRODUCTION

As important as systemic factors, local mechanical milieus influence bone morphology and adaptation. Exercise is one of the key factors influencing the local mechanical milieu and can be a non-pharmacological tactic to decrease the risk of age-related osteoporoses. As bone mineral density in old age is a function of the amount of mineral accrued during growth and the rate of bone loss following growth, exercise can influence bone mass by two different methods. The benefits of exercise in females can be most effective if training was initiated at or before rather than after menarche (Haapasalo et al., 1994). Increased bone mineral density, and hence strength, resulting from exercise has been reported to persist for 20 years following cessation of exercise (Bass et al., 1998). However, the osteogenic response associated with exercise varies with different exercises. Impact exercises have been shown to be more osteogenic than low impact aerobic exercises in humans (MacKelvie et al., 2002; Courtiex et al., 1998), possibly by stimulating a higher bone turnover in favor of bone apposition (Jaffre et al., 2001). Nonetheless, it is apparent that high impact exercises may not be safe for aged individuals with compromised bone structures. Thus, the osteogenic component(s) of exercise regimes must be determined. Impact exercises are characterized by high strain rates but are also associated with higher strain gradients, which may additionally modulate adaptation (Judex and Zernicke, 2000). Furthermore, bone adaptation to mechanical loading has also been shown to vary with the time course of application. Mechanical stimulation prescribed in short discrete bouts has been shown to be more osteogenic than a single, extended bout of mechanical stimulation (Turner and Robling, 2003). Within the context of remodeling, the complex relations between mechanical loading and bone adaptation remain to be fully elucidated.

In vivo controlled exogenous loading regimes are advantageous in that specified aspects of the applied load, such as loading rate, may be isolated and varied. Using these regimes in animals, Hert and colleagues demonstrated that dynamic, rather than static loads were osteogenic (Hert et al., 1971). Moreover, Turner and colleagues showed that the osteogenic response of the rat tibia in response to four-point bending was directly proportional to the strain rate in the bone (Turner and Robling, 1995). Building on the benefits of in vivo controlled loading regimes and reports that higher strain rates are more osteogenic than lower ones, we hypothesized that the osteogenic adaptation of mice tibiae in response exogenous cantilever bending loads would increase with higher rates of loading.

Hence, we applied exogenous loads to tibiae of three groups of mice. Strain was applied at low, medium and high rates; the latter two were comparable to normal ambulation and impact loading recorded in mice (Lee et al., 2002). We found that higher strain rates would effectively stimulate osteogenic adaptive responses. Furthermore, bone adaptation was found with the mature skeleton, emphasizing the positive potential for exercise regimes to counter age-related bone loss.

METHODS

Murine loader- A non-invasive in vivo murine loader was designed and modified from a previously published model (Figure 1; Gross et al., 2002). Programmable waveforms were sent as input to control the linear force actuator (AFX10-200; Motran Industries Inc., USA) output through a 16-bit data acquisition processor (DAP 820; Microstar Laboratories, USA). The data acquisition processor was operated through DAPview on a windows based PC.

Waveform calibration- Five skeletally mature (16wk) C57BL/6 mice were used for waveform calibration. Immediately following euthanization by CO₂ overdose, single element strain gauges (0.25mm width; FLK-1-11, TML, Japan) were secured (CN adhesive, TML, Japan) to the lateral tibia 1 mm proximal to the tibia/fibula junction (that location was flat and allowed secure strain gauge fixation). Mice were then secured in the loader, loaded, and unloaded five times. Trapezoidal waveforms with a frequency of 1 Hz induced peak magnitudes of 1000 με pooled within and across mice. That magnitude was chosen as it was within the bounds of magnitudes encountered during daily loading (Rubin, 1984) Strain rate was the only parameter of the applied load that varied across mice. Loads were applied at low (0.004/s), medium (0.02/s), or high (0.1/s) strain rates.

Animal model- Skeletally mature (16 wk) female C57BL/6 mice were randomized into three groups based on strain rate: low (n=14), medium (n=15), and high (n=14) strain rates. Pilot data showed that securing the tibia in the loader had no effect on static and dynamic indices of bone formation and
that the left limb was representative of the unloaded situation. Mice underwent non-invasive exogenous loading of the right tibia in accordance with their strain rate assignment for 60 seconds, 5 days/week, for 4 weeks. Calcein injections (IP; 10 mg/kg) were administered on days 1 and 18 of the 28 day loading protocol.

Tibial assessment- Upon successful completion of loading regiments, mice were euthanized, right and left tibiae were dissected clean of adherent non-osseous tissue, and 350 µm wafers of bone from the left and right tibiae corresponding to strain gauge position were cut with a diamond wafer saw (Buehler Isomet, USA). Wafers were subsequently ground to 50 µm using 1500 grit wet sandpaper with 70% EtOH used as a lubricant. Sections were mounted in Permount (Fisher, Canada) and viewed at 200X with a mercury vapour fluorescent light source (blue excitation, 400-800 nm; Figure 2). Images were captured with a digital camera (Sony DXC-950P, Tokyo, Japan) and analyzed histomorphometrically with Image J (NIH, USA) following previously established methods (Judex and Zernicke, 2000; Parfitt et al., 1987). All measures were done with the observer blind to the specimen identity.

Statistics- Differences between loaded (right) and unloaded (left) tibiae within each group were assessed using Wilcoxon Signed Ranks Tests. Differences between loaded tibiae in the three groups were assessed using Kruskal-Wallis tests with Mann-Whitney tests used as a post hoc follow up. For all tests, p < 0.05 was considered significant.

RESULTS AND DISCUSSION

Body mass was not significantly different among low (24.9 ± 1.7 g), medium (23.25 ± 1.1), and high (23.3 ± 1.0) strain rate groups.

Figure 2: Exemplar tibial middiaphyseal calcein labeled cross sections in a loaded (right) and an unloaded, control (left) limb. Note the double label in the loaded tibia. This double label is absent from the control bone section. a (anterior), p (posterior), m (medial), and l (lateral).

Four weeks of exogenous loading significantly augmented periosteal mineral apposition rate (MAR) in low (>98%), medium (>42%), and high strain rate loaded tibiae (>62%). Periosteal MAR of loaded tibiae was significantly higher than medium (>29%) and low (>27%) strain rate groups. Periosteal surface referent bone formation rate (BFR•BS-¹) was significantly elevated in the loaded tibia in all three groups and was significantly greater in the high strain rate loaded tibiae than the low and medium strain rate means (Fig. 3a). Medium (>103%) and high (>127%) strain rates significantly increased endosteal MAR. Similarly, only medium and high strain rates significantly enhanced endosteal BFR•BS-¹ (Fig. 3b).

Figure 3: Periosteal (a) and endosteal (b) bone formation rates in low, medium, and high strain rate groups for both the left (L) and right (R) tibial middiaphyses. Values are means ± SD. *Significantly different from left tibia. #Significantly different than high strain rate right tibia.

Resistance to bending had geometrical and material components. Geometrically, the most effective way to minimize bending stress was to concentrate material farther away from the bending axis, and that represented the periosteal surface. As cross sectional moment of inertia is to the fourth power of the radius, a little bone apposition may result in a significant reduction in bending stress. Thus, higher strain rates most effectively stimulated bone cells to adapt.

The precise stimulus mediating bone adaptation remains unascertained but other research suggests that deformation-induced fluid flow can be key for an adaptive response. Bone is a porous media filled with fluid. When mechanically deformed, such as those deformations incurred with exercise, pressure gradients build within the bone tissue. Compressive regions develop high hydrostatic pressures whereas regions in tension develop low hydrostatic pressures. To equalize these pressure differences, fluid within the bone flows from regions of high hydrostatic pressure to regions of low hydrostatic pressure. The resulting fluid velocities during deformation are proportional to strain rate (Turner et al., 1995).

Bone fluid flow may mediate bone adaptation via three mechanisms. Deformation induced fluid flow significantly enhanced the transport of a large molecular weight (1800 Da) tracer within the lanuno-canaliculur system (Knolte Tate et al., 1998). Thus, fluid flow may assist in essential metabolite transport (e.g. nutrients, growth factors) to the cells and waste removal from the cells. Alternatively, the flow of fluid past cell membranes may directly stimulate cells via fluid shear stresses or streaming potentials. Larger strain rates result in a greater velocity fluid flow, which is associated with larger shear stresses (Turner et al., 1995) and streaming potentials (Otter et al., 1992). In vitro, it has been shown that bone cells exposed to larger shear stresses elicit a greater adaptive response as assessed by nitric oxide and prostaglandin E(2) synthesis (Bakker et al., 2001). The flow of an ionic fluid (i.e., bone fluid) confined in a charged porous matrix (i.e., bone matrix) causes an electric potential referred to as a streaming potential. Electric currents have been used with some success to treat fracture non-unions (see Ryaby, 1998 for review) and hence, these streaming potentials may influence local cell activities and modulate bone adaptation. Streaming potentials can increase with strain rate (Otter et al., 1992), but the relation between
streaming potentials and *in vivo* strains are not as simple as anticipated (Beck et al., 2002).

**SUMMARY**

The current study was the first to quantify the effect of strain rate on bone adaptation using controlled cantilever bending. Our experimental system isolated strain rate from other variables implicated in bone adaptation. We demonstrated that higher strain rates, within limits, would effectively stimulate osteogenic adaptive responses. Furthermore, bone adaptation was found with the mature skeleton, emphasizing the positive potential for exercise regimes to counter age-related bone loss.

**REFERENCES**

Beck et al. (2002) *Calcif Tissue Int* 71: 335-43


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