Development of a Knee-Loading Joint Supporter for Potential Use in Preventing Bone Loss during Spaceflight/Aging

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Abstract As a countermeasure for bone loss experienced by astronauts during the microgravity of spaceflight, and by aging patients (e.g., osteoporosis), the designs of devices for the application of lateral loads to the knee joint are described. These “knee-loading joint supporters (KJS)” are intended to clamp onto the human knee and provide variable, intermittent pulses of mechanical load. Based on previous experimentation with mouse models, it is expected that these KJS devices will facilitate human bone strengthening.

Key words; knee, bone formation, tibia, spaceflight

Introduction.

The goal of the present study is to develop and test an innovative “knee-loading joint supporter” (KJS) as a therapy for strengthening bone for possible use by astronauts to prevent bone loss under the weightless conditions of spaceflight, and by aging patients who experience similar bone weakening. With regard to spaceflight, the main load bearing bones are the femur and tibia, and hence they can be expected to be seriously affected by the microgravity of spaceflight. In fact, most bone loss in astronauts has been observed to occur in those leg bones as well as the lumbar vertebrae. Indeed, estimates (e.g., LeBlanc *et al.*, 2000) reveal that bone mass loss can approach dangerous levels (perhaps up to 1-2 % every few months of long duration flights). That bone loss mimics--to a large extent--the bone loss exhibited by bed-ridden patients.

In addition to the direct effects of spaceflight/aging bone loss on the human skeletal system, indirect effects also accumulate. These include--most notably--enhanced renal stone formation as calcium released from bone is accumulated in kidneys (Watanabe *et al.*, 2004).

Although the physiological (e.g., signaling pathways) and cellular (e.g., differentiation) mechanisms which are responsible for bone loss/weakening are not yet understood, insights are slowly emerging. For example,

studies in laboratory model systems reveal that osteoblast number is reduced under simulated-weightlessness conditions. In the rat unloaded hind-limb model, for example, osteoblast number is reduced (Basso *et al.*, 2005). Likely, osteoblast proliferation is also diminished under microgravity conditions (e.g., Wang, Zhang, and Wu, 2003). Similar observations have emerged from histological examinations of bone specimens from spaceflights--not only are osteoblast numbers reduced, but osteoclast numbers are probably increased. Furthermore, gene (e.g., runx2 and AP-1) expression studies indicate that down-expression of osteoblast differentiation markers occurs in the hind-limb unloaded rat model (Ontiveros and McCabe, 2003).

Since bone loss is considered to represent an inherent risk of long duration spaceflight, and because understanding bone loss under weightlessness conditions might eventually yield clues regarding physiological and cellular mechanisms which cause disuse osteoporosis and bone weakening associated with human aging, major research efforts to prevent bone loss are underway. The Table summarizes several of the strategies currently being explored.

Previous studies in our laboratory (e.g., Tanaka *et al.*, 2004) led to the development of a KJS which--in mouse studies--demonstrated enhanced bone formation. Osteogenesis was stimulated in the diaphysis without inducing obvious *in situ* strain at the actual site of bone formation. In the present studies a model of that KJS has therefore been developed for human use. The KJS described herein includes two prototypes: a gear/cam and DC motor control systems and a voice coil-linear actuator system.

Our KJS has the potential for circumventing some of the shortcomings of the various approaches to reducing spaceflight bone loss listed in the Table (see Discussion).

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Table Examples of possible approaches to reducing spaceflight/human aging bone-loss effects.

Approach	Comment	Reference
Pharmacological	Administer hormones such as "glucose-dependent insulinotropic peptide" to stimulate osteoblasts	Bollag <i>et al.</i> , 2001
	Administer vitamin D receptor agonists to improve calcium absorption	Narayanan <i>et al.</i> , 2004
Flight exercise	Various regimens have shown limited effects	Lackner & DiZio, 2000
Whole body vibration	Device design for spacecraft likely to be complicated	Rubin <i>et al.</i> , 2003
Mechanical pulse (KJS)	Preliminary data reveal enhanced bone formation in laboratory model (mouse) system	Tanaka <i>et al.</i> , 2004

Materials and Methods

Mechanical loading

Twenty four C57BL/6 mice (female, ~14 weeks old, body weight of ~20 g) were used in the animal experiments. Their use was approved by the IACUC. The mouse was placed in an anesthetic induction-chamber to induce sedation and mask-anesthetized using 2% isoflurane. Knee loading was applied with a previously developed piezoelectric loader for 3 minutes per day for 3 consecutive days in a lateral-medial direction (Yokota and Tanaka, 2005). The left knee was used for loading, and the right knee was treated as non-loading control (Fig. 1). The mice were randomly divided into 3 loading groups for 3 loading frequencies of 5, 10, or 15 Hz ($n = 8$). A peak-to-peak sinusoidal force of 0.5 N was employed. After loading, the mouse was allowed normal cage activity, and any abnormal behavior, weight loss or diminished food intake was monitored.

Histomorphometry

Mice were given an intraperitoneal injection of a fluorochrome bone label (calcein -Sigma, St. Louis, MO) at 30 $\mu\text{g/g}$ body mass on days 2 and 6 after the last loading. Animals were sacrificed 13 days after the last loading, and the left and right tibiae were harvested for histomorphometric analyses. The samples were dehydrated by immersion in a series of ethanol solutions and embedded in methyl methacrylate. The cross sections (~50 μm in thickness) were cut 25% from the proximal end along the length of the tibia (Fig. 1D).

Using the Bioquant semiautomatic digitizing system (R&M Biometrics, Nashville, TN), we determined an increase in bone formation rate in $\mu\text{m}/\text{year}$ using the procedure previously described (Zhang *et al.*, 2006). In brief, the rate of bone formation was determined for the cross section in the loaded tibia (L) as well as the control tibia (C) and the difference between these two cross sections was defined as $[(L - C)/C]$ in %.

Prototype design and fabrication

Two prototypes for the human KJS were designed and fabricated. Prototype A was a gear/cam and DC motor control system, and Prototype B was a voice coil-linear actuator system (LA1012-027A, BEI Kimco, Vista, CA). Eight design criteria were considered, and included: (1) frequent usage (~30 min daily); (2) operation at high frequencies (up to 30 Hz); (3) application of forces up to 10 N; (4) light weight (100 to 300 g); (5) long (more than 2 years) product life; (6) safety shutoff capability (power switch and on/off lamp); (7) small device size (~100 cm^3); and (8) low manufacturing and operation costs. Fabrication of the prototypes was conducted in the machine shop at IUPUI. AL6061 (McMaster) was used for making mounting plates.

Results

Enhancement of Bone formation by knee loading with the piezoelectric loader

Bone formation in the diaphyseal cortical bone was significantly enhanced. In response to loading at 5, 10, and 15 Hz, the increase in bone formation rate was $62 \pm 4\%$ ($p < 0.001$), $33 \pm 5\%$ ($p < 0.01$), and $27 \pm 4\%$ ($p >$

Fig. 1. Knee loading configuration for the present studies. (A) & (B) Mouse skeleton showing the loading site. (C) Mouse on a loading table for knee-loading. (D) Micro computer tomography image of a mouse tibia showing the position of a diaphyseal cross-section employed for bone histomorphometry.

Fig. 2. Bone histomorphometry. (A) Fluorescent image of a cross-section of the mouse tibia illustrating newly formed bone. (B) Increase in bone formation rate (% relative to non loading control) in response to loads with 0.5 N forces at 5, 10 and 15 Hz. The mean and the standard error mean are shown (n = 8).

0.05), respectively (Fig. 2). The results show dependence of the anabolic responses on loading frequency.

Design of human knee-loading joint supporter (KJS)

Two prototypes of human KJS were designed. Based on the results of previous mouse model bone histomorphometry, the loading frequency was designed to be in the range from 0.5 to 30 Hz. In prototype A (Fig. 3A), the system includes a gear/cam linkage, a DC motor, a loading pad, a pressure sensor, and a supporting belt. The gear/cam linkage converts a motor-driven rotational motion to a sliding motion. The loading pad, attached to the gear/cam linkage, contacts the knee and applies mechanical loads. This pad is designed to avoid a local stress concentration on the knee. The DC motor is regulated by a voltage-mediated controller, and a pilot light was installed to indicate the on/off status of operation. The pressure sensor is used to detect the force applied to the knee and prevent excess loading. The supporting belt is designed to set the loader on the knee in adjustable positions.

In prototype B (Fig. 3B), the system consists of a voice coil-linear actuator, controller, loading pad, mounting plate, and supporting belt. The linear sliding force is generated with the actuator and a spring is included between the loading pad and the knee for safety. The maximum force is 13 N, and the loading frequency is adjustable with the controller.

Force-weight relationship for varying loading modalities

Previous model animal (mouse) studies with varying loading modalities revealed

a double-logarithmic relationship between effective loads for bone formation and body weight (Burr *et al.*, 2003; Gross *et al.*, 2002; Koo *et al.*, 2001; Meade *et al.*, 1984; Meyer *et al.*, 2004; Qin *et al.*, 2003; Recher, 1983; Rubin *et al.*, 2003; Yoshida *et al.*, 2000). Among studies using mice, the required force ranged from 0.5 N with knee loading (Su *et al.*, 2006) to the maximum of 13 N with cantilever bending (Margolis *et al.*, 2004). Figure 4 illustrates those relationships. Knee-joint loading appears to provide effective enhancement of bone formation with minimal loading requirement. The expected loads for human KJS are ~ 10 N. Experimental verification of that approximation is necessary.

Discussion

As documented in Table, various strategies are being tested to counteract the effects of bone loss in astronauts/aging patients. At present, the use of either pharmacological agents or mechanical stimuli offer

Fig. 3. Human knee-loading joint supporter. The scale bar is 2 cm. (A) Prototype A with a gear/cam mechanism. The current design including a 9V battery weighs 0.27 kg. (B) Prototype B with a voice coil-linear actuator. The driving current is designed as ± 5 A. The motor box is 4 x 4 x 6 cm in size, and it weighs 0.26 kg.

Fig. 4. Force-weight relationship for varying loading modalities. WBV represents whole body vibration, and the grey linear zone indicates estimated force with knee loading. In the zone, the force-weight relationship is modeled as “ $\log(\text{force in N}) \sim 0.35 \log(\text{weight in kg}) + 0.30$.”

the most promise as bone loss countermeasures. Both approaches can be tested in model organisms, and for each there exists preliminary evidence that validates their potential usefulness.

Pharmacological approaches ultimately will require identification of appropriate target genes/proteins. Regulatory circuits which control gene transcription in osteoblasts and osteoclasts are, however, exceedingly complex, and likely involve protein-protein interactions as well as protein-gene (DNA) interactions (e.g., see Yu *et al.*, 2005). Thus, it is unlikely that administration of a simple pharmacological agent to astronauts/aging patients will diminish bone loss. Nevertheless, progress is being made on identifying potential gene targets and their cognate protein transcription factors, with the goal of custom synthesizing drugs which interact with those transcription factors (e.g., Krane, 2005).

Side effects of pharmacological agents are, unfortunately, likely to constrain the usefulness of such agents. One such agent, Glucose-dependent Insulinotropic Peptide (GIP), once it has been recognized by its cognate receptor on bone cells, stimulates osteoblast activity and diminishes osteoclast activity. Indeed, transgenic mice with reduced GIP receptor levels exhibit decreased bone mass (Xie *et al.*, 2005). GIP is released from the small intestine after eating, and probably has pleiotrophic effects on human physiology. Although it shows effects on bone formation, its main physiological role is in glucose (energy) metabolism. As well, the possibility exists that human physiological processes will adapt to exogenous GIP levels, and thereby cancel out long term effects on bone strengthening. Despite those potential shortcomings, such research approaches should be considered valid, for if indeed successful, the relative ease of administration of a drug might negate some concern over some of the side effects.

An alternative approach--described herein--views the knee joint, cartilage, and tibia as interdependent components each of which can contribute to the “reading”

of load magnitudes, transmit signals, and respond accordingly. Evidence to support this holistic view of weight bearing skeletal elements was recently provided by Tanaka *et al.*, 2004. The data included in the present report extend those observations and describe initial attempts to develop devices for use with human subjects. The key advance represented by these mechanical devices is the application of lateral loads to the joint, rather than the traditional bone-strengthening approach which employs direct deformation of rigid bone.

That is, instead of applying a load directly to the epiphysis (under the joint). That load to the joint is apparently “read”, which accords well with our view that the knee joint, cartilage, and tibia represent an integrated system that is capable of signal transmission among each of the components (Yokota and Tanaka, 2005).

Two major considerations for design strategies included “effectiveness for stimulating bone formation” and “ease of use” of the KJS. As their descriptions illustrate, both considerations were fulfilled. Thus, future experimentation should be directed at further testing in animal models, and initial trials with human patients.

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