

AD _____

Award Number: DAMD17-01-1-0817

TITLE: Bone Growth, Mechanical Stimulus and IGF-I

PRINCIPAL INVESTIGATOR: Vicente Gilsanz, M.D.

CONTRACTING ORGANIZATION: Childrens Hospital Los Angeles
Los Angeles, CA 90027

REPORT DATE: October 2005

TYPE OF REPORT: Annual

20060315 072

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE (DD-MM-YYYY) 01-10-2005		2. REPORT TYPE Annual		3. DATES COVERED (From - To) 10 Sep 04 – 10 Sep 05	
4. TITLE AND SUBTITLE Bone Growth, Mechanical Stimulus and IGF-I				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER DAMD17-01-1-0817	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Vicente Gilsanz, M.D. E-Mail: vgilsanz@chla.usc.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Childrens Hospital Los Angeles Los Angeles, CA 90027				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Available data indicate that the genetic susceptibility for low bone mass is present very early in life. The aim of this project is to establish whether bone acquisition in teenagers who have sustained a fracture and have low bone mass can be enhanced by changing environmental factors, such as mechanical loading. The effects of two twelve-month interventions on musculoskeletal development will be studied and the results will be compared to matched teenagers undergoing no intervention. This study also examines the possible relations between the cross-sectional properties of bone and circulating levels of IGF-I, IGF-binding protein-3, and IGF-I genotypes, and between bone acquisition induced by interventions and insulin-like growth factors, in teenagers ages 15 to 20 years old. The cross-sectional arm of this project was successfully completed in 144 females and 144 males in August, 2004. Twenty-four females have completed the vibration intervention and calcium intake for one year, 24 female controls have completed the calcium intake for one year, and 24 females are enrolled in the physical exercise intervention for one year. Of the 144 males, 24 are completing the vibration intervention and calcium intake for one year, 24 controls have completed the calcium intake for one year, and 24 are being enrolled in the exercise intervention for one year.					
15. SUBJECT TERMS Mechanical intervention, fractures, IGF-I, teenagers, low bone mass					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	10	19b. TELEPHONE NUMBER (include area code)

Table of Contents

Cover..... 1

SF 298..... 2

Table of Contents..... 3

Introduction..... 4

Body..... 4

Key Research Accomplishments..... 9

Reportable Outcomes..... 9

Conclusions..... 9

INTRODUCTION

Available data indicate that the genetic susceptibility for low bone mass is present very early in life. The aim of this project is to establish whether bone acquisition in teenagers who have sustained a fracture and have low bone mass can be enhanced by changing environmental factors, such as mechanical loading. The effects of two twelve-month interventions on musculoskeletal development in teenagers will be longitudinally studied and the results will be compared to matched groups of teenagers undergoing no intervention. The mechanical intervention consists of brief exposure to low level (0.3g; 1g = earth gravitational field) high frequency (30-Hz) mechanical loading for 10 minutes every day. The resistance exercise intervention consists of 30 minutes of weight-bearing and trunk stabilization exercises three times per week. The cross-sectional properties of the bone make a substantial contribution to its strength. Data indicate that the cross-sectional dimensions of bone are important determinants of low-energy impact fractures in children, stress fractures in military recruits, and osteoporotic fractures in elderly women. Insulin-like growth factor-I (IGF-I), a major regulator of longitudinal bone growth, has also recently been shown to be an important determinant of cross-sectional bone growth. This study will examine the possible relations between the cross-sectional properties of bone and circulating levels of IGF-I, IGF-binding protein-3, and IGF-I genotypes in teenagers with fractures. The possible relations between bone acquisition induced by mechanical stimulus and circulating levels of IGF-I and the IGF-I genotype will also be assessed.

BODY

Cross-sectional Study – Females & Males. The cross-sectional phase of this study was completed in 2004; 144 females and 144 males participated. Subjects underwent physical examinations to confirm completion of sexual development, anthropometric measurements, x-rays of the left hand/wrist for skeletal age, blood draws for IGF-I, IGFBP-3, IGF-I genotyping, measurements of bone and body composition obtained via computed tomography (CT) and dual energy x-ray absorptiometry (DXA), and questionnaires pertaining to dietary intake and physical activity.

Longitudinal Study – Vibration & Controls – Females. In August/September 2003, 24 females began the vibration intervention arm of the study, 24 females served as controls. After six months, telephone contacts were made for questionnaires on dietary intake and physical activity. In August/September 2004, all subjects completed the intervention arm of the study and returned for the short-term post-intervention appointment, which included anthropometric measurements, skeletal age determinations as needed, blood draws, measurements of bone and body composition obtained via CT and DXA, and dietary intake and physical activity questionnaires. After six months, telephone contacts were made for dietary intake and physical exercise questionnaires, and, in September 2005, these subjects began returning for their final visit, the long-term post-intervention analyses. Nineteen subjects from the intervention group and 12 controls have returned as of September 9, 2005.

Findings: Short-Term Post-Intervention Analysis in Females

Table 1. Baseline Values for Anthropometric Parameters, Physical Activity and Calcium Intake

	Control N=24	Intervention N=24	P
Age (yrs)	17.6 ± 1.3	17.3 ± 1.5	0.450
Bone Age (yrs)	17.4 ± 0.7	17.0 ± 1.0	0.118
Height (cm)	164.0 ± 6.1	160.8 ± 3.8	0.037
Weight (cm)	67.5 ± 15	63.3 ± 13.7	0.323
BMI (kg/m ³)	25.1 ± 5.5	24.5 ± 5.5	0.724
Physical Exercise Index (hr/week)	9.9 ± 9.0	11.3 ± 11	0.739
Inactivity Index (hr/week)	8.9 ± 9.3	5.6 ± 3.9	0.114
Calcium Intake (mg/day)	1138 ± 814	1354 ± 1251	0.480

Table 1 illustrates the baseline characteristics of the control and treatment groups. At baseline, the only measure that differed between groups was height; women in the control group were 1.83% taller than those in the experimental group (p=0.037). Study subjects and controls showed identical increases in height (0.39%) and similar increases in weight (2.6% and 2.1%, respectively), BMI (1.9% and 1.4%, respectively) and calcium intake (42% and 36%, respectively), with no differences at follow-up in measures of physical activity or inactivity.

Baseline and follow-up CT values for muscle and bone in the axial and appendicular skeletons are shown in Table 2. Mean baseline values for the musculoskeletal measures were not significantly higher in the treated group than in the controls. While significant increases were present at follow-up for all morphological traits in the treated group, the only substantial change observed in the control group was in the cross-sectional area of the femurs.

Table 2. Baseline and Follow Up CT Values of Musculoskeletal Development in the Axial and Appendicular Skeletons

	Control N=24			Intervention N=24		
	Baseline	Follow-up	P	Baseline	Follow-up	P
Axial Skeleton						
Total Paraspinous Musculature (cm ²)	181.6 ± 26	182.8 ± 27	0.523	167.5 ± 29	177.5 ± 31	<0.001
Psoas (cm ²)	48.7 ± 8.20	48.7 ± 7.70	0.987	45.0 ± 9.5	48.0 ± 10.9	<0.001
Quadratus Lumborum (cm ²)	20.9 ± 5.90	21.9 ± 6.70	0.079	19.1 ± 3.6	21.2 ± 4.30	<0.001
Erector Spinae (cm ²)	112.0 ± 15.0	112.2 ± 15.0	0.892	103.4 ± 21	108.3 ± 21	0.026
Spine Cancellous BD (mg/cm ³)	171.3 ± 17.1	171.5 ± 14.9	0.929	164.8 ± 25	168.6 ± 25	0.025
Appendicular Skeleton						
Quadriceps femoris muscle (cm ²)	112.0 ± 16.0	114.6 ± 14.0	0.141	104.4 ± 13	108.5 ± 15	<0.001
Femur Cross-sectional Area (cm ²)	5.12 ± 0.77	5.17 ± 0.82	0.047	4.82 ± 0.53	4.92 ± 0.52	0.003
Femur Cortical Bone Area (cm ²)	4.18 ± 0.51	4.24 ± 0.58	0.143	3.96 ± 0.43	4.10 ± 0.42	<0.001

BD = Bone density.

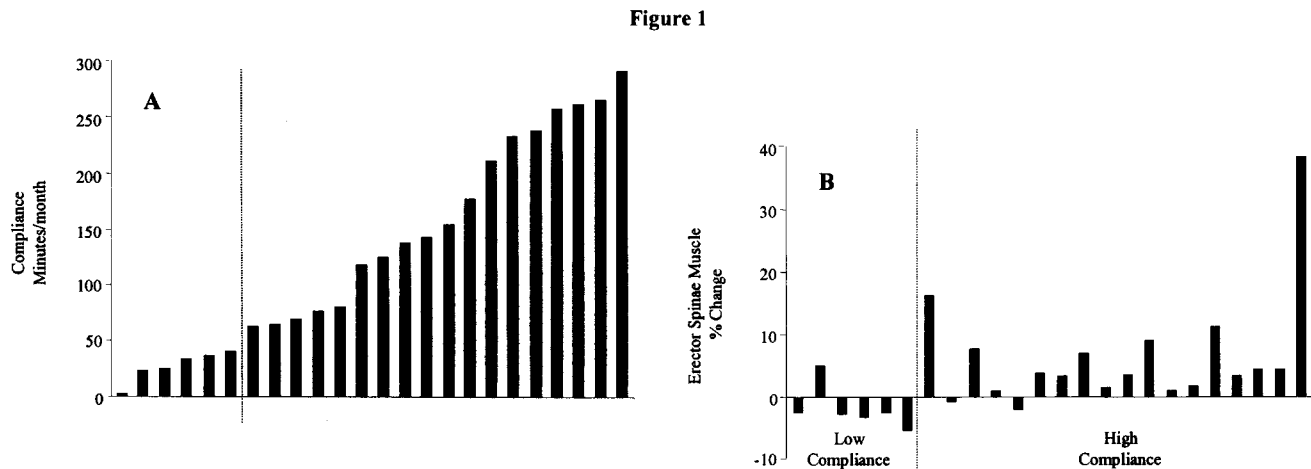
In the axial skeleton, significantly greater increases were present in the absolute and/or percent change of paraspinous musculature of women in the treatment group; 5.9% and 4.3% greater gains in the psoas (p<0.003) and erector spinae (p<0.025) muscles, respectively, were observed. In the appendicular skeleton, treated subjects had 2.3% greater increases in femoral cortical bone area (p<0.039; Table 3). Substantial differences were also found when the changes from all outcome variables were analyzed as a vector of observation using the multivariate Hotelling T statistic; this was true whether the analysis was based on absolute change (p=0.020) or on percent change (p=0.019). When each skeletal site was examined independently, differences were observed using Hotelling's T test for all measures in the axial (p=0.018), but not in the appendicular skeleton (p=0.416).

Table 3. Absolute and Percent Change in CT Musculoskeletal Measurements For Women in the Control and Treatment Groups

Axial Skeleton	Absolute Change			Percent Change		
	Control	Intervention	P	Control	Intervention	P
Total Paraspinous Musculature (cm ²)	1.19 ± 9.00	10.1 ± 12.50	0.002	0.51 ± 5.00	5.38 ± 6.90	0.002
Psoas (cm ²)	0.01 ± 2.87	3.06 ± 3.47	0.002	-0.05 ± .06	5.91 ± 6.74	0.003
Quadratus Lumborum (cm ²)	1.03 ± 2.74	2.20 ± 2.62	0.137	3.03 ± 14.7	8.97 ± 11.70	0.168
Erector Spinae (cm ²)	0.16 ± 5.56	5.29 ± 11.00	0.047	-.004 ± .86	4.30 ± 8.84	0.025
Spine Cancellous BD (mg/cm ³)	0.13 ± 7.73	3.77 ± 7.69	0.108	0.09 ± 4.54	2.14 ± 4.93	0.065
Appendicular Skeleton						
Quadriceps Femoris Area (cm ²)	2.62 ± 8.43	4.11 ± 4.46	0.449	2.17 ± 2.69	3.63 ± 3.63	0.363
Femur Cross-sectional Area (cm ²)	0.05 ± 0.12	0.10 ± 0.15	0.247	0.97 ± 2.15	1.87 ± 3.45	0.283
Femur Cortical Bone Area (cm ²)	0.05 ± 0.17	0.14 ± 0.15	0.079	1.09 ± 3.67	3.35 ± 3.71	0.039

BD = Bone density.

There was a large range in treatment compliance among subjects in the intervention group, (mean compliance: 130.3 ± 92.1 min/month or 4.3 min/day; Figure 1A), and a significant dose effect was observed which was more prominent in the erector spinae muscle (Figure 1B).



Post-hoc analysis indicated the percent change in paraspinous musculature, vertebral cancellous bone density and femoral cortical bone area in subjects with >20% usage to be significantly greater than in subjects with less usage and in controls (Figure 2). Regression analysis indicated that the anabolic effects of the intervention on musculoskeletal development remained significant even after adjusting for difference in baseline weight (Table 4).

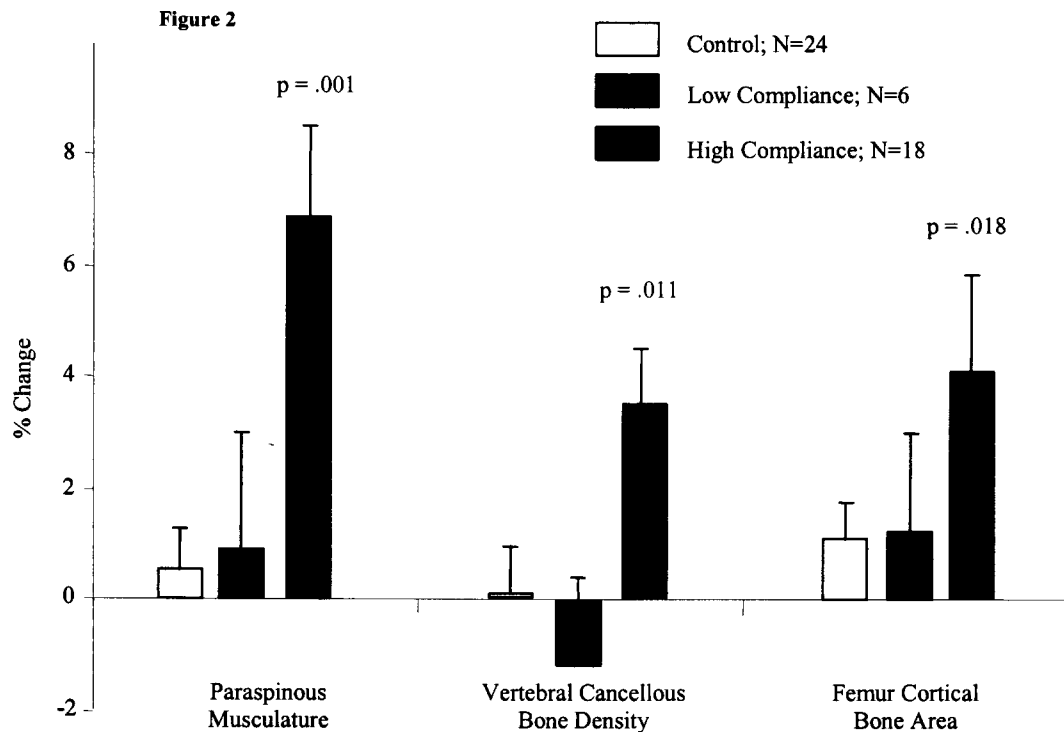


Table 4. Regression Results Estimating the Absolute and Percent Increases in CT Measurements of Musculoskeletal Development After Adjusting for Differences in Baseline Weight

	Absolute Increase			Percent Increase		
	Increase	P	R ² *	Increase	P	R ² *
Total Paraspinous Musculature (cm ²)	12.5 ± 3.22	0.001	0.22	0.08 ± 0.02	0.001	0.20
Psoas (cm ²)	2.90 ± 0.98	0.005	0.19	0.05 ± 0.02	0.011	0.15
Quadratus Lumborum (cm ²)	8.35 ± 2.51	0.002	0.20	0.92 ± 0.03	0.005	0.17
Erector Spinae (cm ²)	1.57 ± 0.81	0.058	0.09	0.09 ± 0.04	0.053	0.08
Spine Cancellous BD (mg/cm ³)	5.67 ± 2.11	0.010	0.22	0.03 ± 0.01	0.011	0.22
Quadriceps Femoris Area (cm ²)	0.86 ± 2.07	0.411	0.01	0.01 ± 0.01	0.729	0.01
Femur Cross-sectional Area (cm ²)	0.07 ± 0.04	0.083	0.07	0.01 ± 0.01	0.124	0.05
Femur Cortical Bone Area (cm ²)	0.11 ± 0.05	0.028	0.18	0.02 ± 0.11	0.018	0.15

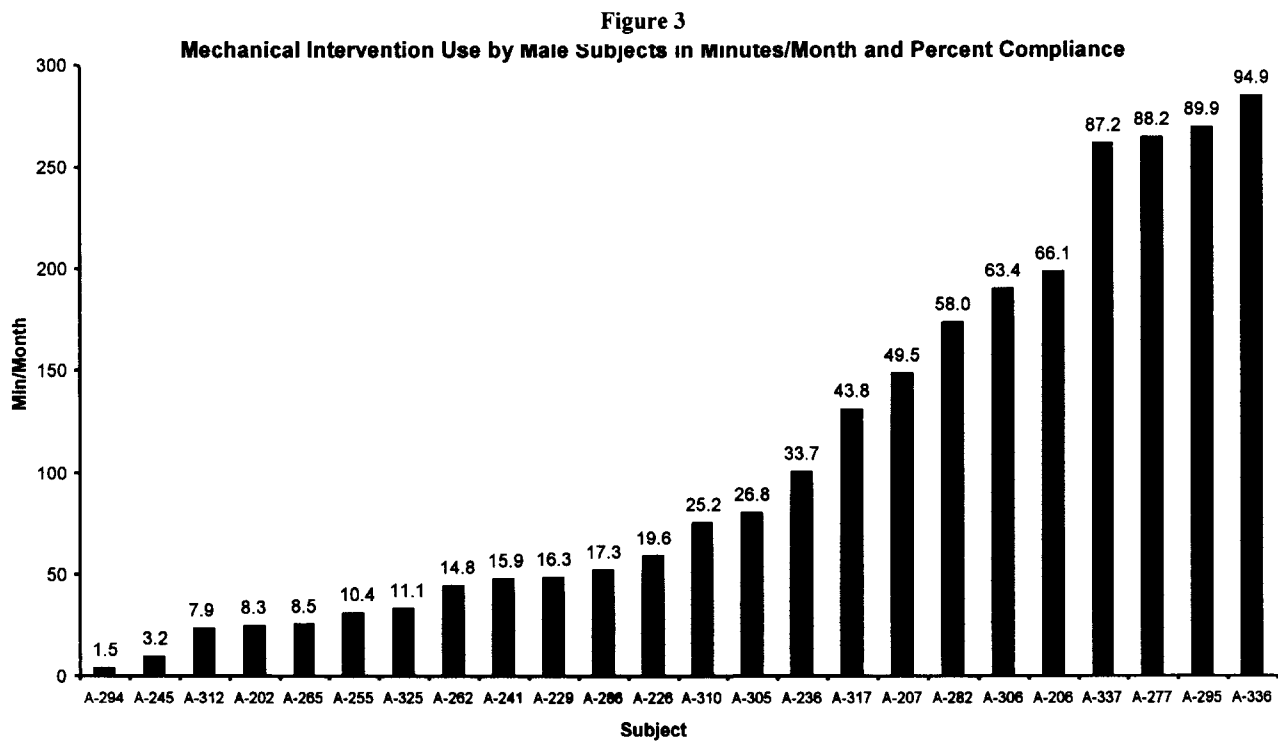
*The coefficient represents the estimated increase from a combined control and low dose (less than 20% usage) to a dose of 20% usage or more. BD = bone density.

Baseline and follow-up DXA values are shown in Table 5. Mean values for spine BMC and aBMD and for total body BMC were significantly higher in both groups at follow-up. In addition, in the treatment group, values for total body aBMD were higher after the intervention. There were, however, no differences measured between groups in the absolute and/or percent change for any of these DXA measures of bone and body composition (Table 6).

Table 5. Baseline and Follow Up DXA Values of Musculoskeletal Development

	Control			Intervention		
	Baseline	Follow-up	P	Baseline	Follow-up	P
Spine BMC (g)	56.1 ± 8.4	58.3 ± 7.8	<0.001	50.7 ± 6.1	52.7 ± 6.0	<0.001
Spine aBMD (g/cm ²)	1.02 ± 0.1	1.04 ± 0.1	0.003	0.95 ± 0.1	0.98 ± 0.8	0.002
Whole Body BMC (g)	1614 ± 258	1676 ± 270	<0.001	1481 ± 184	1535 ± 177	<0.001
Whole Body aBMD (g/cm ²)	0.98 ± 0.08	0.99 ± 0.07	0.145	0.94 ± 0.06	0.95 ± 0.06	0.045
Trunk Lean Mass (g)	19823 ± 2690	20037 ± 2480	0.343	18411 ± 2371	18871 ± 2576	0.067
Total Lean Mass (g)	40114 ± 5922	40816 ± 5615	0.061	37839 ± 5201	38593 ± 5822	0.146

Longitudinal Study – Vibration & Controls – Males. In late 2004, 48 males (24 study subjects and 24 controls) were enrolled in the vibration intervention arm of the study. As of September 9, 2005, 11 males (8 subjects, 3 controls) have completed the intervention year and returned for the short-term post-intervention appointment, as described above in the *Females* section above. Compliance is shown in Figure 3.



Longitudinal Study – Physical Exercise – Females & Males.

The physical exercise arm of this study has not yet been completed. Unfortunately, the inability of obtaining hospital privileges for the exercise trainers, an unexpected prerequisite of our IRB, which was not granted by our medical staff administration, was the key impediment. To overcome this difficulty, we have requested IRB approval for the subjects to carry out the exercise protocol in local athletic clubs.

Positive Findings

Low intensity, high frequency mechanical vibration enhances bone and muscle mass in young women.

Negative Findings

The intervention was not associated to any adverse side effects. There were no associations observed between calcium intake and measures of physical exercise and bone and muscle measures in the control or intervention groups. In contrast to CT, DXA technology was not sufficiently sensitive to identify differences in bone mass between groups.

KEY RESEARCH ACCOMPLISHMENTS

- Baseline studies in 144 females and 144 males completed.
- Mechanical intervention arm of the longitudinal study and short-term post-intervention examinations completed in 24 females. Long-term post-intervention exams are in process.
- Control arm of the longitudinal study and short-term post-intervention examinations completed in 24 females. Long-term post-intervention exams are in process.
- Mechanical intervention arm of the longitudinal study and short-term post-intervention examinations in 24 males are in process.
- Control arm of the longitudinal study and short-term post-intervention examinations in 24 males are in process.

REPORTABLE OUTCOMES

Short-term low intensity, high frequency mechanical vibration has a positive effect on the musculoskeletal development in young women with low bone density who have sustained a fracture. Manuscript has been submitted for publication.

CONCLUSIONS

The results in female subjects indicate that mechanical signals at orders of magnitude below that which might cause damage to bone tissue can have a strong anabolic effect on musculoskeletal development. On average, CT measures of cancellous bone in the axial skeleton and of cortical bone in the appendicular skeleton increased 2.1% and 2.3% more, respectively, in subjects treated with low-magnitude mechanical loading than in controls. Simultaneous to gains in bone, low-magnitude high-frequency vibration significantly increased muscle mass; close to a 5% greater increase in CT values for paraspinous musculature was detected in women in the intervention group, compared to controls. An association was observed between musculoskeletal gains and compliance; women using the vibration system more than 2 min/day had greater gains in cancellous and cortical bone and paraspinous musculature than women using it less than 2 min/day, or not at all.

The findings of the current study are consistent with the premise that adaptive responses of bone to mechanical forces may result from the stimulation of skeletal muscle activity by low-magnitude strains (<10 microstrain); strains similar to those associated with muscle contractions during passive activities, like maintaining posture, and that do not cause microdamage to bone tissue. It should be noted that the anabolic effects of the intervention on muscle and bone were present even after accounting for body weight, despite previous suggestions that low-magnitude mechanical stimulation would be most beneficial in subjects with lesser body weight. However, the intervention used (ten minutes of 0.3g stimulus at 30 Hz daily) induced at ~18,000 cycles/day, was approximately ten times greater than that of the 30 Hz signals arising from the paraspinous musculature while standing. Thus, while the mechanical loads applied in this study were relatively weak when compared with peak events imposed on the skeleton by vigorous exercise, they were significantly greater than those experienced during minimal activity.

The use of CT to obtain measures of muscle and bone in the appendicular and axial skeletons was a significant strength of the present study. DXA cannot fully correct for errors associated with changes in body and skeletal size and does not allow for the independent assessment of muscle mass from other lean tissues. It is noteworthy that significant differences were observed in bone and in muscle values with CT, which were not evident with DXA. Using CT, the cross-sectional area of paraspinous musculature significantly increased from baseline to follow-up in the experimental group, indicating a benefit beyond that specific to bone tissue. Given the importance of muscle function to bone quality and to the risk of falls and fall-related injuries, this intervention may be useful in the rehabilitation of musculoskeletal-deficient patients. Lastly, additional assets of this trial were the relatively long duration of treatment and the daily use of the intervention.

The results of this study suggest that mechanical loading at a level much lower than that associated with vigorous exercise could represent a non-pharmacologic means of augmenting the musculoskeletal system non-invasively. Moreover, low-intensity mechanical signals incorporate all aspects of the complex remodelling cycle and stimulate formation of muscle and bone to improve their quantity and quality. Many questions remain as to whether the effects observed will persist over time, and as to the optimization and usefulness of this type of intervention.