JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 2 (1991) 43–50

Effects of suspension-induced osteopenia on the mechanical behaviour of mouse long bones

S. J. SIMSKE, A. R. GREENBERG, M. W. LUTTGES
Bioserve Space Technologies and Departments of Electrical, Mechanical and Aerospace Engineering, University of Colorado, Boulder, CO 80309, USA

Whereas most studies of tail-suspension induced osteopenia have utilized rat femora, the present study investigated the effects of a 14 day tail-suspension on the mechanical behaviour of mice femora, tibiae and humeri. Force–deflection properties were obtained via three-point bending for long bones from suspended and control mice. Whole bone behaviour was characterized by converting the force–deflection values to stiffness, strength, ductility and energy parameters which were not normalized for specimen geometry. The effects of a systematic variation in the deflection rate over the range 0.1–10 mm min^{-1} were also evaluated. Statistical analysis indicated that the primary effect of the tail-suspension period was lowered bone mass which was manifested mechanically through lower values of the bone strength parameters. These effects were similar in the bones of both the fore and hind limbs. The results also demonstrated that the stiffness, ductility and energy characteristics were much less influenced by the tail-suspension. Whereas a significant dependence of the bone strength values upon deflection rate was observed for the femora and humeri, the other mechanical parameters were less sensitive. Based upon the nature of the physical and mechanical changes observed in the long bones following tail-suspension, the mouse appears to be a suitable animal model for the study of osteopenia.

1. Introduction

Strain generated in bones, as a result of physical activity [1] or applied mechanical loading [2], provides a stimulus for bone growth and remodelling. While the specific mechanism by which strain initiates growth has not been established, the lack of strain may account, in part, for the bone-loss characteristic of bed-rested patients [3, 4]. The removal of normal skeletal loading may also account for the characteristic loss of bone mineral due to spaceflight. Spaceflight-related changes in human and animal bone physiology are well documented and are generally described as microgravity-induced osteopenia [5–8]. Spaceflight can produce significant calcaneus mineral density loss in humans [5] and can affect trabecular bone formation [6] and remodelling [7] as well as periosteal bone formation [8] in rats. In addition to these physiological changes, recent work shows that certain mechanical properties of growing rat bone are adversely affected by spaceflight [9, 10]. For example, Shaw *et al.* [9] reported that a 1 week spaceflight reduced bending stiffness and strength in the tibiae and humeri, while Spengler *et al.* [10] demonstrated that an 18.5 day flight produced a decrease in the torsional stiffness and ultimate torque of rat femora.

Because access to spaceflight is still rather limited, experimental protocols utilizing a variety of hindlimb suspension techniques have been developed to simulate the effects of the microgravity environment during spaceflight. Based upon a comparison of back-suspension and tail-suspension results, tail-suspension has been justified by Wronski and Morey–Holton [11] as an appropriate model for the study of simulated weightlessness. Investigations of the effects of tail-suspension in rats show changes in osteoblastic activity [12] and histogenesis [13] as well as detrimental effects on femoral mechanical properties [14–16]. Turner and Bell [17] demonstrated similar effects in denervation models for hindlimb disuse. Whereas almost all of the tail-suspension studies described in the literature have focused on growing rats, preliminary results show that 2 week tail-suspension of growing mice also induces degenerative bone changes [18]. These adverse effects include lower bone mass and a general decrease in stiffness and strength parameters. Moreover, Fowler *et al.* [19] and Haida *et al.* [20] demonstrated that tail-suspension adversely influences mouse skeletal muscle integrity. These studies suggest that mice may be a viable alternative model to tail-suspended rats.

The overall mechanical behaviour of bone can be quantitatively determined by the relationship between the applied load and the resulting deformation. This relationship governs the structural behaviour of whole bone and reflects the material property distribution within the bone as well as the bone geometry [21]. Of the many techniques available to characterize the mechanical behaviour of long bone, the determination of properties in flexure via three- or four-point bending to break is often utilized due to the relative

0022–2461/91 $03.00 + .12

simplicity of the procedure. Corrections for the cross-sectional area at the break to convert load–deflection to stress–strain values may not improve the degree of precision obtained in such work because geometry at the break may not correlate with that elsewhere along the length of the bone [22]. In general, analysis of bone elastic properties is complicated by the heterogeneous distribution of material and asymmetric geometry exhibited by most long bones [21]. Further difficulties can arise from the consistent difference in porosity with respect to location along the longitudinal axis of the bone [23]. Hence, as indicated by Kusy *et al.* [22], the use of non-normalized load–deflection data obtained from simple bending and the directly derived stiffness, strength, ductility and energy parameters, may provide the most representative assessment of whole bone properties.

Because bone is a viscoelastic material [24], higher values of the strength parameters would generally be expected with increasing strain rates. Based upon the range of deflection rates for long bone flexure tests reported in the literature, there does not appear to be a generally agreed upon standard rate. For example, in two recent studies involving rat femora, deflection rates of 1.8 mm min^{-1} [25] and 51 mm min^{-1} [16] were utilized, corresponding to strain rates of approximately 0.01 and 0.1 sec^{-1}, respectively. These strain rates may have been chosen due to their similarity to reported *in vivo* rates [26, 27]. Because of the generally observed dependence of force–deflection values on deflection rate, it is appropriate to consider the specific nature of this dependence.

The overall objective of this study was to examine comprehensively the effects of tail-suspension on the mechanical properties of mouse femora, tibiae and humeri via three-point bending. These effects are evaluated in comparison to the mechanical properties of non-suspended control mice. The dependence of load–deflection derived mechanical property values upon a systematic variation in the deformation rate is also evaluated.

2. Materials and methods

A total of 18 growing (52 day) female mice (HS strain) were randomly assigned to equally sized suspended (S) and control (C) groups [28]. The S mice were housed in cages identical to those used for the control mice, except that in the case of the former group, suspension wires were strung lengthwise across the tops of the cages. These mice were equipped with a dowel/swivel suspension apparatus attached to the tails with hypoallergenic and duct tape such that the angle of suspension was 30°. Mice could move about the floor of the cage freely through the use of their fore limbs.

The weights of the mice were recorded at the beginning and end of a 2 week experimental period as pre-weight (pre-wt), post-weight (post-wt) and weight loss (wt-loss). Both the C and S mice were an average of 52 days old at the beginning of the experiment, and they were sacrificed over a period of 10 days to allow for mechanical testing without freezing of the bones. Mice matched for age were sacrificed from each group. The mice were closely monitored, and the suspended mice were weighed 5 times per week. The weighing procedure resulted in only 10–20 sec non-suspension per weighing. The weight of the suspension apparatus was subtracted to determine the day to day weights of the suspended mice. The mice were sacrificed by cervical dislocation following treatment (I.P., 90 mg kg^{-1}) with sodium pentobarbital and loss of the corneal reflex.

Following sacrifice, both femora, tibiae and humeri were removed from each mouse. The mice were sacrificed one at a time, and the bones were stored in saline (0.15 N, 24 °C) while the other bones were removed. These bones were cleaned, weighed (wet weight (wet-wt)), measured (length) and mechanically tested within 1 h of their removal. For the mechanical testing, the bones were carefully positioned on a custom-designed anvil with 2 mm diameter supports and an effective span of 8 mm. The femora and humeri were placed flexure side up, while the tibiae, due to bone geometry and their attachment to the fibulae, were oriented at 90° to the flexure side up arrangement used for the femora and humeri. The femora, with their characteristic elliptical cross-sections, were then loaded parallel to the minor axis midway between the supports with a 2 mm diameter load fixture. A similar procedure was utilized for the tibiae and the humeri although geometric considerations prevented such clear definition of axes. The three-point bending was continued through specimen fracture in an Instron model 1331 servohydraulic testing system. The diameters perpendicular and parallel to flexure (d_1 and d_2) at the fracture site were then measured.

The 18 femora, tibiae and humeri derived from the S and C groups were subdivided into three groups of six each that were tested at deflection rates of 10, 1 and 0.1 mm min^{-1}, respectively. Thus, the six groups that were evaluated in these experiments were identified as S10, S1, S0.1, C10, C1 and C0.1. The entire testing procedure took less than 5 min for 0.1 mm min^{-1} tests and approximately 1 min for the 1 and 10 mm min^{-1} tests so that the maximum amount of water lost during the testing was measured to be less than $\simeq$6 wt % (Fig. 1). These small weight losses were not considered to have had a major influence on the

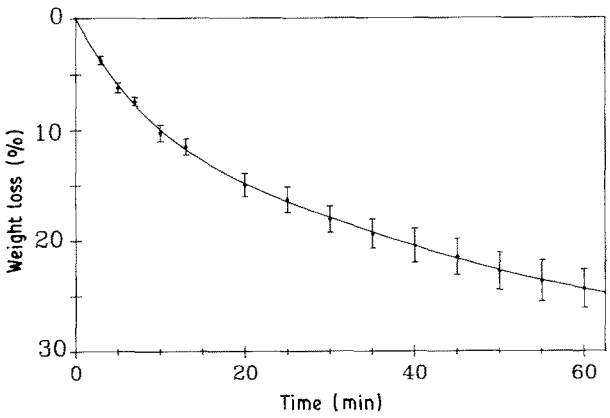

Figure 1 Loss of weight from wet femora (2), tibiae (2) and humeri (2) as a function of exposure to ambient air at 24 °C. Asymptotic weight loss reaches 34% (average for the 6 bones) at 240 min.

measured mechanical behaviour [29]. The breaking curves were digitally sampled at 100, 10 and 1 Hz for the respective deflection rates of 10, 1, and 0.1 mm min^{-1} and stored for later data analysis. The bones were then dried (75 °C) for 24 h and weighed (dry-wt). The ratio of (wet-wt − dry-wt)/wet-wt expressed as a per cent (% Por) was computed as an indicator of whole-bone porosity [30] while the difference between wet-wt and dry-wt represented the water content of the wet bone.

The force–deflection curves were subsequently processed by a BASIC routine that incorporated a correction for machine deflection [31] and smoothed the discretized nature of the digital data. Mechanical measurements were determined with this routine. The elastic protion of the force–deflection curve was fit with a linear regression line, and the remainder of the curve was fit with a cubic spline. The mechanical measurements obtained with the routine were within 2% of hand-made measurements (36 comparisons, 12 for each bone). In view of the non-uniform bone geometry [25], the force–deflection data were evaluated in terms of mechanical parameters not normalized with respect to the original physical dimensions. Stiffness (S) was defined as the slope of the linear or elastic region of the P–δ curve. Forces corresponding to the elastic limit (P_e), the maximum strength (P_m) and the fracture strength (P_f) also were determined from the curves. Corresponding deflection values at the elastic limit (δ_e), maximum load (δ_m) and fracture (δ_f) were calculated. In addition, values of elastic energy or resilience (A_R), energy to maximum load (A_m) and energy to fracture (A_f) were calculated with regard to the respective areas under the curve. These mechanical parameters are defined in terms of the representative P–δ curve shown in Fig. 2. While strength, ductility and energy parameters corresponding to the fracture point were included to facilitate comparison among the suspended, control and deflection rate groups, such values beyond the maximum load have little physical significance with respect to the normal load-carrying capabilities of the bone.

The body and bone weight, weight ratio, length and diameter data were compared with a two-tailed t test

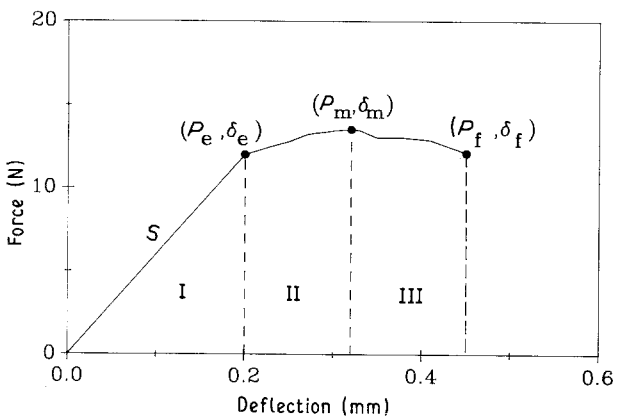

Figure 2 Representative force–deflection curve obtained during a three-point bend test of mouse femur at a deflection rate of 1 mm min^{-1}. Superposed are definitions of the mechanical parameters utilized in this study. Energy parameters A_R, A_m, and A_f correspond to curve areas I, I + II and I + II + III, respectively.

TABLE I Physical characteristics of suspended and control groups. The data are presented as mean ± S.D.

Parameter	Suspended mice	Control mice
Pre-wt (g)	21.8 ± 1.9	21.7 ± 1.7
Post-wt (g)	20.1 ± 0.8[a]	23.3 ± 2.2
Loss	1.7 ± 1.3[a]	− 1.7 ± 0.6
Femora		
Wet-wt (mg)	52.6 ± 3.1[a]	59.0 ± 4.4
Dry-wt (mg)	32.4 ± 2.6[a]	38.2 ± 3.1
% Por	38.4 ± 2.1[a]	35.2 ± 1.0
Length (mm)	13.76 ± 0.35	13.91 ± 0.36
d_1 (mm)	1.42 ± 0.07	1.48 ± 0.10
d_2 (mm)	1.02 ± 0.04[a]	1.05 ± 0.04
Tibiae		
Wet-wt (mg)	42.6 ± 2.7[a]	46.7 ± 2.7
Dry-wt (mg)	28.8 ± 1.9[a]	32.3 ± 2.2
% Por	32.3 ± 1.7	31.0 ± 1.2
Length (mm)	16.52 ± 0.27[a]	16.82 ± 0.29
d_1 (mm)	0.97 ± 0.06	0.99 ± 0.05
d_2 (mm)	0.89 ± 0.06[a]	0.98 ± 0.04
Humeri		
Wet-wt (mg)	25.7 ± 1.6[a]	27.9 ± 2.0
Dry-wt (mg)	16.6 ± 1.2[a]	18.5 ± 1.3
% Por	35.2 ± 1.5[a]	33.5 ± 1.3
Length (mm)	11.18 ± 0.18	11.33 ± 0.25
d_1 (mm)	0.94 ± 0.05	0.94 ± 0.04
d_2 (mm)	1.25 ± 0.09	1.30 ± 0.07

[a] Significantly different from control mice ($p \leq 0.05$).

in which average values for the two femora, tibiae and humeri of each mouse were used. Mechanical property data were analysed with a two-way analysis of variance (ANOVA).

3. Results

Over the 2 week duration of the study, the tail-suspended mice lost weight whereas control mice gained weight (Table I). At the time of sacrifice, body weight differences averaged approximately 14% ($p \leq 0.001$). Correspondingly, bone wet-wt averaged 8%–11% less and dry-wt averaged 10%–15% less in tail-suspended compared to control mice. All losses in bone weight were significant ($p \leq 0.05$).

Only minor differences were observed in bone size as a consequence of tail-suspension. Tibia lengths were reduced approximately 2% and femur and tibia diameters were reduced approximately 4% in the tail-suspended mice. The relatively constant values of estimated bone volume compared to the changes in bone mass are in keeping with the measured porosity changes (Table I). Tail-suspension resulted in 4%–9% increase in % Por.

The mechanical tests were conducted on bones that varied in geometry and mass. The heaviest bones were the femora (control mean wet-wt = 59.0 mg), while the tibiae (46.7 mg) and humeri (27.9 mg) weighed only 79% and 47% as much, respectively. The tibiae were the longest bones (control mean length = 16.82 mm), followed by the femora (13.91 mm) and the humeri (11.33 mm). Thus, the femora were comparatively thicker and shorter than the tibiae. The humeri exhibited length and thickness ratios between those of the femora and tibiae.

TABLE II Mechanical characteristics of suspended and control groups as a function of deflection rate. The data are presented as mean ± S.D.

Mechanical parameter[a]	Suspended mice (S)			Control mice (C)		
	S10[b]	S1	S0.1	C10	C1	C0.1
Femora						
S (N mm^{-1})[c]	87.9 ± 22.5	76.7 ± 19.3	76.2 ± 15.8	100.0 ± 19.6	102.2 ± 22.6	89.9 ± 8.6
P_e (N)[c,d]	11.9 ± 2.4	10.2 ± 3.6	9.7 ± 2.9	14.1 ± 3.3	14.3 ± 1.7	9.7 ± 3.2
P_m (N)[c,d]	16.0 ± 1.9	14.6 ± 2.6	13.8 ± 1.8	19.4 ± 2.6	17.8 ± 1.4	16.6 ± 1.8
P_f (N)[c]	13.7 ± 3.5	13.6 ± 3.1	12.8 ± 2.2	19.3 ± 2.6	17.2 ± 1.5	16.5 ± 1.8
δ_e (mm)	0.14 ± 0.02	0.13 ± 0.02	0.13 ± 0.03	0.14 ± 0.03	0.14 ± 0.02	0.11 ± 0.02
δ_m (mm)	0.28 ± 0.05	0.30 ± 0.08	0.28 ± 0.04	0.31 ± 0.07	0.30 ± 0.06	0.30 ± 0.09
δ_f (mm)[c]	0.42 ± 0.12	0.41 ± 0.10	0.36 ± 0.07	0.33 ± 0.07	0.34 ± 0.06	0.31 ± 0.10
A_R (mJ)[d]	0.82 ± 0.19	0.70 ± 0.32	0.64 ± 0.29	1.02 ± 0.35	1.02 ± 0.15	0.55 ± 0.32
A_m (mJ)[c]	2.81 ± 0.64	2.83 ± 0.95	2.40 ± 0.37	3.94 ± 1.01	3.70 ± 1.09	3.11 ± 1.17
A_f (mJ)[d]	4.80 ± 1.22	4.26 ± 0.78	3.44 ± 0.70	4.25 ± 1.22	4.39 ± 1.10	3.39 ± 1.44
Tibiae						
S (N mm^{-1})	54.1 ± 8.0	51.0 ± 6.5	56.4 ± 9.1	57.4 ± 9.1	58.6 ± 5.5	56.5 ± 10.3
P_e (N)[c]	7.6 ± 1.1	8.4 ± 2.7	6.3 ± 1.2	9.1 ± 1.2	9.1 ± 2.6	8.3 ± 0.4
P_m (N)[c]	9.9 ± 1.8	10.2 ± 2.2	8.5 ± 1.5	11.8 ± 2.4	11.7 ± 2.4	10.4 ± 0.7
P_f (N)[c]	9.8 ± 1.8	8.2 ± 2.4	8.3 ± 1.5	10.9 ± 2.1	11.5 ± 2.3	9.3 ± 1.2
δ_e (mm)	0.14 ± 0.01	0.16 ± 0.05	0.11 ± 0.02	0.16 ± 0.03	0.16 ± 0.04	0.15 ± 0.03
δ_m (mm)	0.21 ± 0.04	0.24 ± 0.04	0.18 ± 0.04	0.27 ± 0.06	0.24 ± 0.06	0.21 ± 0.06
δ_f (mm)	0.21 ± 0.04	0.40 ± 0.24	0.19 ± 0.05	0.37 ± 0.17	0.25 ± 0.06	0.24 ± 0.10
A_R (mJ)	0.54 ± 0.08	0.75 ± 0.46	0.37 ± 0.11	0.75 ± 0.19	0.75 ± 0.45	0.62 ± 0.12
A_m (mJ)[c,d]	1.19 ± 0.53	1.40 ± 0.50	0.87 ± 0.38	1.90 ± 0.70	1.64 ± 0.60	1.19 ± 0.42
A_f (mJ)	1.19 ± 0.53	2.83 ± 2.03	0.94 ± 0.46	3.05 ± 1.85	1.67 ± 0.68	1.48 ± 0.78
Humeri						
S (N mm^{-1})[c]	62.5 ± 8.0	57.6 ± 7.0	53.7 ± 5.5	69.8 ± 7.3	72.6 ± 11.9	64.7 ± 9.4
P_e (N)[c,d]	7.2 ± 0.5	6.1 ± 1.0	6.1 ± 0.5	8.0 ± 1.7	7.0 ± 1.2	7.4 ± 1.2
P_m (N)[c,d]	10.2 ± 0.6	8.9 ± 0.9	8.5 ± 0.4	12.0 ± 1.3	10.3 ± 0.8	9.7 ± 1.7
P_f (N)[c,d]	8.4 ± 1.8	7.6 ± 2.4	5.3 ± 1.6	10.3 ± 1.5	9.0 ± 1.4	8.0 ± 1.7
δ_e (mm)	0.12 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.02	0.10 ± 0.02	0.11 ± 0.02
δ_m (mm)[d]	0.25 ± 0.04	0.20 ± 0.02	0.22 ± 0.03	0.24 ± 0.02	0.21 ± 0.04	0.19 ± 0.03
δ_f (mm)	0.38 ± 0.15	0.28 ± 0.06	0.39 ± 0.11	0.32 ± 0.09	0.28 ± 0.10	0.26 ± 0.05
A_R (mJ)[d]	0.42 ± 0.06	0.32 ± 0.08	0.35 ± 0.05	0.47 ± 0.15	0.35 ± 0.12	0.42 ± 0.10
A_m (mJ)[d]	1.58 ± 0.28	1.07 ± 0.18	1.10 ± 0.20	1.82 ± 0.36	1.33 ± 0.32	1.09 ± 0.34
A_f (mJ)	2.70 ± 1.24	1.62 ± 0.37	2.14 ± 0.55	2.61 ± 1.05	2.05 ± 1.03	1.65 ± 0.62

[a] The parameters correspond to stiffness (S), force (P), deflection (δ) and energy (A). The subscripts e, m and f correspond to elastic, maximum force and fracture regions, respectively. A_R refers to resilience, or elastic energy.
[b] Number refers to deflection rate in mm min^{-1}.
[c] Significant dependence on mouse group (i.e. there are differences between suspended and control mice), $p \leqslant 0.05$.
[d] Significant dependence on deflection rate (i.e. there are differences between 10, 1 and 0.1 mm min^{-1} deflected bones), $p \leqslant 0.05$.

The femora exhibited the largest strength and stiffness values of the three bone types tested (Table II). The tibiae exhibited smaller P and S values. The humeri showed the smallest P values but an intermediate level of S values. Such differences most likely reflect, at least in part, differences in bone geometry, as well as bone axis and flexure orientation with respect to the loading direction.

The effects of tail-suspension are readily seen in differences in the strength parameters P_e, P_m and P_f. The strength parameters were generally higher in the control mice than the suspended mice. The elastic limit was reduced 15%–19% for all three bone types when tail-suspended mice were compared to controls. Maximum strength was reduced 16%–18% and fracture strength was reduced 19%–24%. Thus, the tail-suspension increases in % Por were matched by losses in bone strength. For the stiffness, ductility and energy parameters, tail-suspension generally resulted in smaller differences between the control and suspended mice. Significant reductions in stiffness were observed in the femora and humeri but not in the inherently less stiff tibia. In general, similarities exist between the physical and mechanical changes on a percentage basis reported for rats and those obtained in the present study (Table III).

Many of the strength and energy parameters exhibited deflection-rate dependency. These dependencies were similar in the control and suspended mice. However, the statistical significance of this dependence was different for the three types of long bones. In the case of the femora, P_e, P_m, A_R and A_f all increased significantly with increasing deflection rate. The overall effect of increasing deflection rate is illustrated in the representative femoral force–deflection curves shown in Fig. 3. These curves reflect the mean strength and deflection values in Table II. Similar results were observed for the humeri whereby the parameters P_e, P_m, P_f, δ_m, A_R and A_m all increased significantly with increasing deflection rate. In contrast, only the A_m values in the tibiae were significantly affected by the deflection rate.

Comparison of the femora tested at 10 and 0.1 mm min^{-1} indicated that the strength parameters

TABLE III Effect of degenerative bone changes on physical and mechanical parameters as a result of suspension or lactation. The values for the physical and mechanical parameters are presented in terms of per cent decrease due to suspension or lactation. The mechanical parameters are defined as in Table II

Parameter of interest	[25]	[15]	[16]	This work
Animal	rat	rat	rat	mouse
Age (days)	36	42	120	52
Treatment time (days)	14	14	28	14
Suspension type/lactation	lactation	harness	tail	tail
Mechanical testing[a]				
Deflection rate (mm min^{-1})	10[b]	1.8	51	1.0
Span (mm)	19	[c]	13.3	8.0
L/d ratio	4.8[d]	[c]	4.3[d]	7.7
Physical				
Body mass	[c]	45.9	[c]	13.7
Bone mass	26.0	29.4	[c]	15.2
Bone length	[c]	11.9	[c]	1.1
Mechanical				
S	28.9	[c]	[c]	25.0
P_e	[c]	[c]	22.1	25.0
P_m	29.2	40.9[d]	19.9	23.5
P_f	[c]	[c]	20.3	20.9
δ_m	7.9	[c]	[c]	1.3
A_R	[c]	[c]	20.3	31.4
A_m	39.4	[c]	34.6	23.5
A_f	[c]	[c]	34.0	3.0

[a] All tests three-point bending except [2] which was cantilever bending.
[b] R. P. Kusy, personal communication.
[c] Information not provided.
[d] Value derived from information provided.

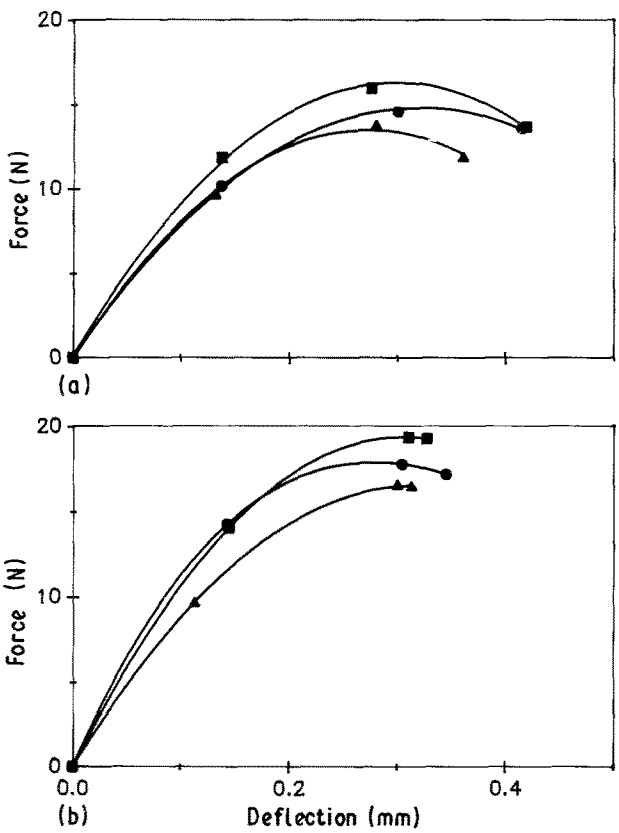

Figure 3 Effect of deflection rate on the force–deflection behaviour of mouse femora: (a) (S) tail-suspended and (b) (C) control group. Deflection rates utilized were (■) 10 (●) 1 and (▲) 0.1 mm min^{-1}.

were an average of 26% and 15% higher at the higher deflection rate for the control and suspended mice, respectively. The femora energy parameters were an average of 46% and 28% higher at the higher deflection rate for the control and suspended mice, respectively.

In general, the observed differences between control and suspended mice were manifested regardless of the deflection rate utilized. Of the 30 mechanical parameters investigated, 10 for each bone, 13 were found to be significantly higher in the control relative to the suspended animals (Table II): 5 in the femora, 4 in the tibiae and 4 in the humeri. For each of these 13 parameters, the ratios of the suspended group mean to the control group mean were calculated for each deflection rate (Table IV). These ratios were generally similar for each of the three deflection rates utilized.

4. Discussion

Osteopenia in the suspended mice was reflected by lower bone mass (Table I) and correspondingly lower mechanical strength parameters (Table II) compared to control mice. A comparison of femora results to those previously reported for rats is presented in Table III in terms of percentage changes in the respective physical and mechanical parameters. In general, both physical and mechanical parameters decrease in the suspended and lactating animals relative

TABLE IV Ratio of suspended group mean to control group mean (as a percentage) for the mechanical parameters significantly affected by suspension (Table II). The mechanical parameters are defined as in Table II

Mechanical parameter	Deflection rate (mm min^{-1})		
	10[a]	1	0.1
Femora			
S	87.9	75.0	84.8
P_e	84.4	71.3	100.0
P_m	82.5	82.0	83.1
P_f	71.0	79.1	77.6
A_m	71.3	76.5	77.2
Tibiae			
P_e	83.5	92.3	75.9
P_m	83.9	87.2	81.7
P_f	89.9	71.3	89.2
A_m	62.6	85.4	73.1
Humeri			
S	89.5	79.3	83.0
P_e	90.0	87.1	82.4
P_m	85.0	86.4	87.6
P_f	81.6	84.4	66.2

[a] Number refers to deflection rate in mm/min.

to the controls. A difficulty with this combined data set is that major differences exist in the nature of the experiments as well as the specific parameters reported. For example, whereas the work of Wunder *et al.* [15] involved suspension-induced osteopenia, the data of Peng *et al.* [25] corresponded to lactation-induced osteopenia. In addition, the former group included total body mass and bone length data with a mixed group of normalized and non-normalized mechanical parameters whereas the latter group included bone mass and non-normalized mechanical properties. Despite this lack of a common data base, the results suggest a similar overall response.

Changes in bone mechanical behaviour have been attributed to material, structural and geometry effects [9, 10, 14, 15, 16, 32]. In the present set of experiments, the changes in the non-normalized mechanical parameters induced by the suspension could have resulted from changes in the inherent material properties of the bone. On the other hand, such changes may have resulted from alterations in porosity or simply due to decreased mass. In order to evaluate the effects of decreased mass, the mechanical parameters that showed statistically significant differences (Table II) were normalized by the respective dry weight data (Table I) and the results presented in Table V. The dry weight, which includes both mineral and bone matrix weight, was utilized because it has been strongly correlated with ash weight in previous suspension studies [31, 33]. Abram *et al.* [14] found that the ash percentage of bone weight was independent of suspension periods of 0, 1, 2 and 3 weeks in rats. The dry weight normalized results (Table V) indicate that the differences due to the suspension-induced osteopenia are almost completely eliminated when the bone mass is taken into account.

If the degree of whole-bone porosity is taken as the measurement % Por, then the femora for the suspended and control groups have porosity values of 38.4% and 35.2%, respectively (Table I). If the mechanical data of Table II are normalized with respect to (1-% Por), then a trend similar to that observed for the dry mass normalization is observed. Hence in the present case, the effects of decreased mass and increased porosity on the mechanical behaviour cannot be distinguished. Although the bones recovered from suspended mice differ from those in control mice in terms of weight and mechanical parameters, the fundamental material and structural characteristics may not differ.

Using the same procedures described above, normalization of mechanical parameters by bone weight

TABLE V Dry weight normalized mechanical parameters.The data are presented as mean ± S.D. The mechanical parameters are defined as in Table II

Mechanical parameter	Suspended mice (S)			Control mice (C)		
	S10[a]	S1	S0.1	C10	C1	C0.1
Femora						
S (N mm^{-1} mg^{-1})	2.62 ± 0.54	2.37 ± 0.39	2.33 ± 0.36	2.64 ± 0.39	2.62 ± 0.43	2.38 ± 0.25
P_e (N mg^{-1})[b]	0.36 ± 0.06	0.31 ± 0.08	0.30 ± 0.09	0.37 ± 0.07	0.37 ± 0.03	0.26 ± 0.09
P_m (N mg^{-1})[b]	0.48 ± 0.04	0.45 ± 0.04	0.42 ± 0.04	0.51 ± 0.03	0.46 ± 0.01	0.44 ± 0.03
P_f (N mg^{-1})[c]	0.41 ± 0.09	0.42 ± 0.05	0.39 ± 0.05	0.51 ± 0.03	0.44 ± 0.02	0.44 ± 0.03
δ_f (mm mg)[c]	13.8 ± 3.5	13.0 ± 2.3	11.7 ± 2.2	12.4 ± 3.0	13.4 ± 2.6	12.0 ± 4.2
A_m (mJ cg^{-1})	0.84 ± 0.02	0.88 ± 0.03	0.74 ± 0.01	1.04 ± 0.02	0.95 ± 0.02	0.81 ± 0.03
Tibiae						
P_e (N mg^{-1})	0.26 ± 0.03	0.29 ± 0.04	0.22 ± 0.04	0.28 ± 0.04	0.27 ± 0.06	0.26 ± 0.02
P_m (N mg^{-1})	0.33 ± 0.05	0.36 ± 0.06	0.29 ± 0.05	0.37 ± 0.06	0.35 ± 0.05	0.33 ± 0.04
P_f (N mg^{-1})	0.33 ± 0.05	0.29 ± 0.07	0.29 ± 0.05	0.34 ± 0.06	0.35 ± 0.05	0.30 ± 0.04
A_m (mJ cg^{-1})	0.40 ± 0.17	0.49 ± 0.16	0.30 ± 0.14	0.59 ± 0.20	0.49 ± 0.16	0.38 ± 0.15
Humeri						
S (N mm^{-1} mg^{-1})	3.60 ± 0.40	3.58 ± 0.47	3.24 ± 0.49	3.78 ± 0.38	3.83 ± 0.46	3.56 ± 0.47
P_e (N mg^{-1})	0.41 ± 0.03	0.38 ± 0.05	0.36 ± 0.04	0.43 ± 0.07	0.37 ± 0.05	0.40 ± 0.05
P_m (N mg^{-1})[b]	0.58 ± 0.02	0.55 ± 0.06	0.51 ± 0.02	0.65 ± 0.05	0.55 ± 0.02	0.53 ± 0.07
P_f (N mg^{-1})[b,c]	0.48 ± 0.10	0.47 ± 0.14	0.31 ± 0.07	0.56 ± 0.08	0.48 ± 0.07	0.44 ± 0.08

[a] Number refers to deflection rate in mm/min.
[b] Significant dependence on deflection rate (i.e. there are differences between 10, 1 and 0.1 mm/min deflected bones), $p \leqslant 0.05$.
[c] Significant dependence on mouse group (i.e. there are differences between suspended and control mice), $p \leqslant 0.05$.

for several rat studies indicated dependencies similar to those reported herein for mice. Although weight data are not available in several investigations of mechanical behaviour [9, 14, 16], in each study where both weight and mechanical data were presented [15, 23, 25, 32] such normalization always decreased the per cent differences for mechanical measurements between groups of rats. In one case [15], such normalization completely removed strength differences between control and suspended rats.

The experimental results indicated that suspension-induced osteopenia occurred in both the fore and hind limbs even though complete mechanical unloading was restricted to the hind limbs. Indeed, a statistically significant decrease in the strength parameters occurred in the humeri (Table II) despite continuous use and loading during the tail-suspension. Bikle *et al.* [33] reported that growing rats (age not given) exhibited similar tibia weight losses of 10.6% after 15 days suspension but no weight loss for the humeri. If the data of Bikle *et al.* [33] are characteristic, then the effects of tail-suspension on mice and rats may significantly differ. The data for the tail-suspended mice may indicate a superposed systemic effect. The similarity of the effects of tail-suspension on mice femora, tibiae and humeri may indicate that either a common stress response exists or that both the fore- and hind-limb loading patterns are altered in tail-suspension. Whereas results reported for mechanical changes in long bones during spaceflight have included data from both the tibia and humerus [9], this has not usually been the case for tail-suspension [14–16], where data for only the femora or the femora and tibiae have been reported. In order to understand better the dynamics of the induced osteopenia, tail-suspension experiments should be designed to evaluate both the unloaded and loaded limbs.

The dependence of strength on strain rate has been reported in a number of bone studies, including compressive [34–36], tension [37, 38] and flexural [39] properties. In addition, studies by McElhaney [40] and Sedlin and Hirsch [41] demonstrated a significant increase in the modulus of elasticity as the strain rate was increased in compression and cantilever bending, respectively. However, in other studies, lesser dependence has been demonstrated [36–38], presumably due to differences in protocol. In general, strength parameters have been observed to increase monotonically as the strain rate is increased over a few orders of magnitude to values of approximately $0.1\,\text{sec}^{-1}$ [34, 36–40]. The specific nature of this dependence is a function of variables such as bone type, mineral content and method of loading. The strain rates utilized in the present investigation (1.7×10^{-4}–$1.7 \times 10^{-2}\,\text{sec}^{-1}$) are within this monotonic range. In the present investigation, the strength parameters P_e, P_m and P_f were highly sensitive to the deflection rate used in the three-point bending test for the femora and humeri. Although the strength parameters for the tibiae increased from the lowest to highest deflection rates, the overall dependence was not statistically significant.

The general deflection rate trend for the strength parameters also applied to the stiffness, ductility and energy parameters. In general, values of S, δ_e, δ_m, δ_f, A_R, A_m, and A_f increased with increasing deflection rate in all bone types for both control and suspended mice. The range of deflection rates utilized here effectively brackets many of those reported in the literature, including those by Wunder *et al.* [15] for cantilever bending and Shaw *et al.* [16] and Peng *et al.* [25] for three-point bending. The deflection rate range of 0.1–$10\,\text{mm min}^{-1}$ corresponds to an approximate strain rate range of 1.7×10^{-4}–$1.7 \times 10^{-2}\,\text{sec}^{-1}$, a range which is likely to include *in vivo* strain rates [26, 27]. Given the widespread use of bone bending tests in tail-suspension studies, the dependence of mechanical parameters on the deflection rate may preclude direct comparison between studies in which deflection rates differ. On the other hand, for each of the femoral, tibial and humeral mechanical parameters in which a statistically significant tail-suspension effect could be documented, the magnitude of the relative differences did not appear to depend upon the deflection rate. Although an optimal deflection rate for comparing treatment and control groups may exist, such an optimum was not indicated by the results of this investigation. For the present protocol such an optimum presumably would be bounded at low rates by water loss considerations and at high rates by instrument response time limitations.

Based upon the degenerative bone changes which occurred during the 2 week tail-suspension period used in these studies, the mouse appears to be a suitable animal for the study of microgravity-induced osteopenia. Despite the attention given to this phenomenon, more study is required to establish clear cause and effect relationships. For example, there is evidence that the rat tail-suspension model is itself not mechanistically analogous to spaceflight [42]. Toward this end, the present results may indicate a potential advantage in the use of mice rather than rats: given the stringent volume requirements imposed on spaceflight experiments, the use of mice would allow significantly more data to be obtained in the space environment. This may allow a clearer determination of important trends which have not been statistically resolved due to the small sample sizes. Despite these advantages, the relatively small size of the mouse bones precludes length/diameter ratios as large as ≈ 10 for flexural testing. Similar constraints exist for rat femora (Table III). Hence, shear effects are likely for both rat and mice bones. The presence of this shear component will influence the absolute values of the mechanical parameters. For example, values of modulus calculated by standard flexural formulae would underestimate the true modulus values by a maximum of $\approx 10\%$ for L/D ratios similar to those of the mouse humeri (≈ 6) and $\approx 20\%$ for ratios typical of rat femora (≈ 4) [43]. However, the magnitude of the correction factor should be verified by appropriate experimental data when possible.

5. Conclusion

The present study has established that the physical

and mechanical behaviour of mouse long bone is significantly affected by suspension. In general, the bones from mice subjected to suspension had lower mass and reduced strength properties. When normalized by mass, the differences between mechanical parameters in the suspended and control groups are almost entirely eliminated. Additional studies are required to determine to what extent geometry and material factors contribute to this reduced mass. The effects of a variation in the deflection rate on the mechanical behaviour were not systematic and varied with bone type. The femora and humeri were similar in that increases in the deflection rate over the range 0.1–10 mm min^{-1} resulted in increases in the strength and energy parameters. This dependence contrasted with that of the tibiae in which only one of the energy parameters was significantly affected. The overall physical and mechanical changes which result from tail-suspension indicate that the mouse is a suitable animal model for the further investigation of osteopenia.

Acknowledgements

The authors greatly acknowledge the support of this research by NASA grant NAGW-1197. The authors also acknowledge helpful discussions with Dr Robert P. Kusy. This paper was presented, in part, at the 22nd International Biomaterials Symposium, Charleston, SC, May 1990.

References

1. S. L. WOO, S. C. KUEI, D. AMIEL, M. A. GOMEZ, W. C. HAYES, F. C. WHITE and W. H. AKESON, *J. Bone Joint. Surg.* **63** (1981) 780.
2. C. T. RUBIN and L. E. LANYON, *J. Orthop. Res.* **5** (1987) 300.
3. C. L. DONALDSON, S. B. HULLEY, J. M. VOGEL, R. S. HATTNER, J. H. BAYERS and D. E. McMILLAN, *Metabolism* **19** (1970) 1071.
4. A. LeBLANC, V. SCHNEIDER, J. KREBS, H. EVANS, S. JHINGRAN and P. JOHNSON, *Calcif. Tissue Int.* **41** (1987) 259.
5. P. C. RAMBAUT and R. S. JOHNSTON, *Acta Astronautica* **6** (1979) 1113.
6. W. S. S. JEE, T. J. WRONSKI, E. R. MOREY and D. B. KIMMEL, *Amer. J. Physiol.* **244** (1983) R310.
7. L. VICO, D. CHAPPARD, S. PALLE, A. V. BAKULIN, V. E. NOVIKOV and C. ALEXANDRE, *ibid.* **255** (1988) R243.
8. T. J. WRONSKI and E. R. MOREY, *ibid.* **244** (1983) R305.
9. S. R. SHAW, A. C. VAILAS, R. E. GRINDELAND and R. F. ZERNICKE, *ibid.* **254** (1988) R78.
10. D. M. SPENGLER, E. R. MOREY, D. R. CARTER, R. T. TURNER and D. J. BAYLINK, *Soc. Exp. Biol. Med.* **174** (1983) 224.
11. T. J. WRONSKI and E. R. MOREY-HOLTON, *Aviat. Space Environ. Med.* **58** (1987) 63.
12. S. B. DOTY and E. R. MOREY-HOLTON, *Physiologist* **25** (1982) S141.
13. P. J. FIELDER, E. R. MOREY and W. E. ROBERTS, *Aviat. Space Environ. Med.* **57** (1986) 1125.
14. A. C. ABRAM, T. S. KELLER and D. M. SPENGLER, *J. Biomechanics* **21** (1988) 755.
15. C. C. WUNDER, K. M. COOK, S. R. WATKINS and W. J. MORESSI, *Aviat. Space Environ. Med.* **58** (1987) 977.
16. S. R. SHAW, R. F. ZERNICKE, A. C. VAILAS, D. DeLUNA, D. B. THOMASEN and K. M. BALDWIN, *J. Biomechanics* **20** (1987) 225.
17. R. T. TURNER and N. H. BELL, *J. Bone Min. Res.* **1** (1986) 399.
18. S. SIMSKE, C. SOMPS, E. GAYLES, L. S. STODIECK, H. WACHTEL and M. W. LUTTGES, *SAE Technical Paper Series* (1989) 891489.
19. W. M. FOWLER, R. T. ABRESCH, N. HAIDA, D. B. LARSON, R. B. SHARMAN, R. G. TAYLOR and R. K. ENTRIKIN, *Exp. Neurol.* **103** (1989) 77.
20. N. HAIDA, W. M. FOWLER, R. T. ABRESCH, D. B. LARSON, R. B. SHARMAN, R. G. TAYLOR and R. K. ENTRIKIN, *ibid.* **103** (1989) 68.
21. T. S. KELLER, D. M. SPENGLER and D. R. CARTER, *J. Orthop. Res.* **4** (1986) 57.
22. R. P. KUSY, T. PENG, P. F. HIRSCH and S. C. GARNER, *Calcif. Tissue Int.* **41** (1987) 337.
23. R. LAZENBY, *J. Biomechanics* **19** (1986) 257.
24. S. A. WAINWRIGHT, W. D. BIGGS, J. D. CURREY and J. M. GOSLINE, "Mechanical Design in Organisms" (Princeton University Press, Princeton, New Jersey, 1976) p. 144.
25. T. PENG, R. P. KUSY, S. C. GARNER, P. F. HIRSCH and M. C. DeBLANCO, *J. Bone Min. Res.* **2** (1987) 249.
26. L. E. LANYON and C. T. RUBIN, *J. Biomechanics* **17** (1984) 897.
27. L. E. LANYON, C. T. RUBIN and G. BAUST, *Calcif. Tissue Int.* **38** (1986) 209.
28. G. E. McCLEARN, J. WILSON and W. MEREDITH, "Contributions to Behavior-Genetic Analysis: the Mouse as a Prototype" (Appleton, Century, Croft, New York, 1970) p. 3.
29. S. J. SIMSKE, A. R. GREENBERG and M. W. LUTTGES, *J. Biomed. Mater. Res.*, submitted.
30. J. L. KATZ and H. S. YOON, *IEEE Trans. Biomed. Engng.* **BME-31** (1984) 878.
31. R. B. ASHMAN, in "Bone Mechanics" (CRC Boca Raton, FL, 1989) p. 75.
32. T. PENG, S. C. GARNER, R. P. KUSY and P. F. HIRSCH, *Bone Min.* **3** (1988) 293.
33. D. D. BIKLE, R. K. GLOBUS and E. R. MOREY, *Physiologist* **25** (1982) S143.
34. D. R. CARTER and W. C. HAYES, *Science* **194** (1976) 1174.
35. D. R. CARTER and W. E. CALER, *J. Biomech. Engng.* **105** (1983) 166.
36. J. D. CURREY, *J. Orthop. Res.* **6** (1988) 32.
37. R. D. CROWNINSHIELD and M. H. POPE, *Ann. Biomed. Engng.* **2** (1974) 217.
38. J. D. CURREY, *J. Biomechanics* **8** (1975) 81.
39. D. M. ROBERTSON and D. C. SMITH, *ibid.* **11** (1978) 455.
40. J. H. McELHANEY, *J. Appl. Physiol.* **21** (1966) 1231.
41. E. D. SEDLIN and C. HIRSCH, *Acta Orthop. Scandinav.* **37** (1966) 29.
42. L. VICO, A. V. BAKULIN and C. ALEXANDRE, *Proc. ESA* (SP-271) **3** (1987) 179.
43. R. P. KUSY and A. R. GREENBERG, *J. Therm. Anal.* **18** (1980) 117.

Received 7 March
and accepted 10 August 1990