N90-26456

EXPERIMENT K-6-02

BIOMEDICAL, BIOCHEMICAL AND MORPHOLOGICAL ALTERATIONS OF MUSCLE AND DENSE, FIBROUS CONNECTIVE TISSUES DURING 14 DAYS OF SPACEFLIGHT

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INTRODUCTION

This is a final report summarizing our findings on the connective tissue response to short-term space flight (12 days). Specifically this report represents data regarding the biochemical, biomechanical and morphological characteristics of selected connective tissues (humerus, vertebral body, tendon and skeletal muscle) of growing rats.

RESULTS

Humerus Cortical Bone:

The vivarium control humeri were significantly longer than either basal controls or flight rats, but were not different in length when compared to synchronous controls (Figure 3A). Using the basal group for comparison, during the 12.5 day period, the humeral lengths increased 4.3% for vivarium controls, 1.4% for synchronous controls, and 0.04% for flight rats.

The humeral cross-sectional geometries showed significant differences among the four groups (Table 1). The synchronous and vivarium controls had greater cortical cross-sectional areas than the flight group by 20% and 13%, but only the synchronous control humeri were significantly different from the flight group (Figure 3B). The endosteal cross-sectional area was not significantly different among groups. Humeral periosteal circumferences were identical both for the basal controls in flight groups and were 6-9% less than the synchronous and vivarium controls.

The lack of middiaphysial growth in the flight humerus bones were evident in several morphological measures other than the overall periosteal circumference (Table 1 and Figure 4). In particular, the second moments of area and cortical (periosteal) diameters for the basal control and flight rats were the same, and synchronous and vivarium control humeri were typically significantly larger. The second moment area is an indicator of the amount of cortical bone and the distance that bone mass is located from the bending axis. The test bending axis (anterior-posterior) and the non-bending axis (medial-lateral), the average area moments of the vivarium and synchronous controls were significantly greater (29-30%) than those of the basal and flight humeri (Figure 4). Concomitantly, the anterior-posterior cortical diameters of the vivarium and synchronous controls were significantly larger (9%) than the basal and flight humeri (Table 1). The middiaphysial cross-sectional densities (Table 1), as well as the regional (anterior, posterior, medial and lateral) cortical thickness, however, were not significantly different among the groups.

The average flexural rigidity (bending stiffness) of flight humeri were significantly less than the vivarium (40%) and synchronous (35%) controls, but the average flexural rigidity of the flight humerus was not different from the basal control group (Figure 5A). The elastic modulus, however, showed no statistically reliable differences among the four groups (Figure 5B). Thus, the geometrical differences in the vivarium and synchronous control humeri vs. the basal control and flight humeri accounted for differences in flexural rigidity, while the elastic modulus (material characteristics) was not different among the humeri of the various groups. The flight group had a non-statistical tendency for lower loads than the vivarium controls at the proportional limit (35% less), maximum (15% less), and failure (17% less) (Table 2). The flight humeri also had a tendency to have a greater (36%) non-linear displacement than the vivarium control humeri.

Vertebral Bone:

The flight group had an average vertebral body (L6) compressional stiffness that was 39% less than vivarium, 46% less than the synchronous, and 16% less than the basal controls (Figure 8). When stiffness was normalized to a vertebral body weight, the flight group's average stiffness remained substantially lower than the vivarium (27% less), the synchronous (33% less), and the basal (7% less) controls (Figure 8). The average initial maximum load of the flight group was 22% of the vivarium, 18% of the synchronous and 6% of the basal controls (Figure 9). However, when normalized for vertebral body weight, the initial maximum load of the flight group was 11% of the vivarium and synchronous controls, but did not differ from the basal controls. The average linear load of the flight group was 34% less than the vivarium, 25% less than the synchronous, but only 4% less than the basal controls (Figure 10). When normalized for vertebral body weight, the flight group was still only 22% of the vivarium, 70% of the synchronous, and essentially the same as the basal controls.

Average calcium (170.0 ± 8.1 μg/mg dry mass) phosphorous (122.3 ± 4.0 μg/mg dry mass) and hydroxyproline (23.0 ± 1.7 μg/mg dry mass) concentrations were not significantly different among groups. The average hydroxypyridinoline crosslink content per collagen molecule of the flight group (0.02472/moles/moles), however, was 35% less than the vivarium, 17% less than synchronous and 15% less than the basal controls.

Nutritional Effects On Bone Biomechanical Properties:

Because of differences between the rodent experiments on U.S.S.R. and U.S. space flights, the present experiment was designed to generate comparative data about the sensitivity of cortical bone (humerus) and trabecular bone (vertebral, T7) to caging environment, diet and rat strain differences. For two weeks (48-62 days), male Taconic-Sprague Dawley and Czechoslovakian-Wistar rats were maintained in flight simulation cages (1-rat/cage = U.S., 10 rats/cage = U.S.S.R.) and fed U.S.S.R. or U.S. diets. On average all rats increased (> 60%) their body mass during the two weeks, and there were no differences among humeral lengths for the different groups. Rats in U.S.S.R. cages had significantly larger total and endosteal cross-sectional areas for the humerus, and the cross-sectional areas for the T7 of Taconic rats were greater than the Czechoslovakian rats. U.S.S.R. caging affects resulted in significantly enhanced structural material properties in rat humeri, while the U.S. diet induced a significantly humeral maximum and failure loads. The bending stiffness of the Czechoslovakian rat humeri were significantly greater than the Taconic rats. Humeri from Czechoslovakian rats on the U.S. diet had significantly greater material properties in the elastic loading region than did the Taconic rats on the U.S.S.R. diet. Also, the humeral failure loads of Taconic rats on the U.S.S.R. diet were more adversely effected by U.S. cages than were the Czechoslovakian rats on the U.S.S.R. diet. The vertebral (T7), had no significant structural differences among the different groups, but material properties were influenced by all three factors; generally, the combination of factors that produced significantly greater material properties were U.S.S.R. caging, U.S.S.R. diet, and the Czechoslovakian strain of rat.

Soft Dense Fibers Connective Tissue Response:

Acute exposure to space flight did have a tendency to modify the composition in the tendon matrix. Data summarized in Table 10 showed that patellar tendons obtained from flight animals had consistently lower amounts of mature collagen crosslinks, collagen concentration and DNA concentration. Decreases in both the level of collagen maturity and concentrations of fibroblasts and structural protein would range from 8-22%. However, there were no significant trends reported in Achilles tendon obtained from flight animals. These data seems to suggest that there is a hetrogeneous response of tendon types to spaceflight in rodents. This hetrogeneity can reflect differences in load history of each tendon.

There seems to be no significant effect upon the collagen concentration in various types of skeletal muscles. Data summarized in Table 12 showed that collagen concentration was not significantly modified by space flight. However, the concentration of collagen is slightly higher in soleus muscle of flight animals as compared to basal and synchronous groups. However it should be noted that we were unable to obtain whole muscles in order to determine total collagen content. These data only reflect muscle connective tissue composition on the basis of concentration.

APPENDIX:

RAT DIET COMPOSITION

1. USSR Cosmos Diet *

Ingredients: (quantities in g)	
Casein (milk)	3.0
Cornstarch	3.0
Sucrose	6.7
Sunflower seed oil	1.7
Dry Brewers yeast	1.0
Salt mixture	0.6
Water	24.0
Food Content (quantities in g)	
Protein	3.06
Fats	1.79
Carbohydrates	9.61
Mineral Content (quantities in mg)	
Sodium	60.900
Chlorine	15.500
Potassium	67.100
Phosphorus	86.300
Calcium	84.260
Iron	3.190
Iodine	0.070
Zinc	0.080
Copper	0.080
Cobalt	0.008
Flourine	0.130
Aluminum	0.0008
Magnesium	6.960
Sulfur	11.170
Manganese	0.900
Vitamin Content (quantities in μg)	
B1	64.8
B2	62.4

B6	50.5
Pantothenic acid	240.0
Nicotinic acid	493.6
E	1380.0
A	20.0
D	6.0
Folic acid	32.0
Inosine	800.0
B15 Biotin	16.0
P-amino benzoic acid	800.0
B12	480.0
Choline	16000.0
K	16.0

* Sorbic acid 0.5% to weight of feed added as a preservative.

Quantities are for 40 g of diet, wet weight.

2. **USA Diet** (TEKLAD TD85348, values are g/kg, *fed for first week of experiment*)

Casein, High Protein	100.0
DL-Methionine	3.0
Wheat Gluten	120.0
Wheat Flour, durum 2nd clear	225.0
Corn Syrup (supplied by customer)	100.0
Sucrose	100.0
Corn Oil	40.0
Cellulose (fiber)	50.0
Mineral Mix, AIN-76 (#170915)	35.0
Calcium Carbonate, $CaCO_3$	5.0
Vitamin Mix, AIN-76A (#40077)	20.0
Choline Bitartrate	2.0

USA Diet (TEKLAD L-356, values are g/kg, *fed for second week of experiment*)

Casein, "Vitamin-Free" Test	200.0
Autolyzed Yeast Powder, Low Sodium	20.0
Liver, Desiccated (Whole Liver Substance)	20.0

Rice, White Polished (Finely ground)	582.8293
Corn Starch (Diluent for Vitamin Mix)	2.1743
Corn Oil	67.7
Non-Nutritive Fiber (Cellulose)	50.0
Calcium Carbonate, $CaCO_3$	15.0024
Potassium Phosphate, Dibasic, K_2HPO_4	11.2518
Sodium Phosphate, Dibasic, Na_2Hpo_4	10.0016
Magnesium Sulfate, $MgSO_4 \cdot 7H_2O$	3.7506
Ferric Citrate (16.7% Fe)	3.7506
Calcium Phosphate, Dibasic, $CaHPO_4$	2.7505
Sodium Chloride, NaCl	2.5004
Manganese Sulfate, $MnSO_4 \cdot H_2O$	0.6251
Cupric Sulfate, $CuSO_4$	0.192
Zinc Sulfate, $ZnSO_4 \cdot 7H_20$	0.05
Potassium Iodide, KI	0.0375
Aluminum Potassium Sulfate, $AlK\ (SO_4)_2 \cdot 12H_20$	0.0375
Cobalt Chloride, $CoCl_2 \cdot 6H_2O$	0.025
Sodium Borate, $Na_2B_4O_7 \cdot IOH_2O$	0.025
Asorbic Acid	2.0
Inositol	1.0
Choline Chloride	1.9934
Pyridoxine HCl	0.0202
Pyridoxamine Dihydrochloride	0.0041
Thiamin HCl	0.0606
Riboflavin	0.0303
Niacin	0.0504
Niacinamide	0.0504
Calcium Pantothenate	0.303
Biotin	0.001
Folic Acid	0.0101
p-Aminobenzoic Acid	0.0504
Vitamin B12 (0.1% trituration in mannitol)	0.2518
Dry Vitamin A Palmitate (500,000 U/g)	0.016
Vitamin D3 triturated in Vft Casein (3000 U/g)	0.3333
Mixed Tocopherals (372 U/g)	1.0214
Manadione	0.1

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TABLE 1

MIDDIAPHYSIAL CROSS-SECTIONAL MORPHOLOGY OF HUMERUS

	Basal	Synchronous	Vivarium	Flight
Periosteal circumference (mm)	8.3±0.2*	9.1±0.3	8.8±0.3	8.3±0.3*
Endosteal circumference (mm)	4.1±0.3	4.4±0.6	4.4±0.3	4.2±0.3
Medial-lateral cortical diameter (mm)	2.4±0.1	2.5±0.1	2.4±0.2	2.3±0.2
Medial-lateral medullary diameter (mm)	1.2±0.1	1.2±0.2	1.2±0.1	1.2±0.2
Anterior-posterior cortical diameter (mm)	2.7±0.1†	3.0±0.2	3.0±0.1	2.8±0.3†
Anterior-posterior medullary diameter (mm)	1.3±0.1	1.4±0.2	1.5±0.2	1.3±0.1
Density (mg/mm^3)	1.58±0.24	1.79±0.48	1.74±0.07	1.76±0.11

Values are means ± S.D. for 5 rats in the basal and synchronous control groups and for 4 rats in the vivarium and flight groups.

* Synchronous group is significantly ($p \leq 0.05$) different from the basal and flight groups.
† Synchronous and vivarium groups are significantly ($p \leq 0.05$) different from the basal and flight groups.

TABLE 2

MECHANICAL CHARACTERISTICS OF HUMERUS

	Basal	Synchronous	Vivarium	Flight
Load at yield (N)	70.2±12.4	71.1±12.5	87.0±16.6	64.5±18.3
Load at maximum (N)	91.0±15.5	96.9±18.3	117.1±17.6	100.3±21.6
Energy to yield load (N•s)	13.2±4.5	11.9±5.1	17.5±6.6	12.3±6.0
Energy to maximum load (N•s)	33.3±10.4	37.2±8.2	44.3±11.6	42.0±14.2
Tensile yield stress (N/mm^2)	298±49	251±40*	346±47	329±48
Nonlinear displacement (mm)	0.20±0.05	0.25±0.04	0.21±0.01	0.29±0.06

Values are means ± S.D. for 5 rats in the basal and synchronous control groups and for 4 rats in the vivarium and flight groups.

* Synchronous group is significantly ($p \leq 0.05$) different from the vivarium group.

TABLE 3

BIOCHEMICAL CHARACTERISTICS OF HUMERUS

	Basal	Synchronous	Vivarium	Flight
Calcium (µg/mg dry mass)	297±10*	306±9	322±10	314±10
Phosphorous (µg/mg dry mass)	155±7	152±4	152±4	157±4
Hydroxyproline (µg/mg dry mass)	22.0±9*	20.0±0.7	20.0±0.9	20.0±0.9
Hydroxypyridinoline (nMoles/mg dry mass)	0.0345±0.008	0.365±0.014	0.0310±0.021	0.044±0.018
Ratio of hydroxyproline to collagen (moles/moles)	0.059±0.020	0.054±0.018	0.054±0.037	0.064±0.020

Values are means ± S.D. for 5 rats in the basal and synchronous control groups and for 4 rats in the vivarium and flight groups.

* Basal group is significantly ($p \leq 0.05$) different from all other groups.

TABLE 4

CAGE EFFECTS ON HUMERUS

	10-Rat/Cage	1-Rat/Cage
Load at proportional limit (N)	66.33±13.12	56.82±10.77*
Load at maximum (N)	82.03±16.51	76.51±15.11
Energy to proportional limit (N•s)	13.59±4.93	10.42±3.88*
Energy to maximum load (N•s)	29.93±12.75	30.25±11.71
Energy to failure load (N•s)	46.19±21.27	44.24±19.58
Flexural Rigidity (kN/mm)	10.45±2.41	10.05±2.59
Elastic Modulus (GPa)	3.59±1.34	2.64±1.40*
Tensile stress at proportional limit (MPa)	206.8±69.2	143.4±53.1*

* $p \leq 0.05$

TABLE 5

DIET EFFECTS ON HUMERUS

	USA	USSR
Load at proportional limit (N)	63.34±11.60	58.92±13.70
Load at maximum (N)	85.03±12.57	73.17±16.79*
Energy to proportional limit (Ns)	12.23±5.43	11.51±3.68
Energy to maximum load (Ns)	34.93±12.80	25.01±8.92*
Energy to failure load (Ns)	52.63±20.58	37.23±16.71*
Flexural Rigidity (kN/mm)	10.84±2.49	9.59±2.38
Elastic Modulus (GPa)	3.27±1.13	2.86±1.71
Tensile stress at proportional limit (MPa)	179.9±56.2	164.9±79.7

*$p \leq 0.05$

TABLE 6

RAT-STRAIN EFFECTS ON HUMERUS

	Taconic	Czech
Load at proportional limit (N)	61.04±13.25	61.32±12.52
Load at maximum (N)	77.17±15.76	81.05±15.93
Energy to proportional limit (Ns)	13.32±5.32	10.66±3.62
Energy to maximum load (Ns)	30.57±12.93	29.72±11.53
Energy to failure load (Ns)	48.82±23.42	42.01±16.79
Flexural Rigidity (kN/mm)	9.15±1.75	11.16±2.68*
Elastic Modulus (GPa)	2.84±1.40	3.27±1.47
Tensile stress at proportional limit (MPa)	174.4±72.5	171.0±66.1

$p \leq 0.05$

TABLE 7

CAGE EFFECTS ON RAT THORACIC VERTEBRA (T7)

	10-Rat/Cage	1-Rat/Cage
Load at proportional limit (N)	70.8±22.7	63.3±19.5
Maximum load (N)	112.8±23.7	99.2±22.8
Compressional stiffness (N/mm)	690.7±265.5	691.1±207.9
Elastic modulus (MPa)	1.89±0.66	1.81±0.58
Stress at proportional limit (MPa)	15.50±6.35	12.99±4.28
Strain energy density at maximum stress (kNmm/ml)	1.38±1.35	1.04±0.75

TABLE 8

DIET EFFECTS ON RAT THORACIC VERTEBRA (T7)

	USSR	USA
Load at proportional limit (N)	72.6±27.0	60.9±9.9
Maximum load (N)	110.5±27.9	100.8±18.4
Energy at maximum load (N•s)	48.04±30.86	41.46±20.43
Compressional stiffness (N/mm)	718.6±286 6	661.6±165.3
Elastic modulus (MPa)	1.96±0.74	1.73±0.44
Stress at proportional limit (MPa)	15.88±7 16	12.44±1.51*
Strain energy density at maximum stress (kNmm/ml)	1.43±1.35	0.96±0.67

* $p \leq 0.05$

TABLE 9

RAT-STRAIN EFFECTS ON RAT THORACIC VERTEBRA (T7)

	Taconic	Czech
Load at proportional limit (N)	66.01±18.66	67.90±24.01
Maximum load (N)	107.9±18.1	103.5±29.3
Compressional stiffness (N/mm)	723.4±192.3	656.5±273.2
Elastic modulus (MPa)	2.07±0.48	1.61±0.66*
Stress at proportional limit (MPa)	15.25±5.86	13.11±4.92
Strain energy density at maximum stress (kNmm/mm)	1.36±1.23	1.03±0.91

* $p \leq 0.05$

TABLE 10 COSMOS #1887 PATELLAR TENDON COMPOSITION

Group	Hydroxyproline concentration (μg/mg)	Hydroxypyridinoline collagen crosslinks (mole/mole)	DNA concentration (μg/mg)
Flight	66.23±15.01	0.0489±0.020	8.93±3.47
Basal	71.57±10.52	0.0335±0.008	9.76±5.39
Synchronous	71.43±8.45	0.0245±0.003	10.69±4.37
Vivarium	71.82±16.29	0.0625±0.042	9.53±3.31

Values represent mean and standard deviations. * denotes a statistical significant difference, $P < 0.05$ from vivarium control group. There were no significan differences, however, values associated with flight animals were consistently lower than vivarium rats (8%-22%).

TABLE 11 COSMOS #1887 ACHILLES TENDON COMPOSITION

Group	Hydroxyproline concentration (μg/mg)	Hydroxypyridinoline collagen crosslinks (mole/mole)	DNA concentration (μg/mg)
Flight	63.15±17.18	0.0328±0.018	8.39±3.14
Basal	64.74±12.55	0.0347±0.015	8.81±2.26
Synchronous	58.56±15.07	0.0256±0.008	8.93±2.76
Vivarium	58.99±9.86	0.0274±0.010	7.18±1.87

Values represent mean and standard deviation. * denotes a statistical significant difference, $P < 0.05$ from vivarium control group. There were no significant differences among the experimental group.

TABLE 12 CCSMOS #1887 SKELETAL MUSCLE COLLAGEN CONCENTRATION (μg/mg)

Group	Soleus	Medial Gastroenemius	Tibialis Anterior
Flight	9.17±1.8	5.37±0.60	7.12±3.8
Basal	5.33±2.6	6.94±2.1	7.16±2.7
Synchronous	8.50±2.3	5.08±1.4	6.73±0.9
Vivarium	9.46±3.9	5.43±1.0	4.79±1.5

Values represent mean and standard deviations. * denotes a statistical significant difference, $P < 0.05$ from vivarium control group. There were no significant differences.

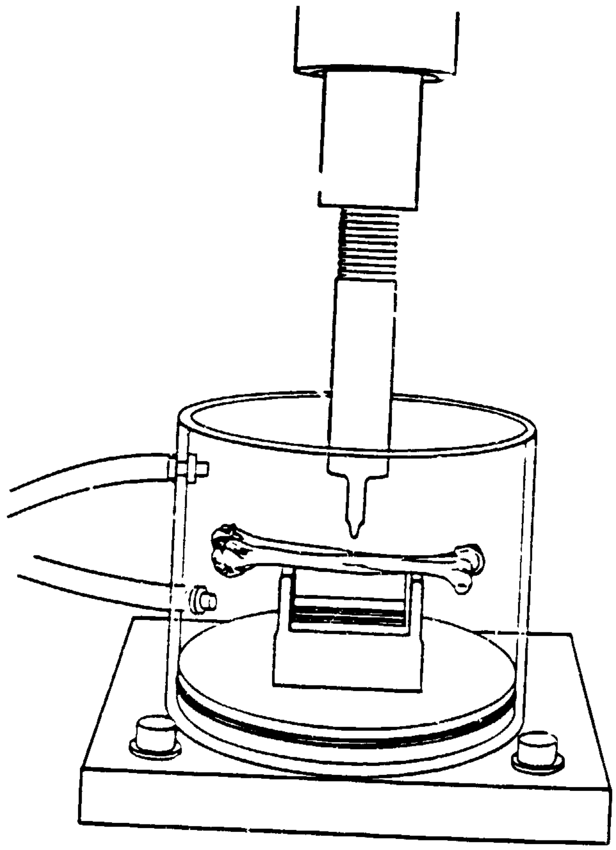

Figure 1. Apparatus used for three-point bending of the humerus.

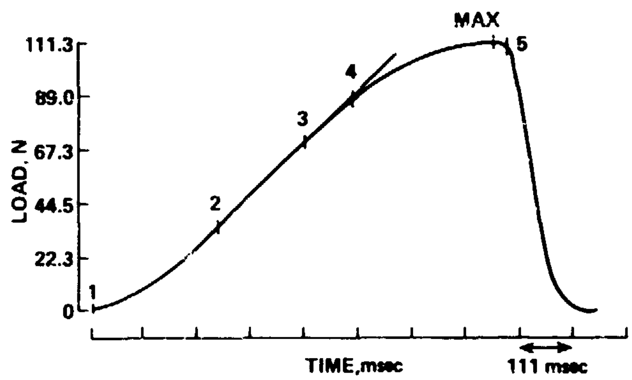

Figure 2. Exemplar load-time curve for humerus test. Point 1 indicates the application of the initial load. Points 2 and 3 are boundary marks for the linear regression line that is calculated to estimate the slope of the load-deflection curve. Point 4 is the proportional limit. MAX indicates the maximum load, and Point 5 is failure load.

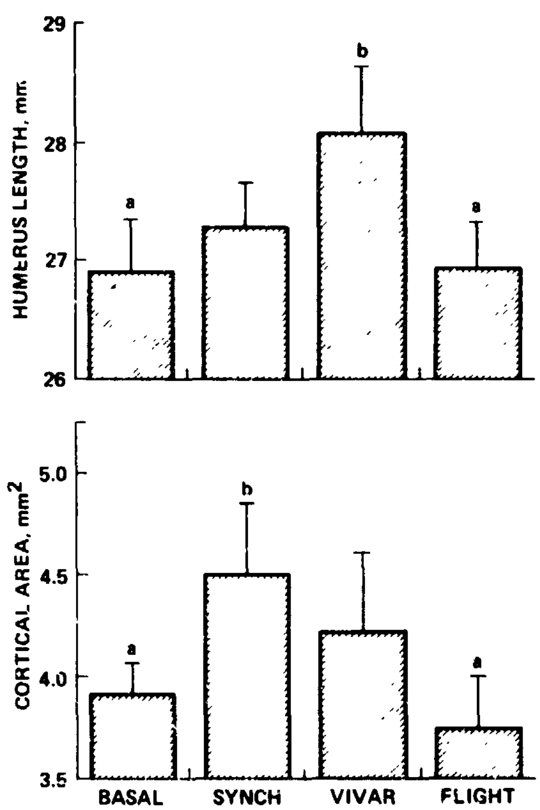

Figure 3. Humeral length (a) and middiaphysial cortical cross-sectional area (b). Mean values and SD error bars are indicated. Statistically significant ($p \leq 0.05$) relationships include the following: b > a.

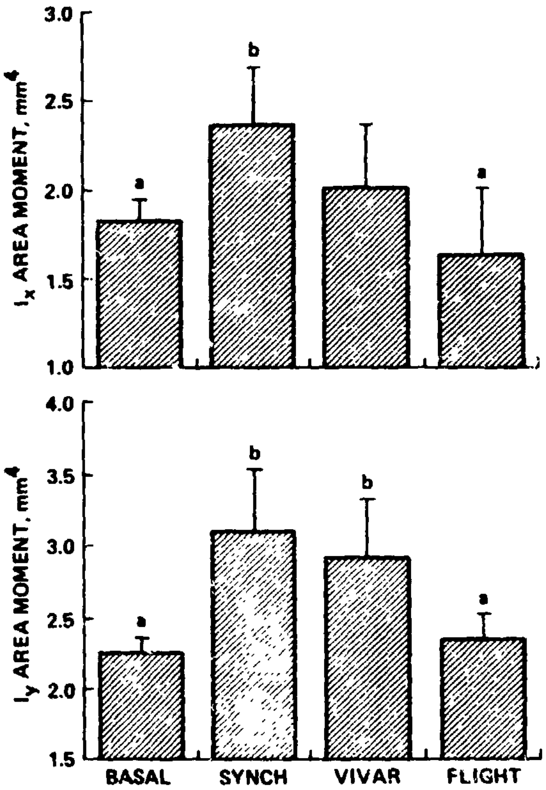

Figure 4. Humeral second moment of area with respect to anterior-posterior axis (Ix, Fig. 4a) and medial-lateral axis (Iy, Fig. 4b). Mean values and SD error bars are indicated. Statistically significant ($p \leq 0.05$) relationships include the following: b > a.

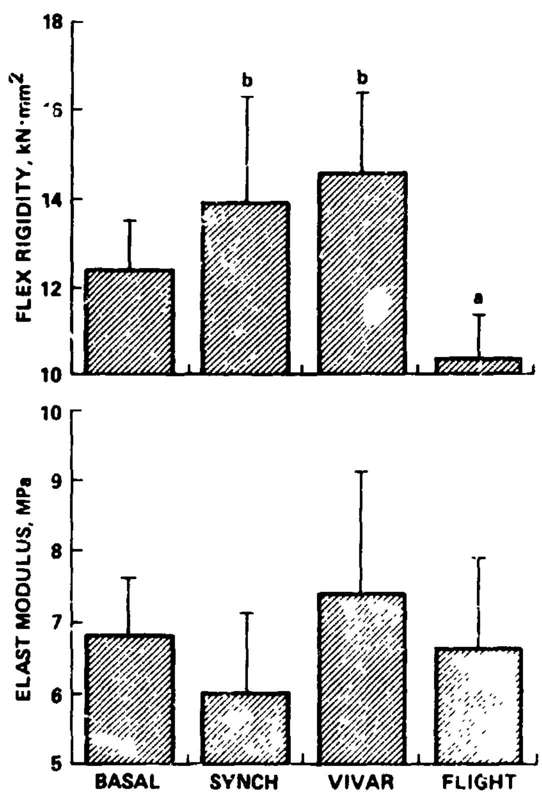

Figure 5. Humeral flexural rigidity (a) and elastic modulus (b). Mean values and SD error bars are indicated. Statistically significant ($p \leq 0.05$) relationships for flexural rigidity include the following: b > a. No statistically reliable differences were found among the elastic moduli of the groups.

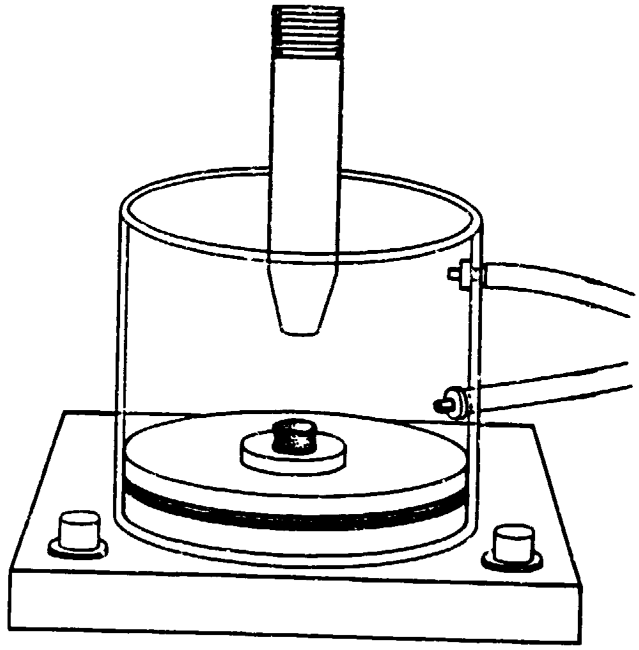

Figure 6. Set-up for the rat vertebral body compression tests. The vertebral body is shown fixed to a cylindrical stainless-steel plate while immersed in a warmed, circulating buffer solution.

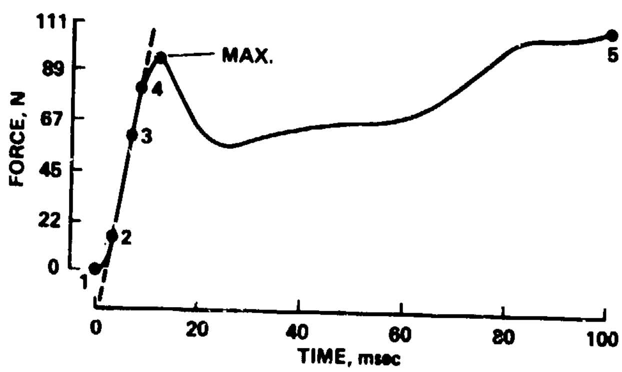

Figure 7. Exemplar load-time curve for a compression test of a rat L6 vertebral body. (1) denotes the point of initial loading, (2 and 3) are arbitrary points in the linear load region, (4) is the proportional limit, (5) is 50% strain, and MAX represents the initial-maximum load.

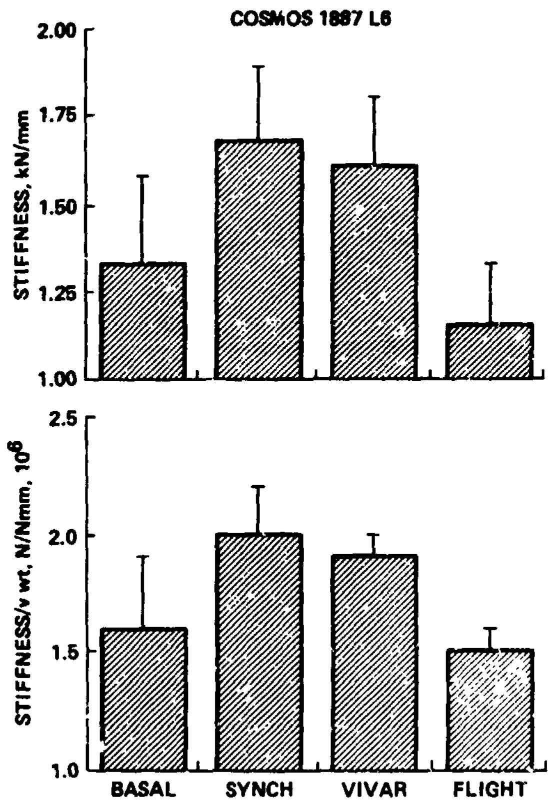

Figure 8. Compressional stiffness and normalized stiffness (per unit vertebral-body weight) for rat L6. Mean and SD values are indicated for the flight, vivarium, synchronous, and basal control groups.

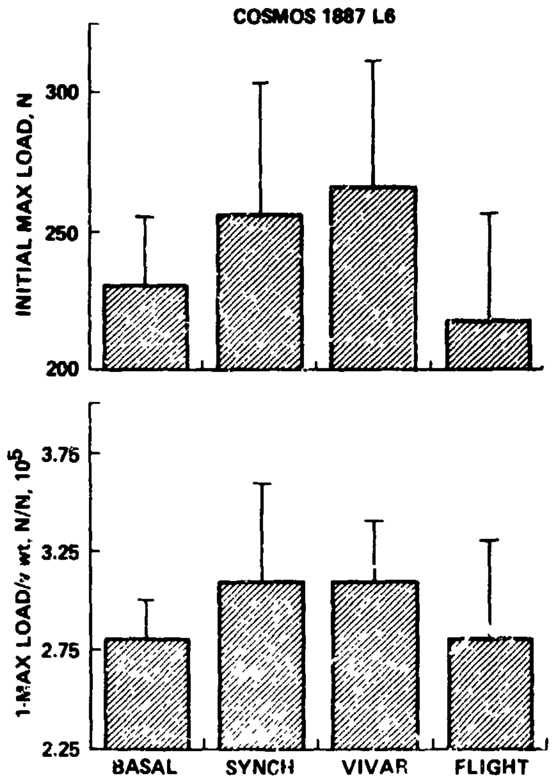

Figure 9. Initial-maximum load and normalized initial-maximum load (per unit vertebral-body weight) for rat L6. Mean and SD values are indicated for the flight, vivarium, synchronous, and basal control groups.

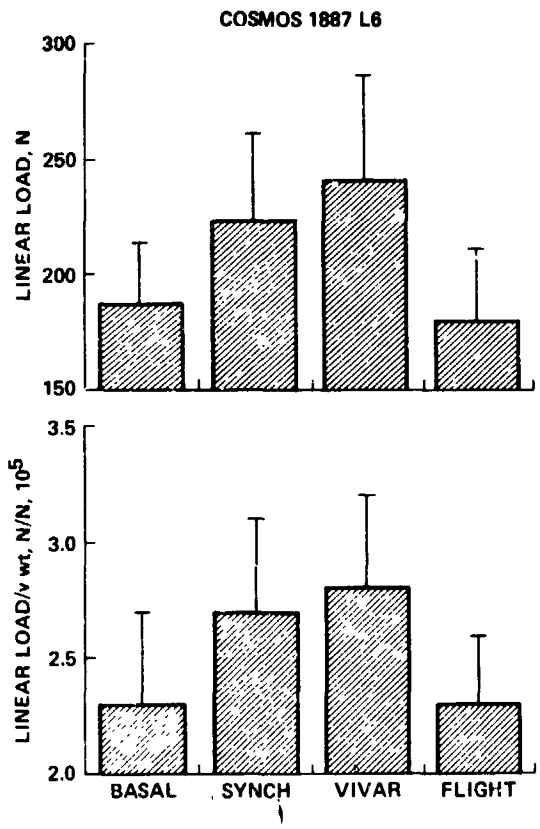

Figure 10. Linear load and normalized linear load (per unit vertebral-body weight) for rat L6. Mean and SD values are indicated for the flight, vivarium, synchronous, and basal control groups.

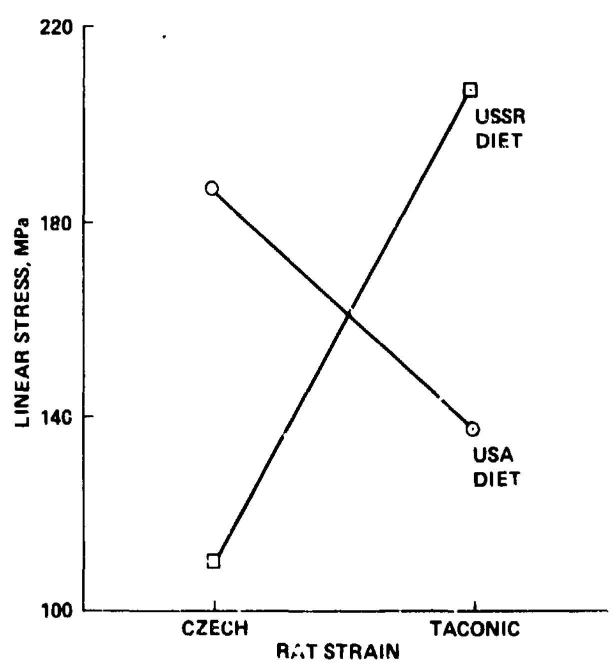

Figure 11. Humerus linear stress two-way significant interaction (diet x strain).

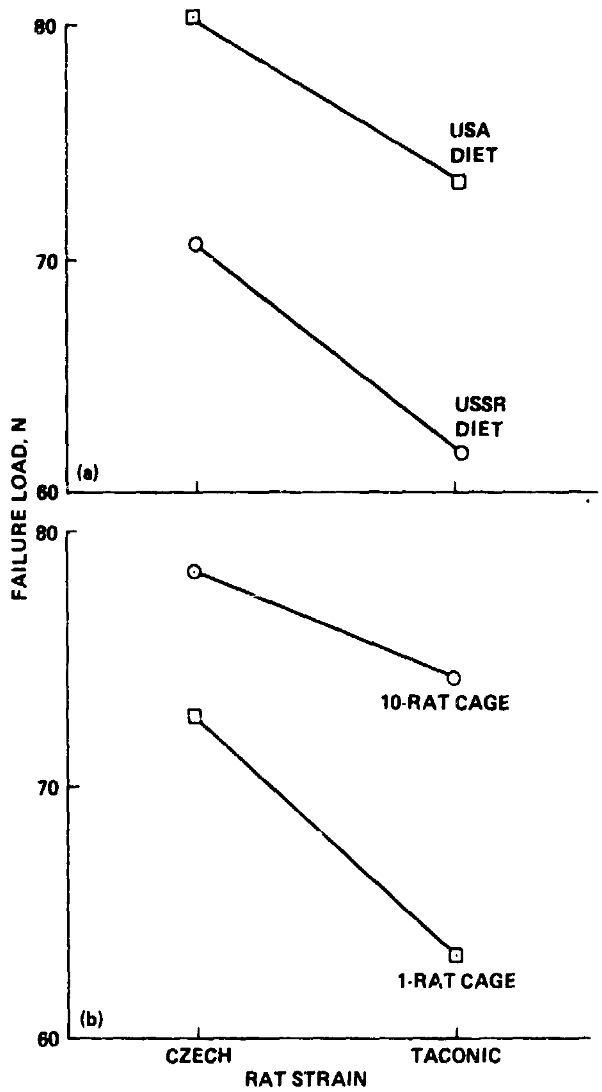

Figure 12. Humerus failure stress three-way significant interaction (diet x cage x strain). (a) illustrates diet x rat strain and (b) illustrates cage x rat strain effects.

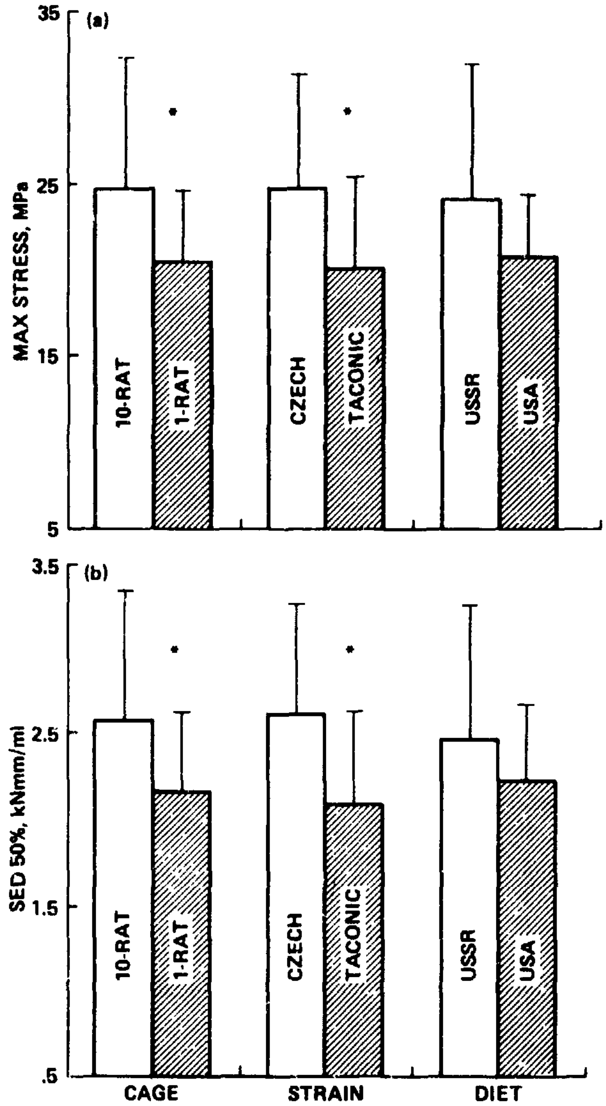

Figure 13. T7 vertebral-body differences for maximum stress (a) and strain energy density at 50% strain (b). Both the cage and rat-strain effects were statistically significant differences (*). The diet effect was not significant.