Indian J Physiol Pharmacol 2000; 44 (3) : 359–362

BONE CHANGES DURING SIMULATED WEIGHTLESSNESS IN RATS

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(Received on October 15, 1999)

Abstract : Weightless environment due to prolonged Space mission results in decreased mineralisation of the weight bearing bones. Hind limb unweighting (HU) in rats by tail suspension was used to simulate the effect of weightlessness on tibia. Adult male albino rats were divided into two groups as (i) Control (CON, n = 12) and (ii) HU for 15 days (HU, n = 18). After 15 days of HU tibia from all the animals were removed and subsequently dried and ashed. The calcium content of these bones were then determined. HU resulted in atrophic changes in the weight bearing bone, tibia, due to the reductions of water content (–35.8%), organic matrix (–12.2%) and calcium content (–33.4%). The reduction in the dry wt of tibia (–13.5%) was due to proportionate reductions in the organic matrix and total mineral content of the bone. The reduction in the mineral content was solely due to the reduction in calcium content of the bone.

Key words : hind limb unweighting mineralisation bone weightlessness

INTRODUCTION

Biomedical data obtained from space missions demonstrate that bone and mineral metabolism are altered during space flight. Calcium balance becomes increasingly negative throughout flight and the bone mineral content of the weight bearing bones decline. Loss of total body calcium and skeletal changes have been observed in animals and people who have flown from 1 week to more than 365 days in space (1, 2). Rats have been the commonest animal used for studying the changes in bone and its mineral content during actual space mission and ground based simulation models (1). In general, ground based models for simulating weightlessness emphasize cephalad fluid shift and disuse of limbs by immobilisation/ unloading of weight bearing limbs (1, 3).

Hindlimb unweighting (HU) by tail suspension in rats has proven to be an useful ground based animal model to simulate the effects of weightlessness on the various systems of the body (3, 4). The present study was undertaken to see the changes in weight bearing bone tibia after 15 days of tail suspension in rats.

METHODS

Male Wistar albino rats, aged 90 to 180 days (d) weighting 150–210 gm, were used in this study (3, 5). They were housed individually in 'Weightlessness Simulation

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Cages' (WSC) with food (pelleted) and water provided *ad libitum* (3). After 7 d of adaptation to WSC and the feed they were divided randomly in 2 groups (3). Group 1 (CON) rats were left in WSC for another 15 d without any treatment. Group 2 (HU) rats were given simulated weightlessness (S-W), continuously for 15 d, by using tail suspension technique of hind limb unweighting (3). After 15 d of experimentation rats were anaesthetized by pentobarbital sodium (50 mg/kg body wt, ip). Rats were sacrificed and their tibia removed, stripped of adhering muscle and connective tissue and weighed for their wet bone wt. Bones were dried in individual steel container at 100°C for 24 h in a forced air drying oven, removed to a covered tray containing desiccant and were reweighed to obtain their constant dry bone wt as per method used by others (6). Organic matrix of the dry bone was removed by incinerating it and converting it in to ash. Bones were ashed in individual crucibles for 24 h at 600°C in a muffle furnace and the ash wt determined. Ash, consisting of only inorganic component, was then transferred to a 100 ml flask and dissolved in 10 ml of 2N HCI. The sample was then diluted to 100 ml in double distilled water and its Calcium concentration was measured by Baginski's Cresolphthalein complexone (CPC) method (7). Water content of the bone was determined by subtracting dry bone wt from wet bone wt and organic matrix component of bone was determined by subtracting ash wt from dry bone wt. All these parameters of bone were expressed as mg/100 gm body wt (BW) as done by other authors (6, 8).

Student's unpaired 't' test was used to compare means of various bone parameters of HU with CON group. In all cases, the minimum level of significance was set as $P<0.01$.

RESULTS

The effects of S-W on various bone parameters are shown in Table I. HU group showed reductions in the wt of wet bone by 20.9%, water content by 35.8%, organic matrix by 12.2%, total inorganic content

TABLE I : Bone changes during simulated weightlessness (S-W).

Parameters	*CON (n = 12)*	*HU (n = 18)*	*% Change*
Wet tibia wt (mg/100 gm, BW)	214.0±24.2	169.3±12.3	–20.9**
Tibia water (mg/100 gm, BW)	70.4±10.7	45.4±6.6	–35.8**
Dry tibia wt (mg/100 gm, BW)	143.4±13.7	123.9±10.0	–13.5**
Tibia organic matrix (mg/100 gm, BW)	64.5±6.8	56.6±7.7	–12.2*
Ash wt (mg/100 gm, BW)	78.9±7.3	67.3±6.2	–14.6**
Tibia Calcium (mg/100 gm, BW)	33.8±3.9	22.5±4.8	–33.4**

Values are Mean ± SD; * = P<.01; ** = P<.001; BW = Body weight; Con = control; HU = S-W by hind limb unweighting.

(ash wt) by 14.6% and calcium content by 33.4% of tibia.

DISCUSSION

Bone is a modified connective tissue consisting of cellular elements like osteoblasts and osteoclasts, organic intercellular matrix rich in collagen, mucopolysaccharides and lipids and inorganic mineral component like calcium and phosphorus (2). Results of CON group show that wet tibia consists of 1/3 water while 2/3 of it is dry bone consisting of orgnaic matrix and mineral content. Analysis of dry bone, revealed that 45% of dry bone is organic matrix and 55% of it is mineral component. These findings are in agreement with the data available in literature (2, 9).

HU for 15 d resulted in reduction of wet bone wt. This reduction in wet bone wt may be the result of reduction in water/organic matrix/mineral content of bone. On comparing water content in tibia of HU group with CON group, it was found 35.8% reduced in HU group. Reduction in the water content of bone may be due to reduction in collagen matrix leading to less osmotic binding of water molecule or as a part of reflex reduction in blood volume and body water due to cephalad fluid shift as induced during S-W by HU (2, 8).

HU group also showed reduction in dry bone wt by 13.5%. It may be the result of reduction in organic matrix and/or mineral component of the bone. HU group showed proportionate reductions in the organic matrix (12.2%) and mineral component (14.6%). As majority of the organic matrix in a bone is made up of collagen fibres (2, 9), reduction in organic matrix can be interpreted as reduction in collagen fibres of the bone. Reduction in collagen matrix of bone in space flight has also been reported by others (6). It is also possible that some collagen fibres of bone were replaced by lipid material (10).

The reduction in mineral content of bone may be due to reductions in calcium, phosphorus or some other mineral of the bone. The difference between the CON and HU for total mineral content (11.6% mg/100 gm, BW), and calcium content (11.3 mg/100 gm, BW), of tibia appears to be equal. Therefore, it can be concluded that the reduction in the mineral content of the bone was solely due to reduction in the calcium content of the bone. Reduction in mineral content of bone during weightlessness has also been reported by others (10). Reduction in calcium content of bone may be the result of either less mineralisation of collagen fibres of bone and/or replacement of some collagen fibres by lipid materials in the bone (10). Lipid material of bone are normally not calcified in contrast to collagen fibres which are calcified as soon as these are formed by osteoblast (2, 9). A 33.4% decrease in calcium of tibia as compared to 12.2% decrease in organic matrix is suggestive of replacement of some of the collagen fibres of bone by lipid materials during S-W. Reductions in the collagen matrix and calcium content of the bone have also been reported by various other workers (10, 11, 12).

As greatest changes in bone mass during space flight occur in weight bearing bones, changes in mechanical loading are undoubtedly important. Bone is deposited in proportion to the compressional load that the bone must carry (2). Space flight and S-W are known to result in atrophy of extensor muscles in the hind limb (3, 8). Therefore, changes in muscle forces could affect periosteal surfaces of bone by causing localised changes in bone formation and resorption (13). Decrease in the bone mass could also result from decrease in blood volume and microvascular blood pressure leading to less nutrient supply to bone (2, 6). Electron microscopic study in adult rats subjected to 14 d space flight in Soviet biosatellite Cosmos 2044 showed vascular inclusions and reduced bone cell activity (5). All these factors are likely to disturb bone formation/resorption. The changes in the tibia are not likely to be due to stress of the experiment as Thomason DB et al reported only minimal and transient stress during 1st week of HU but no signs of stress were seen after 1st week of HU (14). Whatever the mechanism involved, this study demonstrates that S-W by tail suspension in rats resulted in reduction of water, collagen and calcium content of tibia.

CONCLUSION

S-W by HU in rats resulted in atrophic changes in the weight bearing bone, tibia, due to reduction of its content of water, collagen matrix and calcium. The reduction in the dry tibia wt was due to proportionate reduction in its collagen matrix and total mineral content. The effect on the mineral content was solely due to the reduction in calcium content of the bone.

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