*Original Article*

# Modification of bone atrophy by daily 2 hour weight support during simulated weightlessness in rats

Wg Cdr PK Jain [$] Dr EM Iyer [@] Dr PK Banerjee[#] Gp Capt NS Baboo[*]

**Hind limb unweighing (HU) in rats for 15 days (d) simulates the deconditioning effects of weightlessness on the weight bearing bones. The present study evaluates the effects of daily 2 hour (h) weight support (WS) during simulated weightlessness (S-W) in preventing the atrophic changes in tibia. Adult male albino rats were divided into three groups as (i) control (CON, n=12), (ii) S-W by tail suspension for 15d (HU, n=18), (iii) HU with daily 2h WS (2HRWS, n=10). After 15 d tibia from all the animals were removed and subsequently dried, ashed and then calcium content of the bones were determined. HU showed reductions in the water content by 35.8% organic matrix by 12.2% and calcium content by 33.4% of tibia. Daily 2h WS during S-W resulted in complete prevention of water loss in tibia. However, organic matrix and calcium content did not show significant improvement in 2HRWS when compared with HU. 2HRWS showed reductions in organic matrix by 10.4% and calcium content by 27% when compared to CON. These findings indicate that daily 2h WS during S-W in rat is not an effective countermeasure to prevent the atrophic changes in bone.**

**Keywords: Hind limb unweighing, osteroporosis, bone mineralisation.**

Weightlessness during space flight results in decrease in mineral content of weight bearing bone and an increase in urinary and focal calcium loss [1,2,3]. The possibility that these calcium losses may not abate with time of exposure has raised concerns of serious biomedical risk during prolonged exposure to hypogravic environments. Different workers have attempted to prevent these physiological alternations in space by using methods viz diet supplementation, drugs, exercise and lower body negative pressure but without achieving any effective countermeasure [2,4,5]. Only severe exercise regimens have been found effective in preventing physiological deconditioning of cosmonauts in very long duration spaceflights [6]. However, as the time required for such a programme is more and it leads to muscular pain, it is not an ideal countermeasure for long term space flights and undoubtedly some form of artificial gravity, such as by rotating space station or the use of human centrifuge in space station is required for such missions [6,7]. A recent study has shown that the increase in urinary calcium output in continuous bed rest subjects were reduced to nearly pre bed rest levels with three hours of daily standing at 1 G. But neither daily supine bicycle ergometry up to 4 hours (h) per day, nor sitting at 1 G for 8 h per day had any effect on increased urinary calcium from

[$] *Classified Specialist (Physiology), No. 10 Wing, Air Force, C/o 99 APO*

[@] *Scientist 'E', Institute of Aerospace Medicine*

[#] *Scientist 'E', Defence Institute of Physiology & Allied Sciences, Delhi*

[*] *Professor (Physiology), Institute of Aerospace Medicine*

bed rest [6]. In our earlier study 4 hr of daily weight support (WS) during simulated weightlessness (S-W) in rats was found as a partially successful countermeasure in preventing the bone demineralisation [8].

Anti orthostatic hypokinetic posture by tail suspension in rats is an accepted model for simulating effects of weightlessness on skeletomuscular system [9,10]. This study was undertaken to see the usefulness of daily 2 h WS during S-W in rats in preventing the atrophic changes in bone.

## Material and methods

Wistar strain of male albino rats, aged 4 months to 8 months and weighing 150-210 gm, were used in this study [11,12]. They were adapted to the environment in 'Weightlessness Simulation Cages' (WSC) with food (pelleted, Gold Mohur feed) and water provided ad libitum [13]. After 7 d of adaptation to WSC and the feed they were divided randomly in 3 groups. Group 1 (CON, n=12) rats were left in WSC for another 15 d without any treatment. Group 2 (HU, n=18) rats were given S-W by hind limb unweighing (HU) by tail suspension [5,13] for 15 d. Group 3 (2HRWS, n=10) rats were given S-W for 15 d but released from tail suspension for 2 h daily from 0800 h to 1000 h, to bear their own weight.

After 15 d of experimentation rats were anaesthetized by pentobarbital sodium (50 mg/kg body wt, ip) and hind limb muscles were studied for their contractile properties. After this, rats were sacrificed and their tibiae removed for their wet wt, dry wt, ash wt, and calcium content (bone paper) [8]. Water content and organic matrix component of bone were determined indirectly [8]. All these parameters of bone were expressed as mg/100 gm body wt (mg/100 gm BW) [5,8,10]. Bone calcium was also expressed as mg/100 mg dry bone [5,8].

## Results

Bone changes during S-W and the effects of daily 2 h WS on these are presented in Table 1. HU group showed reductions in the wt of wet bone, water content, collagen matrix, total inorganic content (ash wt) and calcium content of tibia. These findings are in agreement with the reports of other workers [5,8,10]. 2 HRWS group showed significant improvement in the bone water content when compared to HU, however, Organic matrix and calcium content were not found to be significantly improved.

Student's unpaired t test was used to compare means of various bone parameters of HU and 2 HRWS groups with CON group and 2 HRWS with HU. In all cases, the level of significance was set as $P < 0.05$.

## Discussion

Bone is a modified connective tissue consisting of living cells (viz. osteoblast, osteoclast), organic intercellular matrix (viz. collagen, mucopolysaccharides and lipids) and inorganic (minerals viz. calcium, phosphorus) component. Results of CON group show that tibia consists of 1/3 water and 2/3 of organic matrix (45%) and mineral content (55%) [8,14,15].

S-W by tail suspension in rats for 15 d resulted in reduction of wet bone wt by 20.9%. 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. The HU group also showed reduction in the dry wt by 13.5%. This reduction in the dry wt may be due to reductions in the organic matrix or mineral content of the bone. The wt of organic matrix was found to be reduced by 12.2% in HU group. As majority of the organic matrix in a bone is made

**Table 1. Modification of bone atrophy by daily 2 hour weight support during simulated weightlessness in rats**

| Parameters | Con (n=12) | HU (n=18) | Difference (HU-CON) | 2 HRWS (n=10) | Difference (2HRWS-CON) | (2HRWS-HU |
|---|---|---|---|---|---|---|
| Wet tibia wt | 214.0 | 169.3 | -20.9% | 189.7 | -11.4% | 12.0% |
| (mg/100gm BW) | ± 24.2 | ± 12.3 | *** | ± 12.4 | ** | *** |
| Tibia water | 70.4 | 45.4 | -35.8% | 64.3 | -9.0% | 41.7% |
| (mg/100gm BW) | ± 10.7 | ±6.6 | *** | ± 8.2 | NS | *** |
| Dry tibia wt | 143.4 | 123.9 | -13.5% | 125.4 | -12.5% | 1.2% |
| (mg/100gm BW) | ± 13.7 | ± 10.0 | *** | ± 4.8 | ** | NS |
| Tibia organic | 64.5 | 56.6 | -12.2% | 57.7 | -10.4% | 2.0% |
| matrix | ± 6.8 | ± 7.7 | ** | ± 3.8 | * | NS |
| (mg/100gm BW) | | | | | | |
| Ash wt | 78.9 | 67.3 | -14.6% | 67.7 | -14.2% | 0.5% |
| (mg/100gm BW) | ± 7.3 | ± 6.2 | *** | ± 1.8 | *** | NS |
| Tibia calcium | 33.8 | 22.5 | -33.4% | 24.7 | -27.0% | 9.6% |
| (mg/100gm BW) | ± 3.9 | ± 4.8 | *** | ± 1.3 | *** | NS |
| Calcium (mg/100mg | 23.4 | 18.2 | -22.4% | 19.7 | -16.1% | 8.2% |
| Dry Bone) | ± 1.6 | ± 3.8 | *** | ± 0.7 | *** | NS |

* = p<.05, **=p<.01, ***=p<.001, NS= Not Significant, BW=Body weight, CON=Control, HU=S-W by hind limb unweighing, 2HRWS=HU with daily 2h wt support.

up of collagen fibres [14,15], 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 [10]. HU group also showed reduction in total mineral content of bone by 14.6%. Reduction in mineral content of bone during weightlessness has also been reported by others [1]. The reduction in mineral content of bone may be due to reductions in calcium, phosphorus or any other mineral of the bone. Calcium content of tibia was found 33.4% reduced in HU group. Calcium in relation to dry bone was also reduced by 22.4% in HU group. This finding is suggestive of more calcium loss in a dry bone as compared to its collagen matrix by S-W. Reduction in the calcium content of the bone associated with increased calcium, phosphorus and other mineral loss in line during actual and simulated weightlessness has also been found by various other workers [1,2,16]. These observations suggest that S-W by tail suspension in rats resulted in reduction of water, collagen and calcium content of tibia.

2 h WS during 15 d of S-W resulted in partial improvement of wet tibia wt (by 12% as compared to HU). This improvement in bone wt may be the result of improvement in water/organic matrix/mineral content of the bone. On further analysis water content of tibia was found to be improved. Although water content in 2 HRWS was still 9% less than CON but difference was not found significant. Dry bone wt in 2 HRWS did not improve as compared to HU. On further analysis organic matrix content in 2 HRWS did not show improvement when compared to HU and was still 10.4% less as compared to CON. Total mineral content of the tibia (ash wt) also did not improve and it was still 14.2% less in 2 HRWS as compared to CON. Total calcium content (mg/100 gm BW) of the bone was still 27% less in 2 HRWS as compared to CON and it also did not show significant improvement as compared to HU. Calcium content in relation to the dry bone was 16.1% less in 2 HRWS as compared to CON. Although, it showed improvement by 8.2% in 2 HRWS as compared to HU, it was insignificant. These findings are suggestive of more calcium loss as com-

pared to the collagen matrix loss in tibia inspite of giving daily 2 h WS during S-W. Thus 2 h WS during S-W was not effective in preventing the calcium loss and reduction in collagen matrix of the bone, however, it was found sufficient in fully preventing the water loss, from tibia as induced by S-W.

## Conclusion

HU in rats simulates deconditioning effects of weightlessness on weight bearing bone tibia resulting in atrophic changes in the form of reduction of water content, organic matrix and calcium content of bone. 2 h WS during S-W resulted in complete prevention of water loss form tibia but was found inadequate for prevention of collagen matrix and calcium loss. 2 h WS during S-W is not an effective counter measure to prevent the atrophic changes in above.

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