Original Article

Intermittent weight support as a countermeasure to hind limb muscle atrophy in simulated weightlessness

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

Hind limb unweighting (HU) by tail suspension in rats simulates the effects of weightlessness and results in atrophy of muscles, especially of those having a load bearing function which consist primarily of slow twitch fibres. The present study was designed to evaluate the effects of 2 and 4 hour (h) weight support (WS) in ameliorating this atrophic response to HU. Adult male albino rats were assigned to control (CON), HU, HU with 2h daily WS (2HRWS) and HU with 4h daily WS (4HRWS) groups. After 15 days, weight of gastrocnemius (G), plantaris (P), both predominantly having fast twitch fibres and soleus (S) predominantly with slow twitch fibres were studied. HU group showed significant weight reductions of G (-17.9%), P (-13.3%) and S (-41.9%). 2h WS during HU was sufficient in preventing atrophic change in P while 4h WS during HU resulted in prevention of atrophy of G and P. S muscle weight showed significant improvement even in 2HRWS group although no further improvement in the weight of S muscle was observed in 4HRWS group. These findings indicate that 4h daily WS during HU is a better countermeasure as compared to 2h daily WS and it results in the prevention of atrophic changes in the muscles having predominantly fast twitch fibres while muscles having primarily slow fibres improve partially.

Keywords: **Weightlessness, muscle atrophy, tail suspension models, simulated microgravity**

Weightlessness during space missions brings about functional changes in various systems of the human body including disturbances in the skeletomuscular system [1]. Hind limb unweighting (HU) by tail suspension [2] has been proved to be a useful ground based model to simulate the effects of weightlessness on the skeletomuscular system. Simulated weightlessness by HU results in atrophy of antigravity muscles of hind limbs. Muscles characterized by a tonic activation pattern and composed predominantly of slow oxidative fibres (type 1) are affected to the greatest degree [3]. Muscles serving in an antigravity role such as soleus (S), plantaris (P) and gastrocnemius (G) are affected to a greater extent than the muscles not serving an antigravity role such as extensor digitorum longus (EDL) and tibialis anterior (TA) [4]. This differential effect also can be seen in fibres that stain light (slow twitch) *vs* those that stain dark (fast twitch) for

$ *Classified Specialist Physiology IAM, IAF*
@ *Scientist 'F' DIPAS*
+ *HOD, Environmental & Space Phyisology IAM, IAF*
# *Scientist 'E', IAM IAF*

myosin ATPase at an alkaline pH.

Scientific evidence suggests that the body does not require continuous exposure to gravity for bodily processes and a short duration of gravitational stimulation on a regular daily or perhaps less frequent basis is sufficient to maintain these processes [5]. Recently, it has been shown that the mass of S can be maintained if the rats were removed from non weight bearing and subjected to WS for 4 times per d, lasting 10 min each [6,7,8]. To apply it as a counter measure for antigravity muscle atrophy of space, astronauts will be required to undergo gravity exposure for number of times in a day and thus would spend much more time in the centrifuge, out of a limited time available during an actual space mission [5].

It was hypothesized that probably a one time +1 Gz exposure by simple WS for few h per d in HU rat model may be an effective counter measure for preventing muscle deconditioning. This study was undertaken to find the usefulness of 2h/d and 4h/d exposure to gravity in preventing the muscle atrophy in tail suspended rats.

Material and methods

Wistar strain of male albino rats, aged 4 months to 8 months and having body weight (BW) 150-210g, were selected from our own laboratory [9, 10]. They were housed individually in identical, 18 ´18 ´18 inch Weightlessness Simulation Cages (WSC) with food (pelleted, Gold Mohur feed) and water provided ad libidum. They were allowed to adapt in WSC for 7d [9]. After 7d of adaptation to WSC they were divided in 4 groups. Group 1 (CON) rats were left in WSC for another 15d without any treatment. Group 2 (HU) rats were tail suspended for 15d [9]. Group 3 (2HRWS) rats were tail suspended for 15d but released from tail suspension for 2h daily from 1000h to 1200 h, to bear their BW. Group 4 (4HRWS) rats were tail suspended for 15d but released from tail suspension for 4h daily from 0800 h to 1200 h, to bear their BW. Rats were checked daily for signs of tail lesions or discoloration, unusual breathing patterns or undue discomfort. Any animal exhibiting any of these signs was immediately removed from the study [9]. Their daily feed intake and BW were also monitored.

At the conclusion of 15d, the rats were anaesthetized with pentobarbital sodium (50 mg/kg BW, intra peritoneal) and gastrocnemius (G) plantaris (P) and soleus (S) muscles were excised and weighed to the nearest 0.1 mg on Mettler balance [9]. The net weight of muscles was normalized for the BW and expressed as g/100 g of BW [2].

Student's unpaired t test was used to compare means of wet muscle weights of HU, 2HRWS groups with CON group. Parameters of 2HRWS and 4HRWS groups were also compared with HU group. In all cases, the level of significance was set as $P < 0.05$.

Results

Table 1 presents weights of G, P, S muscles under four conditions including control. Table 2 shows the changes in the muscle weight between varying conditions. HU group showed reductions in the weight of all the postural antigravity G (-17.9%), P (-13.3%) and S (-41.9%) muscles. 2HRWS group showed significant improvement in the weight of P and S muscles when compared with HU group, however, G weight showed no significant improvement. 4h WS during HU resulted in complete prevention of atrophic

Table 1: Effect of intermittent weight support (WS) during 15 days of simulated weightlessness by hind limb unweighting (HU) in rats on the weight of antigravity muscles

Muscle weight	CON	HU	2HRWS	4HRWS
Gastrocnemius	482.4± 49.6	396.3± 48.3	403.8± 39.5	445.4± 46.3
Plantaris	83.8± 8.3	72.6± 8.5	82.7± 8.5	81.1± 9.9
Soleus	41.0± 4.2	23.8± 4.2	31.9± 4.1	31.8± 6.2

Values are Mean± SD; CON = control; 2HRWS = HU with 2 hour daily WS; 4 HRWS = HU with 4 hour daily WS; BW = body weight

Table 2 : Effect of intermittent weight support (WS) during 15 days of simulated weightlessness by hind limb unweighting (HU) in rats on the weight of antigravity muscles

Muscle weight	(mg/100g BW)	(2HRWS-CON)	(2HRWS-HU)	(4HRWS-CON)	(4HRWS-HU)
Gastrocnemius	-17.9%(***)	-16.3%(***)	+ 1.9% (NS)	- 7.7% (NS)	+ 12.4% (**)
Plantaris	-13.3% (**)	-1.3% (NS)	+ 13.9% (**)	- 3.2% (NS)	+ 11.7% (*)
Soleus	-41.9% (***)	-22.2% (***)	+33.9% (***)	-22.4% (***)	+33.5% (***)

Con = control; 2HRWS = HU with 2 hour daily WS; 4 HRWS = HU with 4 hour WS; * * * = P < 001; ** = P < 01; * = P < .05; NS = Not significant; BW = body weight

changes in G and P muscles as their net weights were not significantly different from CON. 4HRWS group also showed significant improvement in the weight of S muscle, however, it was still -22.4% less than CON group.

Discussion

Skeletal muscle is composed of 75% water 20% protein and 5% other component viz, inorganic material (eg. calcium, magnesium), carbohydrate (eg. glycogen) and other organic extractives [11]. All the muscles of the body are continually being remodelled to match the functions that are required of them. Their diameters, vascular supplies and even the types of muscle fibres are altered. This remodelling process is often quite rapid and takes only few weeks. Muscle contractile protein can be totally replaced in as little as 2 weeks (1).

The S, P and G muscles are synergistics. They are primary ankle extensors, but they have very different type of fibre composition and recruitment patterns. The S is composed primarily of slow twitch fibres [12,13], where as G and P are composed of a mixture of fibre types and have predominantly fast twitch fibres [14]. Functionally S is near maximally activated even during simple WS, whereas G and P become highly active only when high activity (and presumably, force) demands are required [15].

HU group showed decrease in G, P and S weight when compared with CON. Maximum weight reductions were found in S (-41.9%) followed by G (-17.9%) and P (-13.3%). Our finding are in agreement with the findings of other workers in the field [2,4,16].

2 HRWS group showed significant improvement in P and S weight; P weight was not found significantly different from CON but S weight was still 22.2% less from CON. G weight did not show significant improvement in 2HRWS when compared with HU and it was still 16.3% less than CON. These findings indicate that 2h WS during 15d of HU resulted in full prevention in reduction of contractile protein and/or water of P, partial prevention in S muscle while it was not sufficient in preventing the atrophic changes in G muscle.

4HRWS group showed increase in G weight as it was found 12.4% more than HU. G weight in 4HRWS group was not found significantly different from CON group. Changes in P weight and S weight were similar to 2HRWS group as P weight was found similar of CON group, while S weight was still 20% less than CON group. These findings are suggestive of complete prevention of reductions in contractile proteins and/or water of G and P muscles by giving 4h WS during HU. The S muscle was the only one in 4HRWS group which showed reduction in weight as compared to CON. Our findings find support of D'Aunno et al who reported normal content of protein in soleus inspite of its weight being 84% of control when intermittent acceleration of 1.2 G for 15 min was given 4 times in a d during 7d of HU [17]. The mechanisms under acceleration stress and weightlessness are different and need to be discussed. Therefore, we can conclude that 4h WS during HU was sufficient in completely preventing the atrophic changes in muscles, which predominantly contains fast fibres (G and P) while it was not sufficient in complete prevention of the atrophic changes in muscle predominantly consisting of slow twitch fibres (S).

Conclusion

Simulated weightlessness by tail suspension in rats resulted in atrophy of all the antigravity muscles (G, P and S). Atrophy was seen in the areas of muscle consisting predominantly of slow fibre (S). 2h daily WS showed complete prevention of atrophic changes in P muscle and partial prevention of atrophic changes G and S muscles. 4h daily WS completely prevented atrophic changes in G and P muscles while prevention of atrophic changes in S muscle was no more seen as compared to 2h daily WS. 4h WS was found a better countermeasure as compared to 2h WS during simulated weightlessness. 4h WS during simulated weightlessness was sufficient in fully preventing atrophic changes in those antigravity muscles which consist predominantly of fast twitch fibres while it was found only partially successful in preventing the atrophic changes in muscle consisting predominantly of slow twitch fibres.

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