Original Article

Effects of 2 hour weight support during simulated weightlessness on contractile properties of hind limb muscles

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Anti orthostatic hypokinetic posture in rats by tail suspension simulates the deconditioning effects of weightlessness on the skeletomuscular system. It results in decrease in mass and contractile function in the hind limb muscles. The present study evaluates the effects of 2 hour (h) daily weight support (WS) during simulated weightlessness condition in reducing this atrophic response. Adult male albino rats were divided into three groups: (1) control (2) hind limb suspended for 15 days (HS) and (3) HS with daily 2 h WS (HS + WS). The HS group showed significant reductions in the weight of GPS muscle (gastrocnemius– plantaris–soleus) and its contractile properties, viz., peak isometric twitch tension and peak isometric tetanus strength. The HS + WS group showed completed reversal of values for both the contractile properties of GPS and significant improvement in GPS weight. These findings indicate that 2 h daily WS during simulated weightlessness results in amelioration of functional atrophy in GPS muscle.

Keywords: Twitch tension, tetanic tension, fibre type population, hind limb suspension, atrophy, fast and slow muscle.

Prolonged space mission and confinement in space cabin bring about functional changes in various systems of the human body including disturbances in the muscular system. Hind limb unweighing (HU) by tail [1] or harness suspension [2] has proven to be useful ground based animal model to simulate the effects of weightlessness on musculoskeletal system. Weightlessness, produced by space flight or HU, does not affect hindlimb muscles equally. Muscles, characterised by a tonic activation pattern and composed predominantly of slow oxidative fibres (type 1) are affected to the greatest degree [3]. Thus an order of responsiveness to decreased weightbearing is observed within the muscles of the rat hindlimb [3–6]. Muscles serving in an antigravity role as soleus (S), plantaris (P) and gastrocnemius (G) are affected to a greater extent than the muscles not serving an antigravity role as extensor digitorum longus (EDL) and tibialis anterior (TA). This differential effect also can be seen in fibres that stain light (slow twitch) vs those that strain dark (fast twitch) for myosin ATPase at an alkaline pH. For example, the cross sectional area of light, ATPase fibres in the S appear to be more sensitive than the dark ATPase fibre to both HU [7,8] and space flight [9]. This atrophy represents a serious health risk for long duration space missions and results in decrease in the amount of endurance capability [10]. It is feared that the effectiveness and safety of extra vehicular activities as well as the strength and ability to maintain and operate spacecraft may be compromised. The concern generated from these data, in part, have lead to the addition of a small animal centrifuge to the space station so that the effectiveness of countermeasures studies and other gravity related experiments can be determined [11]. Therefore, it is imperative to see the effectiveness of intermittent weight support (WS) as a countermeasure to simulated weightlessness on earth and also obtain sufficient level of data and expertise in preparation for the expensive and realistically infrequent space based animal centrifugation experiments. 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 a day (d), lasting 10 min each [8,12,13]. To apply it as a counter measure for muscular deconditioning in space, astronauts will be required to undergo gravity exposure for number of times in a day and thus spending much more time in preparing and coming out of centrifuge, out of a limited time available during actual space mission.

We hypothesised that full 24 h WS is not necessary in the maintenance of normal muscular functions of antigravity muscles and probably one time 1 gravity exposure by simple WS for few hours (h) per d in hind limb suspended (HS) rat model may be an effective countermeasure for muscular deconditioning in HU rats.

Material and methods

Adult male albino rats, aged 4 months to 8 months and weighing above 150 g were selected from the breeding cages of our own laboratory. Rats were assigned randomly to one of the following 3 groups; control (CON, n = 14), HS (n = 22) and HS with daily 2 h WS between 1000 h to 1200 h (HS + WS, n = 11). Rats from each group were housed individually in identical, 45 × 45 × 45 cm, suspension cages with food (pelletted, Gold Mohur feed) and water provided ad libitum and were allowed to acclimatise there for 7 d. After 7 d of acclimatisation CON rats were continued to be maintained in the cages for another period of 15 d without any further treatment while HS and HS + WS group rats were subjected to HU for 15 d by tail suspension method [1,14]. HS + WS rats were released from tail suspension and allowed to bear their weight for a period of 2 h daily during their HU. Rats were checked daily for signs of tail lesions or discoloration, unusual breathing patterns or undue discomfort. An animal exhibiting any of these symptoms was immediately removed from the study. Their daily feed intake and body weights (BW) were also recorded.

At the conclusion of 15 d, the rats were anesthetized with pentobarbital sodium (50 mg/Kg BW, ip) and GPS muscle with its sciatic nerve was exposed [4]. The GPS muscle was dissected free of surrounding tissues. The common tendon of the GPS muscle was removed from the calcaneus. Animal and its dissected limb was secured at knee and ankle to the dissection tray with the help of pins leaving behind GPS muscles. GPS tendon was attached to force transducer (Recorder and Medicare, Chandigarh) in a horizontal position with the help of noncompliant silk thread (4–0). A bipolar stimulating electrode was attached to the sciatic nerve. Kreb's solution, maintained at 37°C, was frequently poured on the exposed muscle nerve preparation during whole of the procedure. Peak isometric twitch (Pt) of GPS muscle at its optimal length was elicited by applying supramaximal 0.2 ms square wave pulse from an Electronic stimulator (Recorder and Medicare, Chandigarh) to sciatic nerve. Three such twitch contractions were recorded at 50 mm/sec moving paper on polygraph (Recorder and Medicare, Chandigarh). Then peak isometric tetanic contraction (Po) was elicited by 0.2 ms, supramaximal pulses at 250 Hz for a period of 2 sec [14,15]. Three such maximal tetanic contractions were recorded at 1 mm/sec moving paper. Best of the curves were selected for calculating Pt and Po. After completion of recording, GPS muscle was excised from limb, freed from all the tissues, dried by blotting paper and weighed. GPS weight was also expressed as g/100 g of BW. Student's unpaired t test was used to compare means of various parameters of HS and HS + WS group with CON. In all cases, the level of significance was set as $P < 0.01$.

Table 1. Effect of 2 hour weight support during simulated weightlessness on contractile properties of hind limb muscles

Characteristics	*CON (n = 14)*	*HS (n = 22)*	*Difference (CON-HS)*	*HS + WS (n = 11)*	*Difference (CON-HS + WS)*
Body wt (g)	197	172	–12.7%	189	–1.6%
GPS wt (mg)	1230 ± 185	850 ± 132	–30.9% ***	985 ± 144	–19.9% ***
GPS wt (mg/100 gBw)	622 ± 57	493 ± 56	20.8% ***	518 ± 41	–16.7% ***
Pt(g)	85 ± 10.2	56.5 ± 21.8	–33.5% ***	75.2 ± 16.4	–11.6% NS
Po(g)	126.4 ± 17.2	95.9 ± 20.0	–24.1% ***	125.6 ± 17.3	–0.6% NS

Values are mean ± SD; n = number of rats; CON = control; HS = 15 days hind limb suspended; HS + WS = HS plus 2 hour daily weight support; GPS = gastrocnemius-plantaris-soleus muscle; Pt = peak isometric twitch tension, Po = isometric tetanus strength; *** = significantly different from control by t test ($P < .01$); NS = Not significant; BW = body weight.

Results

The mean BW of CON group increased by 9.8% while HS and HS + WS group showed reduction in the BW by 10.3% and 6.7% respectively during 15 d study. Average food intake for CON group was 14.3 g/d during the study while HS and HS–WS group consumed 21% and 12% less than the Con group.

In agreement with previous reports [4,5,16–18] HS group showed atrophic changes in the postural antigravity GPS muscle (Table I). GPS weight when expressed per 100 g BW reduced by 20.8% in HS group as compared to CON. HS group also showed reductions in Pt and Po when compared with CON. HS + WS group although showed reduction in GPS weight by 16.7% as compared to CON but Pt and Po were not found significantly decreased from CON.

Discussion

Reduction in weight bearing activity results in considerable changes in normal muscle structure and function and severity of these effects is dependent, at least in part, on the normal function of muscle. Generally, muscles having strong antigravity function such as extensors (GPS) are affected to a greater degree than those muscles having less of an antigravity function, such as the flexors (EDL and TA). Further, within the cross section of a muscle, the effects of unloading appears to be related to both the region of the muscle and to the type of fibre, *i.e.*, fibres in the deep (close to the bone) areas are more affected than fibres in the superficial areas, and fibres that stain lightly for myosin ATPase, alkaline pre incubation (presumably slow fibres, Type 1) are more affected than fibres that strain darkly (presumably fast fibres, Type 2) [6,19].

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 [7,20], whereas G and P are composed of a mixture of fibre types and has predominantly fast twitch fibres [19]. The GPS, as a whole, is predominantly fast twitch fibres (66%) on a fibre mass basis [4] and because they have the greatest capability for isometric tension production, account for 80 to 85% of the total GPS tension development. 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 [21].

HS group showed decrease in GPS weight by 30.9% when compared with CON. When GPS weight was expressed per 100 g BW it was still found 20.8% less than CON. As GPS is primarily antigravity muscle, its weight loss on HS can be well explained by the mechanism of disuse atrophy, due to nonweight bearing by hind limbs during HU. HS group also showed reductions in Pt by 33.5% and Po by 24.1%. When reductions in Pt and Po was seen along with reduction in GPS weight, it was observed that contractile parameters decreased almost in the same proportion as reduction in weight of muscle. These results indicate equal structural and functional atrophy of antigravity muscles during simulated weightlessness, probably involving both fast and slow twitch fibres. This theory has the support in the findings of Bell Gordon and Thomas *et al* [22] who observed reductions in the no. of mitochondria in both slow and fast type of fibres during actual space flight.

HS + WS group also showed reduction in GPS weight when compared to CON but its magnitude was less than the HS group. When contractile parameters were compared among HS and HS + WS group, it was observed that although GPS weight was 15.9% more in HS + WS group, Pt and Po were found to be 33.1% and 30.9%, respectively, more in HS + WS group. When Pt and Po of HS + WS group were compared with CON, Pt was 11.5% less in HS + WS group, although it was not statistically significant. Po in CON and HS + WS was found to be same. These findings indicate full functional recovery in GPS muscle inspite of some decrease in its weight by giving daily 2 h WS during 15 d of HU in rats.

Whether this reduction in weight of GPS during HU is also contributed by reductions in connective tissue or fluid changes in the muscles, remains to be answered and if it is so then we can conclude that 2h WS was sufficient in preventing atrophic changes of both slow and fast twitch fibres in antigravity muscles but not enough for preventing connective tissue or fluid changes of muscle. Probably increased period of WS and/or some period of exercises may prove useful in preventing the muscle weight loss also.

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