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PHYSIOLOGICAL PROPERTIES OF RAT HIND LIMB MUSCLES AFTER 15 DAYS OF SIMULATED WEIGHTLESS ENVIRONMENT

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Abstract: Weightlessness during space mission results in atrophic changes in those muscles which have maximum weight bearing function and consist primarily of slow twitch fibres. In the present study an animal model was designed to evaluate the effects of 15 days of hindlimb unloading (HU) in rats by tail suspension on the (i) weight of gastrocnemius (G), plantaris (P), both predominantly having fast twitch fibres and soleus (S) muscle, predominantly having fast twitch fibres and (ii) contractile properties viz peak twitch contraction (Pt) and peak tetanic contraction (Po) of GPS muscle. HU rats showed significant weight reductions of G (–17.9%), P (–13.3%) and S (–41.2%) muscles. Pt and Po were also reduced in HU group but when these were expressed per gm of GPS muscle, no significant changes in Pt and Po were observed. These findings confirm that HU in rats result in maximum atrophic change in those muscles which have predominantly slow twitch fibres and reductions in contractile properties of muscles are in proportion to reduction in muscle weight. Also, HU by tail suspension provides a good ground based model for developing the deconditioning of muscles as applicable to weightlessness of space and offers a scope for the development of various countermeasures.

Key words : weightlessness, tail suspension models simulated, muscle atrophy, simulated microgravity weightlessness

INTRODUCTION

Prolonged space mission and confinement in space cabin bring about functional changes in various systems of the human body including disturbances in the skeletomuscular system (1). Hind limb Unweighing (HU) by suspension from the tail has proven to be a useful ground based animal model to simulate the effects of weightlessness on skeletomuscular system (2). Weightlessness does not affect hind limb muscles equally. Muscles serving in an antigravity role [soleus (S), plantaris (P) and gastrocnemius (G)] are affected to a greater extent that the muscles not serving an antigravity role viz. extensor digitorum longus (EDL) and tibialis anterior (TA) (3, 4). The relative infrequency and prohibitive expense of space experimentation emphasize the need to develop ground based models of weightlessness. Bed rest has been used to mimic space flight in humans. Although skeletomuscular deconditioning occurs in adults subjected to prolonged bed rest, restrictions on the use of invasive techniques (muscle biopsy etc.) in humans make it difficult to determine the pathogenesis of antigravity muscles' atrophy. For this reason, animal models to study the effects of weightlessness are of considerable interest. No earth bound experiments eliminate gravity, so true weightlessness on earth is not

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achieved. HU by tail suspension in rat results in cephalad fluid shift and decreased weight bearing of hind limbs, as it happens during actual space missions.

This study was undertaken to determine the effect of 15 days (d) of simulated weightlessness (S-W) using hind limb unweighing on physiological properties of antigravity muscles.

METHODS

Wistar strain of male albino rats, aged 90d-180d and having body weight (BW) 150-210 gm. were selected for the study. They were housed individually in identical, 18x18x18 inch Weighlessness Simulation Cages (WSC) with food (pelleted, Gold Mohur feed) and water provided *ad libitum*. They were allowed to adapt in WSC for 7d and observed daily for their feed intake and weight gain. Rats showing any unusual signs of adaptation to the cage (no food intake in the preceding 24 hours, BW <150 gm, loss of shining hair/skin, diminished activity at the time of daily cleanliness of the cage/change in feed and water) were discarded from the study. After 7d of adaptation to WSC and the feed they were divided in 2 groups. Group 1 (CON) rats were left in WSC for another 15d without any treatment. Group 2 (HU) rats were given S-W by tail suspension (Fig. 1) for 15d (5). Rats were checked daily for signs of tail lesions/ blackening of tail, unusual breathing patterns and undue stress (no food intake, market wt loss (BW < 150 gm), loss of shining hair on the skin, diminished activity at the time of daily cleanliness of the cage/change in feed and water and haemorrhagic spots at face and fore limbs). An animal exhibiting any of these signs was immediately removed from the study. Their feed intake and BW were also monitored daily.

After 15d of HU, the rats were anaesthetized with pentobarbital sodium (50 mg/kg BW, intra peritoneal) and Gastrocnemius Plantaris Soleus

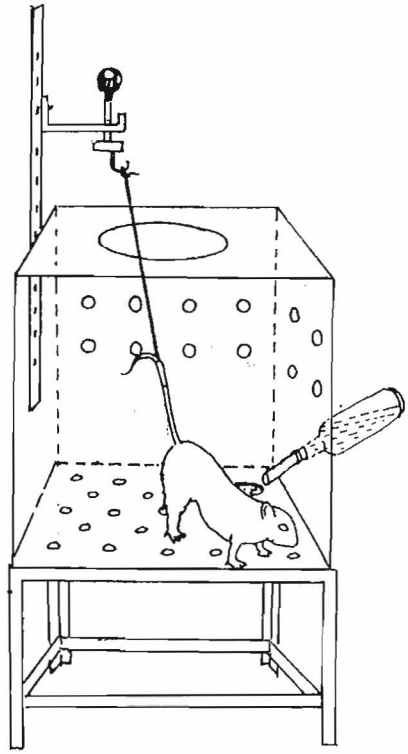

Fig. 1: Weightlessness simulation by Hind Limb unweighing in rat

(GPS) muscle with its sciatic nerve was exposed (3). The GPS muscle was dissected free of surrounding tissues. The common tendon of the GPS muscle was removed from the calcaneus. The animal and its dissected limb were secured at the knee and ankle to the dissection tray with the help of pins leaving behind GPS muscle. Before the start of every dissection, a force transducer (Recorder and Medicare, Chandigarh) was fixed on a micrometer stand (improvised from the microscopic stand) and calibrated for various wts for reading the isometric contraction of GPS muscle subsequently. GPS tendon was attached to this precalibrated force transducer in a horizontal position with the help of noncompliant 4-0 silk thread passing through a pulley. A bipolar stimulating silver electrode was attached to the sciatic nerve. Kreb's solution, maintained at 37°C, was frequently poured on

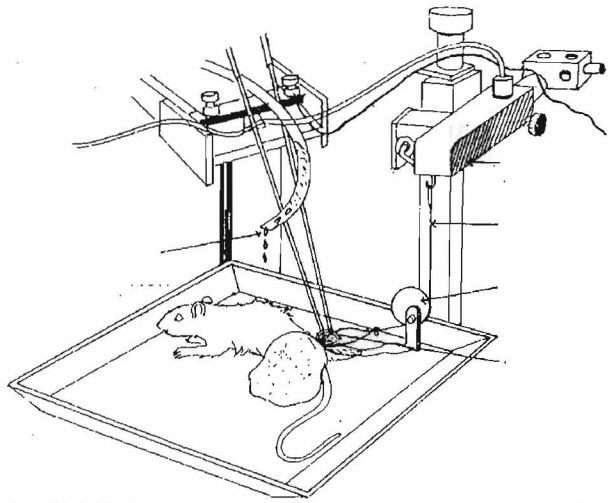

Fig. 2: Recording of contractile properties of gastrocnemius plantaris soleus muscle.

the exposed muscle nerve preparation during whole of the procedure (Fig. 2). An isometric muscle twitch contraction was recorded by applying a single 0.2 ms square wave pulse from an Electronic stimulator (Recorder and Medicare, Chandigarh) to the sciatic nerve. A maximum stimulus was determined for each muscles by gradually increasing the voltage of the stimulus till a maximum height of twitch contraction was achieved. A voltage of 5 times of it was used as supramaximal stimulus. Then optimal length of the muscle was found out by adjusting the resting tension of the muscle by microadjustment knob of the stand and applying the supramaximal stimulus every time. Peak isometric twitch contraction (Pt) of GPS muscle at its optimal length was elicited by applying supramaximal 0.2 ms square wave pulse. Three such twitch contractions were recorded on a polygraph (Recorder and Medicare, Chandigarh) at a paper speed of 50 mm/sec. Then peak isometric tetanic contraction (Po) was elicited by 0.2 ms, supramaximal pulses at 150 Hz for a period of 2 sec (5,6). Three such maximal tetanic contractions were recorded at a paper speed of

TABLE I: Effect of 15 days of Simulated Weightlessness by Hindlimb unweighing (HU) in rats on the weight (Mean ± SD) of Antigravity muscles (mg/100 gm body weight)

Muscle	*Control (n = 22)*	*HU (n = 22)*	*Difference (HU - CON)*
GPS	607.2 ± 57.8	492.8 ± 56.5	–18.9%***
Gastrocnemius	482.4 ± 49.6	396.3 ± 48.3	–17.9%***
Plantaris	83.8 ± 8.3	72.6 ± 8.5	–13.3%**
Soleus	41.0 ± 4.2	23.8 ± 4.2	–41.9%***

GPS = Gastrocnemius Plantaris Soleus muscle;
***P < 0.001; **P<0.01

TABLE II : Effect of 15 days of Simulated Weightlessness by Hindlimb unweighing (HU) in rats on the peak twitch (Pt) and peak tetanus (Po) contractions (Mean ± SD) of GPS muscle.

Parameters	Control (n=22)	HU (n=22)	Difference (HU-CON)
Pt (gm/100 gm BW)	42.9 ± 7.2	31.4 ± 10.8	–26.8%***
Po (gm/100 gm BW)	62.1 ± 10.0	54.1 ± 12.5	–12.9%*
Pt (gm/gm GPS)	70.9 ± 11.6	64.3 ± 23.4	–9.4% (NS)
Po (gm/gm GPS)	102.7 ± 16.5	110.9 ± 28.4	+7.9% (NS)

GPS = Gastrocnemius Plantaris Soleus muscle ; BW = body weight ;
***P < 0.001; *P < 0.05; NS = Not significant

1 mm/sec. Best of these curves were selected for calculating Pt and Po respectively. After completion of recording, GPS muscle was excised from limb, freed from all the tissues, dried by blotting paper and weighed to the nearest 0.1 mg on Mettler balance, for G, P and S muscle wet weights (wts) separately. GPS wet wt was derived by adding the wts of G, P and S muscles. All the wet wts of muscles, Pt and Po were expressed as gm/100 gm of BW (2). Pt and Po were also expressed as gm/gm of GPS wt (5).

Student's unpaired 't' test was used to compare means of various muscle parameters of HU group with CON group. The level of significance was set as $P < 0.05$.

RESULTS

HU group showed reductions in the wt of all the postural antigravity G (–17.9%), p (–13.3%) and S (–41.9%) muscles. Meanwhile, GPS muscles as a whole was reduced by 18.9% (Table I). HU group also showed reductions in Pt (–26.8%) and Po (–12.9%) when expressed per 100 gm BW. However, when Pt and Po were expressed per gm of GPS muscle, no significant reductions were observed (Table II).

DISCUSSION

All the muscles of the body are continually being remodelled to match the functions that are required of them. Their diameters, vascularity and even the types of muscle fibers are altered. This remodelling process is often quite rapid and can be completed in as little as 2 weeks (1). Reduction in wt bearing activity by HU in rats 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 degrees that those having less of an antigravity function, such as the flexors (EDL and TA). Further, in a muscle, the effects of unloading appears to be related to both the region of the muscle and to the type of fiber. Fibers in the deep (close to the bone) areas are more affected than fibers in the superficial areas, and fibers that stain lightly for myosin ATPase, alkaline pre incubation (presumably slow fibers, Type I) are most affected than fibers that stain darkly (presumably fast fibers, Type 2) (7, 8).

The S, P, and G muscles in rats are synergistics. They are primary ankle extensors, but they have very different type of fiber composition and recruitment patterns. The S is composed primarily of slow twitch fibers (9,10), where as G and P are composed of a mixture of fiber types and has predominantly fast twitch fibers (8). The GPS, as a whole, is predominantly fast twitch fibers (66%) on a fiber mass basis (3) and because they have the greater 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 (11).

HU group showed decrease in GPS wt by 18.9% when compared with CON. However, when G,P and S muscles of HU group were compared with CON group, maximum reductions were found in S muscle wt (–41.9%) followed by the decrease in wt of G (–17.9%) and P (–13.3%) muscles. Our finding are in agreement with the findings of other workers in the field (2, 3, 12). The reduction in the wet wts of these muscles may be due to reduction in the water content and/or protein content (contractile filaments viz actin and myosin filaments) of these muscles. HU in rats would result in cephalad shift of fluid leading to reflex reduction in blood volume and body water content (1). This is likely to

result in reduction in the water content of muscles. As G,P and S muscles are primarily antigravity muscles, their wt loss can also be well explained by the mechanism of disuse atropy due to nonweight bearing during HU, resulting in decreased number of contractile protein filaments. Thomason et al reported 40% loss of protein in S and 14% loss of protein in G after 14d of HU (13). Reduction in the number of subsarcolemmal mitochondria, responsible for protein synthesis in muscle, was seen in the S muscle of rats after 12.5d of actual spaceflight in COSMOS-1887 mission (14). Reduction in the contractile protein content of these muscles is indirectly confirmed in our study by finding the reductions in Pt (-26.8%) and Po (-12.9%) of GPS muscle in HU group, as strength of contraction is directly proportional to contractile protein content of muscle fiber (1). Reduction in Po of the soleus to 79% of control was also seen by Thomason et al (13). McDonald et al observed that 15d of HU did not affect blood flow to soleus muscle while blood flow to the muscles consisting of predominantly fast twitch glycolytic fibers was found greatly increased by HU (6). Therefore, decrease in the wet wt of GPS and its contractile parameters can not be attributed to diminished blood flow and nutrient supply to it. Atrophic changes of GPS also do not appear to be due to stress response of the animal to HU as Thomason et al reported only minimal and transient stress during 1st week of HU (13). Reduction in the contractile protein content of the GPS muscle appear to be in proportion to the reduction in GPS wt as Pt and Po, when expressed as gm/gm GPS, were not found significantly different in HU and CON groups. Fell et al also did not find reduction in Pt and Po of soleus and gastrocnemius muscle, when expressed as per gm of muscle (15). Our finding of same Pt and Po per gm of GPS muscle does not agree with observations of some workers in the field who observed reduction in these parameters after HU (5).

As contractile parameters viz Pt and Po of GPS muscle in HU group were found reduced as compared to CON group, there is also possibility of reduction in the amount of calcium ion released with each stimulus from sarcoplasmic reticulum (SR) of GPS muscles. In SR of atrophied soleus muscle of HU rats, the amount of calcium uptake and calcium release had been found higher than the control muscles by Stevens et al, thereby suggesting that after HU soleus muscle acquired SR properties of a faster muscle (16). Therefore reduction in the release of calcium ion from SR does not appear to be a valid explanation for the reduction of contractile parameters of GPS muscle in HU group. Reduction in the contractile parameters in HU group also does not appear to be due to change in the calcium affinity of the contractile proteins of GPS muscle as Thomason et al reported no change in the calcium affinity of the contractile proteins in atrophied soleus muscle after 15d of HU (13). Reductions in Pt and Po in HU group also do not appear to be due to decrease in ATP and glycogen content of GPS muscle as Thomason DB et al reported 38% increase in ATP and 69% increase in glycogen concentration in soleus muscle after 7d of HU (13). However, Marsh et al observed that total ATP production, glycogenolysis and glycolysis are unaffected by HU in soleus, plantaris and white gastrocnemius muscles (3).

These findings indicate that S-W by HU in rats results in atrophy of all the antigravity (G,P and S) muscles with maximal atrophy in muscles having predominantly slow twitch fibers (S muscle). Muscle atrophy during HU appears to be mainly due to reduction in the contractile protein content of GPS muscles. HU by tail suspension offers a good ground based model for developing the deconditioning of muscles as applicable to weightlessness of space and offers a scope for the development of various countermeasures.

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