Original Article

Atrophy of Hind Limb Muscles in Tail Suspended Rats

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The tail suspended Anti-Orthostatic Hypokinetic (AOH) rat model was established and evaluated for its efficacy in simulating skeletomuscular deconditioning as seen in rats after exposure to actual microgravity of space flight. Ten Wistar rats of more than 120 days of age and 200 g of weight were subjected to tail supension for 15 days with weight matched litter mates acting as a pair-fed control. Both AOH and control rats incurred significant body weight loss during the experiment. AOH rats lost more body weight compared to controls and the difference between the two groups became significant since 6th day of suspension. Atrophy of hind limb muscles in AOH rats was measured from the differences in muscle weight per 100 g of body weight weight in comparison with the control. Qualitatively, the muscle atrophy was similar to that reported in previous studies: soleus with predominant antigravity action showed the largest changes (-23.5%, $p<0.001$), followed by gastrocnemius (-13.3%, $p<0.005$) while EDL, predominantly composed of type II fast fibres, showed minimum changes (-5%, $p>0.05$).

Key Words : Anti-orthostatic hypokinesia, disuse atrophy, simulated weightlessness

Atrophy of anti-gravity muscles is a well known consequence of long term space flight[1]. Suspension hypokinesia of rats results in muscle atrophic changes comparable to those seen in the weightlessness of space, as this model contains the same critical components as encountered during space flight, ie unloading of hind limbs and a cephalad fluid shift[2]. Based on this principle, a model of producing hindlimb unweighting was developed by Morey-Holton[2] using tail suspension of rats. This model has popularly been called the Anti-Orthostatic Hypokinetic (AOH) model.

Most of the studies with the AOH model have reported either a normal rate of weight gain or a reduction in the rate of weight gain as a result of suspension[2,3]. Booth[4] put forth the argument that if growing rats are used for such studies, the difference between actual atrophy and a mere arrested growth may not become apparent and has advocated the use of non growing rats for the studies on muscle atrophy.

The present investigation was undertaken with the object of establishing the tail suspended AOH model in our laboratory using "non growing" rats of age above 120 days and body weight more than 200 g and observing the changes in the weights of Gastrocnemius(GN), Soleus(SOL), and External Digitorum Longus(EDL) muscles in suspended rats vis a vis pair-fed controls.

Material and Methods

Ten pairs of adult rats (Wistar) of more than 120 d of age and weighing more than 200 gm were used for this study. Litter mates weighing within 5 gm of each other were selected as experimental and control pairs.

The experimental rats were subjected to hind limb unloading by method described by Morey-Holton[2]. Both AOH and control rats were housed in Plexiglas cages of similar dimensions (400 X 200 X 200 mm). In AOH rats a nylon thread was intertwined spirally over the tail and fixed with adhesive tape. This nylon cord was then tied to the suspension bar ensuring a head down tilt of approximately 30°. A complete mechanical unloading of the hind limbs was achieved with the forelimbs remaining in contact with the floor to allow mobility in 360° and free access to food, provided ad lib, as preformed pellets(Goldmohur, Lipton India) in a shallow dish. The control rats were pair-fed with the same amount of food as consumed by the corresponding AOH rat on the previous day. Water was provided ad lib to both AOH and control rats. A daily record of food consumption and an alternate day record of body weight of both the control and AOH rats was maintained.

On 16th day, final measurement of body weight was taken following which the rats were

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sacrificed under Urethane anaesthesia (USP, 1g/Kg i.p.). GN, SOL and EDL muscles were carefully dissected out and cut at the tendinous portions gaining prominence. The muscles were blotted dry on a filter paper and weighed in a physical balance to the nearest mg. Students 't' test was used for comparison of various parameters between AOH and control group of rats.

Results

Body Weight Changes and Feeding Habits : Initial food intake in the AOH rats was 7.5 g per day for the first two days. It fell to 5.2 g on the third day . There was a gradual recovery to 8.5 g on the 7th day and thereafter fluctuated between 7.5 and 9 g per day for the rest of suspension period (Fig 1). However, the average daily food intake during the suspension period showed a lower value as compared to the average reported value of normal intake of 10 to 12 g per day for the adult Wistar rat[5].

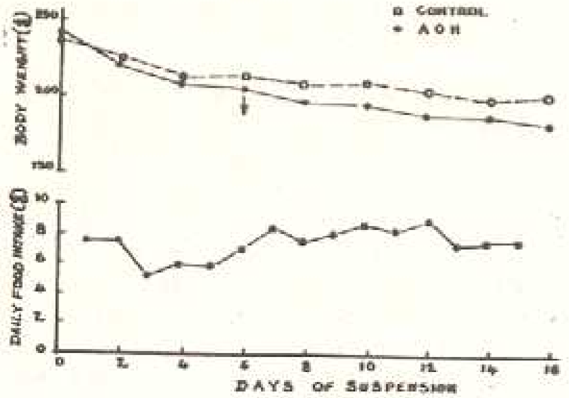

Fig-1. Mean values of daily food intake and body weight in AOH and pair fed control rats.

The rats showed a significant decrease in body weight over the 15 days of AOH suspension (Fig 1). The mean body weight of tail suspended rats decreased from 242.5 ± 29.4 g to 181.2 ± 10.4 g as compared to 239.6 ± 28.7 g and 198.8 ± 17.2 g in control rats. The loss in body weight was more rapid in AOH rats as compared to the control group and this difference between the body weights of the two groups became significant from day 6 onwards($p < 0.05$).

Muscle Weight Changes: Table I shows the weights of GN, SOL, and EDL muscles in AOH and control rats after 15 days of suspension. The weights are expressed as absolute values as well as per 100 g of body weight on the day of sacrificing.

Table I Hind limb muscle weight changes in control and AOH (m ± sd, n=10)

Parameter	Groups	Hind Limb Muscles SOL	GN	EDL
Muscle Wt (mg)	Control	111± 17	1523 ±124	130 ±14
	AOH	76 ± 10	1194 ±128	110 ±15
	p	<0.001	<0.001	<0.05
	% change	-32.1%	-21.6%	-13.1%
Muscle wt (mg/100 g body wt)	Control	51 ±6	701 ±69	60 ±4
	AOH	39 ±4	607 ±76	57 ±5
	p	<0.001	< 0.05	NS
	% change	-23.5%	-13.3%	5%

The GN showed a mean weight of 1523 ± 124 mg in control rats as against 1194 ± 128 mg in AOH rats, the differences being highly significant ($p<0.001$). The corresponding values for SOL were 111±17 mg and 76±10 mg ($p<0.001$) and for EDL 130±12 and 112 ±11 mg ($p<0.05$) respectively. Muscle weights expressed in mg/100 g body weight were 701±69 in control and 607±76 in AOH rats for GN ($p<0.05$), 51±6 (control) and 39 ±4 (AOH) for SOL($p<0.001$) and 60±4 (control) and 57±5 (AOH) for EDL ($p>0.05$).

Discussion

The AOH rat model has been used to mimic changes in skeletomuscular system as produced by space flights with increasing frequency. Most authorities agree that the changes in AOH rat in physiological, morphological and biochemical characteristics of muscles credited with weight-bearing function are similar to those seen in rats flown aboard biosatellites[2,6].

The muscle atrophy seen in the AOH model could be due to either disuse brought about by hind limb unloading, or may be the result of the stress of suspension procedure itself[3,6]. An absence of change in food habits and maintenance of body weight or rate of gain of body weight have generally been accepted as indicators of animal's tolerance to the experimental stress[3]. Wronski and Morey-Holton[3] observed that 42 day old rats of average weight of 130 g subjected to tail suspension showed a rate of weight gain comparable to that of control animals.

In the present study, the observed rapid loss in body weight can be attributed to a reduced food intake in the initial 3-5 days (Fig 1), since similar changes were noticed in pair fed controls. Subsequent increase in food intake was associated with a relatively lower rate of reduction in body weight in both the AOH and pair fed control rats. However, from the 6th day onwards, the AOH rats showed significantly lower body weights as compared to the control rats for the rest of the suspension period. In some of the studies on growing rats, a reduction in the rate of weight gain in AOH rats as compared to control rats has been reported[2,7]. However, absolute loss of weight was not reported in these studies except in the initial few days of acclimation to suspension, where it was attributed to low food intake and dehydration[7]. Flynn and Max[8] observed only a marginal reduction in weight in AOH rats after two weeks of suspension. Jaspers and Tischler[9] have reported a significant difference in the weights of suspended and control animals by 7th day of suspension, which is in good agreement with the present study. They observed a normal growth of suspended animals only in those with initial body weight below 200 g. With larger animals they observed an actual loss of body weight by 10-20 g in the first 6 days. The absolute body weight loss as seen in the present study may be due to the use of 'non growing' rats above 200 g of weight[9].

In humans subjected to simulated weightlessness by 30 day bed rest, Convertino et al have reported a continuous significant loss of body weight with a large reduction seen in the first four days and a smaller and more gradual reduction throughout the remainder of the bed rest period[10]. Though the magnitude of body weight loss observed in the present animal model was much higher (25.3%) as compared to the values reported by Convertino et al for human subjects (3.2%), the temporal pattern of weight loss had striking similarities in the two studies. Also, 3.8% loss in absolute body weights were reported in Skylab 2 astronauts in 28 d mission[1].

In the present model, all the hind limb muscles studied showed significant atrophy following 15 day AOH suspension when expressed in terms of absolute muscle weights. The greatest difference from control rats in absolute weight was seen in SOL (-32.1%, $p<0.001$) followed by GN (-21.6%, $p<0.001$) and EDL (-15.1%, $p<0.05$). However, when the muscle weights were expressed in mg/100 g final body weight, which is a more appropriate expression of muscle weight changes in circumstances where whole body weight may change[8], AOH rats showed a highly significant difference compared to controls for SOL (-23.5%, $p<0.001$), also a significantly lower value for GN (-13.3%, $p<0.05$) with EDL showing no significant difference (-5%, $p>0.05$). These findings are in general agreement with values reported earlier on tail suspended AOH rats. Morey-Holton and Wronski[2] have reported a 40%, 20% and 5% lower values for weights of SOL, GN and EDL respectively per 100 g body weight in AOH rats as compared to controls. Mussachia et al[11] reported a significantly lower value for the weight of soleus(42% less than control), as against 10.5% lower value compared to control for GN. While both these differences were significant, EDL did not show a significant difference from control. Jaspers and Tischler also reported similar extent of changes in the above muscles[9]. Rats flown aboard Cosmos-936 showed -35%, -20% and -15% change from control for SOL, GN, and EDL respectively per 100 g of body weight[2]. Ilyna-Kakueva reported an average post flight loss of weight in SOL by 32% and EDL by 12% as compared to controls[12].

From the foregoing discussion, it is apparent that soleus, an antigravity muscle with predominance of slow twitch fibres, is most seriously affected in all the experimental conditions of hypokinesia/hypodynamia cited above. On the other hand, EDL, a predominantly fast twitch muscle, shows the minimum atrophic changes, while gastrocnemius, a mixed fibre muscle, shows an intermediate degree of tissue loss.

It seems that the use of "non growing" rats above 120 day of age and more than 200 g of weight in the present study was responsible for a different whole body weight loss pattern seen on AOH suspension, as compared to the studies with younger rats of lower body weights used. Nevertheless, the muscle atrophy seen in our model is essentially comparable with those seen in AOH models and space-flight experiments using younger rats of lower body weight. Also, the temporal feature of body weight loss seen in this study was more like the pattern reported for human subjects upon weightlessness simulation by bed rest.

It is concluded that AOH model as followed in this study is a valid simulation analogue of skeleto-muscular deconditioning of hypogravity. Still, in view of a greater magnitude of whole body weight loss observed in the present set up, there is a scope of further evaluating the experimental condition towards reducing the overall stress/trauma of the experimental procedure.

References

1. Nicogossian AE : Space Physiology and Medicine, 2nd edition, Washington DC, Lea and Febiger, 1988.

2. Morey Holton E, Wronski TJ : Animal models for simulating weightlessness. The Physiologist, 1981; 24 : S45-S48.

3. Wronski TJ, Morey Holton ER: Skeletal responses to simulated weightlessness - A comparison of suspension techniques. Aviat Space Environ Med 1987; 58 : 63-68.

4. Booth FW : Effect of limb immobilization on skeletal muscle. J Appl Physiol 1982 ; 52 : 1113-1118.

5. Farris EJ : The Care and Breeding of Laboratory Animals. 3rd edition, New York, John Wiley and Sons, 1957;p 43.

6. Mussachia XJ : The use of suspension models & comparison with true weightlessness : A resume. The Physiologist 1985; 28 : S 237-240.

7. Mussachia XJ, Deavers DR, Meininger GA et al: A model for hypokinesia : effects on muscle atrophy in the rat. J Appl Physiol 1980; 48 : 479-486.

8. Flynn DE, Max SR : Effects of suspension hypokinesia/hypodynamia on rat skeletal muscle. Aviat Space Environ Med 1985; 56 : 1065-1069.

9. Jaspers SR, Tischler ME : Atrophy and growth failure of rat hind limb muscles in tail cast suspension. J Appl Physiol 1984; 57 : 1472-1479.

10. Convertino VA, Doerr BF, Mathes KL et al: Changes in volume, muscle compartment and compliance of the lower extremities in man following 30 days of exposure to simulated microgravity. Aviat Space Environ Med 1989; 60 : 653-658.

11. Mussachia XJ, Steffen JM, Deavers DR : Rat hind limb muscle responses to suspension hypokinesia/hypodynamia. Aviat Space Environ Med 1983; 54 : 1015-1020.

12. Ilyina - Kakueva EI, Portugalov VV, Krivenkova NP: Space flight effects on skeletal muscle of rats. Aviat Space Environ Med 1976; 47 : 700-703.