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Application of Air Suction System to Mouse Soleus Muscle During Weightlessness

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ABSTRACT

The purpose of this study was to investigate the possibility of preventing muscle atrophy due to weightlessness by means of an air suction system. The soleus muscle of the mice were examined following a period of two weeks hindlimb suspension with or without a 20-minute-a-day relief period of standing on their hindlimbs in the air suction cage connected to a vacuum cleaner which removed the effects of gravity. The results showed that the body weight and the cross-sectional area of the muscle fibers decreased in all the mice. However the change in the proportion of histochemical muscle fiber types due to hindlimb suspension was prevented by the air suction system. These findings indicate that a chronic exposure of air suction during weightlessness can be provided for the next step.

KEY WORDS

Muscle atrophy, Hindlimb suspension, Air suction treatment, Muscle fiber types

INTRODUCTION

The atrophy of skeletal muscle that occurs during flights in space is one of the major problems of prolonged weightlessness, and it is well known that a rat after the flight shows a greater decrease of muscle mass in the slow muscles such as in the soleus muscle than in the fast muscles[1-4]. Moreover, the percentage of type I fibers of the rats soleus muscle decreases and this is counterbalanced by a simultaneous increase in type II fivers[5,6]. The animal and human studies have contributed the development of electrical stimulation to the skeletal muscles or physical exercise program in order to prevent disuse muscle atrophy due to weightlessness. However, with regard to applying them in space, an electrical stimulation producing a maximum contraction is intolerable to most subjects, and the electrodes applied to the skin would require an innumerable number to the muscles required for standing[7,8]. Vigorous physical exercise during short-term weightlessness, ie. up to two weeks, retards the rate and degree of muscle atrophy[9,10]. However, the best solution to the problem of prolonged space flights over three weeks may be afforded by artificial gravity generated by spacecraft rotation. The effects of chronic artificial gravity during a space flight which was generated by a centrifugal force resulted in moderate atrophic change of the rats muscle and strength of the soleus muscle was not altered[11,12]. A rotating space station that produces artificial gravity will probably be developed in the future. So an idea of a loading force on the muscles was suggested in this study ; that is continuous air suction applied to the cage in which a floating animal is fixated on the mesh floor. The best animal model for stimulating the hypokinesia and hypodynamia of weightlessness appears to be one in an head-down tilted body suspension that unloads the hindlimbs[9,13,14]. This study was undertaken to determine whether a hypo-

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kinetic mouse model would provide some information on the atrophy of skeletal muscle under conditions of an air suction environment.

MATERIALS AND METHODS

Eight-week-old male Slc : ICR mice, body weight 34.0~38.4g, were divided into three groups ; the suspension group, the air suction group and the control group. In the suspension group, the gravitational force of the body weight was removed from the hindlimb for 14 days, using the whole body suspension model modified by the method of Morey et al.[15]. The mouse was put in a leather jacket fastened with a Velcro tape during suspension and permitted to use its forelimbs to obtain food and water. In the air suction group, the whole body was suspended in the same way as the suspension group except for 20 minutes a day, five days a week, it was relieved from the jacket to be placed in an air suction cage. The cage was a pipe of 6cm in diameter and 17cm in length with an open end, and the inner surface coated with plastic (Fig. 1). The other end was sealed by wire mesh at the side. It was connected to a vacuum cleaner (VC-1050P, Toshiba, Japan). The suction power of the vacuum was made variable from 600g to 1,100g. The mesh end of the cage was positioned at an angle of 45° to 90° to the horizontal plane, and the experimental mouse was placed so as to be held by suction with its hindlimbs on the wire mesh. The mouse could move with its forelimbs within the cage, and the suction power was generated sufficiently for the mouse not to drop from the cage on the slippery inner surface. The mice in the control group received no treatment.

Following 14 days of experiment, all mice were sacrificed by an overdose of anesthesia using ether, and the soleus muscles were removed bilaterally and frozen in liquid nitrogen. Serial cross-sections, 10μm thick, were cut in a cryostat at -20°C and stained for myofibrillar adenosine triphosphatase activity using Round et al.[16] method. Muscle fibers were classified into type I, IIa and intermediated stained II (int II) fibers. Cross-sectional areas of each fiber type were calculated by the digitizer on stained sections with aid of a computer (PC-9801, NEC, Japan).

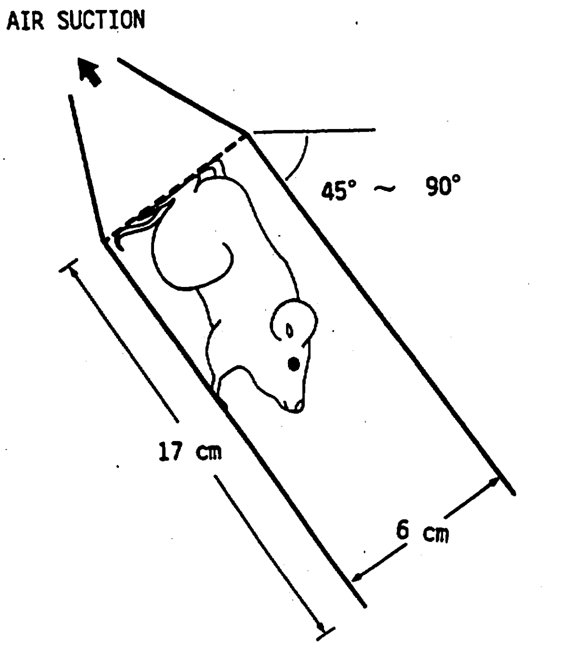

Fig. 1. Mouse in cage with air suction. Animal is able to move on the wire mesh but not to drop from the cage by regulating the strength of the suction power.

RESULTS

The body weight of the suspended mice decreased, and the decrease was not altered by the air suction treatment, whereas mice in the control group increased their body weight (Table 1).

Table 1. Change of body weights

Group		Body Weight, g	
		Before	After
Control	(n=3)	35.8±0.7	42.2±0.6*
Suspension	(n=4)	36.2±1.3	32.1±1.4*
Air Suction	(n=5)	35.7±0.9	31.9±1.2*

Values are means±SD. n, number of mice.

* Significantly different between before and after experimental data, P<0.01.

Table 2. Cross-sectional fiber areas in soleus

Group	Fiber Areas, μm^2		
	I	IIa	int II
Control	2,395±665	2,116±551	1,617±320
Suspension	1,389±498*	993±380*	838±380*
Air Suction	915±752*	1,009±378*	914±272*

Values are means±SD of 100 fibers / muscle.

* Significantly different between contol and experimental groups, P<0.01. No significant difference was found between suspension and air suction groups.

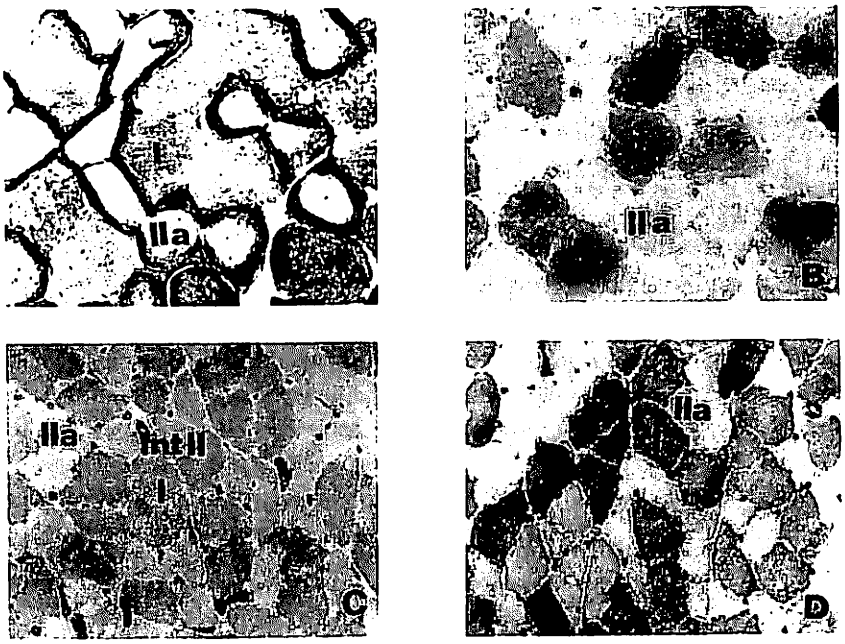

Fig. 2. Cross sections of mouse soleus muscle stained for myofibrillar adenosine triphosphatase activity.

×186. A : control group at pH 9.4. B : control group at pH 4.6. C : suspension group at pH 4.6. D : air suction group at pH 4.6. I, type I muscle fiber ; IIa, type IIa musle fiber ; int II, intermediate type II muscle fiber. The size of muscle fibers was reduced due to suspension with or without air suction treatment.

Table 3. Proportion of fiber types in soleus

Group	% Distribution		
	I	IIa	int II
Control (n=6)	46.0± 4.2	51.5±4.0	2.7±1.5
Suspension (n=6)	59.8±10.5*	38.4±9.0*	1.9±1.7
Air Suction (n=7)	45.9± 7.0	51.9±6.6	2.3±1.7

Values are means±SD. n, number of muscles.

* Significantly different between contol and experimental groups, $P<0.05$.

As shown in Table 2 and Fig. 2, suspension resulted in a significant reduction in the size of type I, II and int II fibers of the soleus muscle, and this atrophy was not prevented by the 20-minute air suction. The proportion of fiber types of the mice soleus muscle has been reported as a mixture of both type I and II fibers by previous investigators[17,18] compared with the rats soleus muscle in which type I is predominant. In this study the percentage of type I fibers in the control soleus muscle was as low as 46% (Table 3). A significant increase in the percentage of type I fibers and decrease in the percentage of type IIa fibers were found after suspension without air suction. However, there were no significant changes in proportion of fiber types in the soleus muscle of the air suction group compared with the control group.

DISCUSSION

The air suction system requires a large flow power to fixate the mouse hindlimbs on the wire mesh in opposition to gravity. But when the power of the vacuum was set extremely strong, the mouse could not stand on its hindlimbs in the cage and lay down on the wire mesh. In this study the power of the vacuum cleaner was ajusted to maintain the mouse on the mesh, but not disturb its breathing and movement inside the cage. The vacuum power was regulated by varying the size of the air hole on the vacuum hose during the experiment, so as the mouse remained in the cage even if it moved. Oganov et al.[12] carried out to expose the rat to artificial gravity with the aid of continuous centrifuge with a 20-minute stop once a day, and this chronic exposure did not alter the strength of the soleus muscle. The loss of body weight and the muscle atrophy of the suspended animal have been observed[17,19-27], and 20 minutes relief from suspension did not have any effect to prevent these changes. D'Aunno et al.[28] reported that the one-hour centrifugation showed no lessening of atrophy in the non weight bearing soleus muscle of the rat, however, the two-hour daily treatment exhibited a reduction in the muscle atrophy. The stimulation time of the air suction in this study was seemed too short to prevent the atrophy, and a chronic application program would be necessary to find the effect of the air suction system on the muscle atrophy.

The proportion of muscle fiber types in mice is different from that of rats due to their species difference. The investigations using rats showed a predominance of type I fibers in the soleus muscle and a decrease in the number of the type I fibers by hindlimb suspension[29]. Whereas type II fibers predominate in mice, it has been reported that this predominance was lost with suspension[17]. The control soleus muscle in this study also showed a mixture of both type I fibers and II fibers with the latter predominating, and the predominance of fiber types was reversed after two weeks suspension. As this histochemical change resulting from the loss of weight bearing was thought to affect the contractile characteristics of skeletal muscles[17,30], the fact that the air suction treatment prevented the conversion of muscle fiber types suggested validity of this new idea. As the mice in the cage with the air flow did not seem to need extra effort to stay for 20 minutes, when the problems of excretion, cleaning and food and water supply have been solved without disturbing the air flow together with an adequate power vacuum pump, chronic application of the air suction system can be done on the animals without enforcing a high load exercise.

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廃用性マウスヒラメ筋に対する空気吸引装置使用の試み

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要　旨

マウスの後肢を２週間懸垂免荷するとヒラメ筋は廃用性変化を呈するが，その間１日20分間空気吸引装置にてマウスの後肢を重力に逆らって吸引し，後肢が吸着面に押しつけられることにより筋の変化が抑制できるかどうか試みた。その結果，マウスの体重とヒラメ筋の筋線維横断面積の減少は吸引装置の使用の有無にかかわらず後肢懸垂すると同程度に認められ，その変化を防止することはできなかった。しかし，筋線維タイプの構成比率については，吸引装置を使用すると正常筋と同じ比率に保たれ，廃用性変化の１つとして知られる，筋線維タイプがタイプⅡからタイプⅠに変化する現象は認められなかった。以上のことから空気吸引装置の使用は免荷の条件下でヒラメ筋に部分的に有効に作用することがわかった。今後の課題として，装着使用時間の延長をはかりその効果について検討したい。