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EEEFECTS OF TOWER CLIMBING EXERCISE ON MUSCLE MASS AND HEMATOLOGICAL STATUS IN GLUCOCORTICOID-INJECTED RATS

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Abstract

To determine the effects of tower climbing exercise on loss of muscle mass and hematological status in glucocorticoid-injected rats, 29 male Sprague-Dawley rats, 10 weeks of age, were assigned to three groups : a saline control (C, n=9), a glucocorticoid-sedentary (GS, n=10) group and a glucocorticoid-exercise (GE, n=10) group. The GS and GE groups were given 2 mg/kg/day of prednisolone, and the C group was given 2 ml/day of saline injected daily subcutaneously. Each group was meal-fed commercial rat chow in equal quantity at 7 : 30 am to 8 : 30 am and 7 : 30 pm to 8 : 30 pm and given free access to water for 8 weeks. The GE group voluntarily climbed the 200-cm tower to drink water from the bottle set at the top of it. Weight gain duri ng the 8-week experimental period was greater in the C group than in the GS and GE groups. Liver, heart, kidney, spleen and abdominal adipose tissue weights were not different among the three groups. The hindlimb muscles and total muscle mass were heavier in the C group than in other groups. The weight of each muscle relative to body weight did not differ among the three groups, except quadriceps and forearm muscles. Hematological status was not influenced by glucocorticoid administration with or without climbing exercise. These results suggest that 8 weeks of climbing exercise did not attenuate glucocorticoid-induced muscle atrophy. It is suggested that this exercise may be too light to prevent loss of muscle.

Introduction

Glucocorticoids are commonly used therapeutically in patients with cardiac and pulmonary pathologic conditions for their potent antiinflammatory or immunosuppressive effects.[1] Unfortunately, long-term glucocorticoid administration has many side effects.[2,3] One such side effect of glucocorticoid treatment is skeletal muscle atrophy. This has led the medical community to search for ways to attenu ate muscle atrophy in patients receiving glucocorticoids. The role of exercise in preventing glucocorticoid-induced atrophy in patients is of great interest because exercise has a general anabolic effect on skeletal muscle.[4] In rodent studies, aerobic exercise, including running and swimming on a nonvoluntary basis, has been applied to study the effects of exercise on glucocorticoid-induced muscle atrophy[5-7] but rarely has resistance exercise been applied.[8-10] These previous studies examined the effects of resistance exercise induced by electric stimulation on the floor of a metal cage[8] or used an ablation model of functional overload.[9,10] Though these previous studies found positive effects of resistance exercise on glucocorticoid-induced muscle atrophy, the rat training models did not exclude the effects of electrical stimulus and/or a nonvoluntary training regimen.[5-10]

We modified the resistance exercise models, using the rat climbing technique reported by Yarasheski et al.[11] and Duncan et al.[12] In our model, rats voluntarily climbed the 200-cm tower to drink water from a bottle set at the top. We previously reported that 4-week voluntary climbing exercise using our model increased hindlimb muscle weight in normal rats.[13] However, we did not know whether or not our exercise model would improve glucocorticoid-induced muscle atrophy. The purpose of this study was to discover the effects of voluntary resistance exercise on glucocorticoid-induced muscle atrophy in growing rats. Furthermore, it was reported that glucocorticoid administration increased red blood cells and reduced lymphocytes.[14] Therefore, we examined the effects of glucocorticoid administration and resistance exercise on rat hematological status, which had yet to be studied in detail.

Materials and methods

All procedures involving animals were approved by the Experimental Animal Care Committee of the University of Tsukuba.

Animals and experimental design.

Male Sprague-Dawley rats were purchased from Japan

CLEA, Inc. (Tokyo) and were acclimatized for 2 weeks under standard laboratory conditions (22±2℃, 60% humidity). The light/dark cycle was 12 h with lights on from 7 : 00 am to 7 : 00 pm. Twenty nine rats, 10 weeks of age, were randomized by body weight to three groups. One group was saline control (C, n=9) and the other groups were glucocorticoid-sedentary (GS, n=10) and glucocorticoid-exercise (GE, n=10). The GS and GE groups were given 2 mg/kg/day of prednisolone (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The C group was given 2 ml/day of saline injected daily subcutaneously at 8 : 30 am. Each group was meal-fed commercial rat chow (CE-2, Japan CLEA, Tokyo) at 7 : 30 am to 8 : 30am and 7 : 30 pm to 8 : 30 pm and given free access to water for 8 weeks. To avoid any difference in food intake, all groups were given the same amount of food, using the feeding methods described in our previous reports.[15] We measured the minimum amount of food consumed by the groups, and the amount of food was then reduced to the minimum amount on the next day. If the food was consumed completely, the amount of food was increased by 4-6 g/day. Therefore, at 8 weeks, the amount of food consumed by the C, CS and CE groups was equal.

At the end of the experiment, the rats were killed by exsanguination under ether anesthesia. Immediately after death, the hindlimb muscles (gastrocunemius, plantaris, soleus, tibialis anterior, extensor digitorum longus (EDL) and quadriceps), liver, heart, kidney, spleen and abdominal adipose tissues (epididymal, perirenal, mesenteric) were isolated and weighed. Blood hematological tests (hemoglobin (Hb) concentration, hematocrit, red and white blood cell numbers, reticulocyte percentage) were requested from Scripps Reference Laboratory (SRL Co., Ltd., Tokyo). Total Hb was calculated with equations described previously[16] : total Hb (g) =Hb concentration (g/100 mL) $\times[-1.80\times\log(\text{body weight (g)})+10.21]\times$body weight (g) $\times 10^{-4}$. Erythrocyte glucose 6-phosphate dehydrogenase (G6PD, EC1.1.1.49) and glutathione peroxidase (GSHPx, EC 1.11.1.9) activities were determined by methods reported previously.[17,18]

Resistance exercise.

The GE group was housed in metal cages with a wire meshed tower with two water bottles set at the top (Fig. 1). At the beginning, the bottles were set at a height of 20 cm. The set point of the drink bottles was elevated gradually to 200 cm over a week. The rats were monitored for 24 hours every 2 weeks using a CCD camera (CCD-TRV 95, Sony, Tokyo). The daily distances climbed were obtained from the monitoring records.

Data analysis.

All values are expressed as mean and SEM. Data were analyzed by a one-way ANOVA and Scheffe's test.[19] Differences with P less than 0.05 were considered significant.

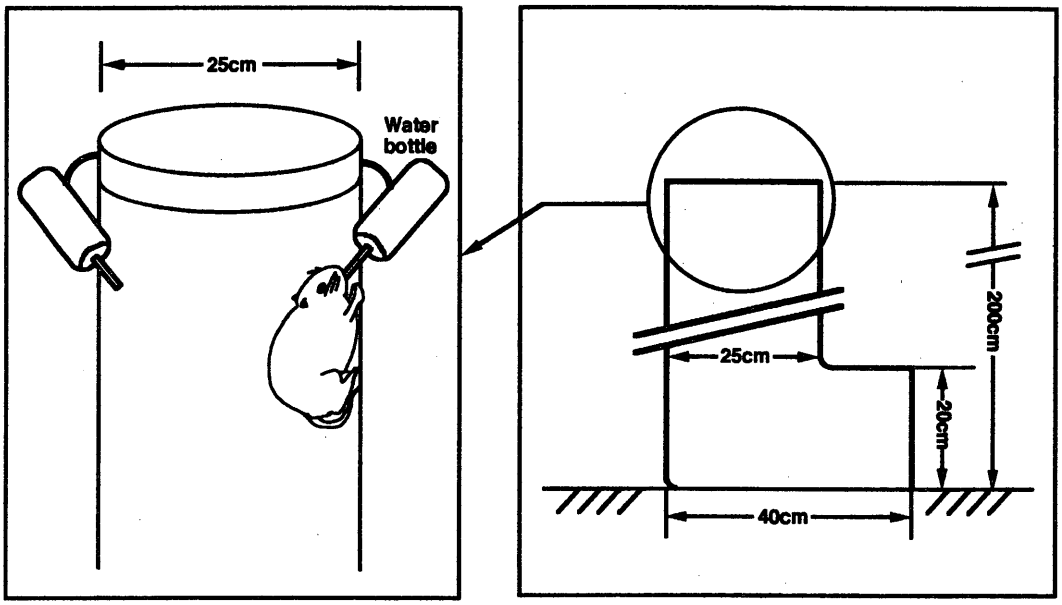

Fig. 1 Diagram illustrating the resistance exercise apparatus.

Results and Discussion

Final body weight and weight gain during the 8-week experimental period were greater ($p<0.05$) in the C group than in the GS and GE groups (Table 1). Liver, heart, kidney, spleen and abdominal adipose tissue weights were not different among the three groups (Table 1). When the splanchnic tissue weights were expressed as final body weight, liver, heart, kidney and abdominal adipose tissue weights were greater in the GS and GE groups than in the C group (Table 2). Hypertrophy of splanchnic tissues and abdominal fat deposition were recognized possible side effects of glucocorticoid administration.[20,21] Voluntary climbing exercise tended to improve abdominal fat accumulation, but not significantly (Table 2). We previously reported that voluntary climbing exercise reduced abdominal fat weight in normal rats.[13] These findings suggest that glucocorticoid administration reduced the effect of voluntary resistance exercise on abdominal fat accumulation.

The hindlimb muscles and total muscle mass at the final stage of the experiment were heavier in the C group than in other groups, although the soleus, plantaris and EDL muscle weights did not differ significantly (Table 1). The weight of each muscle relative to body weight did not differ among the three groups, except quadriceps and forearm muscles (Table 2). These results suggest that 8 weeks of climbing exercise did not prevent glucocorticoid-induced muscle atrophy in most skeletal muscles. The forearm muscles were probably affected by this exercise, but the difference between the GS and GE groups was not significant. We speculate that "climbing" places a larger load on the front legs supporting the idea that this climbing involved insufficient resistance. Current animal research indicates that resistance exercise training initiated with or before

Table 1 Body weight and tissue weights after 8 weeks of exercise[1]

	Control	Glucocorticoi	
		Sedentary	Exercise
Body weight (g)			
Initial	284±7	287±5	286±5
Final	377±6[a]	338±5[b]	330±5[b]
Gain	93±9[a]	51±6[b]	44±5[b]
Splanchnic tissues (g)			
Liver	10.0±0.2	9.8±0.2	9.6±0.3
Heart	0.95±0.01	0.92±0.03	0.93±0.02
Kidney	2.47±0.07	2.38±0.05	2.40±0.05
Spleen	0.74±0.04	0.67±0.04	0.60±0.03
Abdominal adipose tissues	13.2±0.7	15.3±0.6	13.5±1.2
Skeletal muscles (g)			
Gastrocnemius	3.92±0.10[a]	3.46±0.07[b]	3.23±0.06[b]
Soleus	0.29±0.01	0.28±0.01	0.27±0.01
Plantaris	0.66±0.01	0.63±0.01	0.64±0.04
Tibialis anterior	1.56±0.08[a]	1.35±0.03[b]	1.36±0.04[b]
EDL[2]	0.39±0.01	0.33±0.02	0.34±0.01
Quadriceps	5.88±0.16[a]	5.37±0.10[b]	4.99±0.08[c]
Forearm muscle[3]	2.68±0.09	2.52±0.05	2.55±0.04
Total	15.4±0.4[a]	13.9±0.2[b]	13.4±0.2[b]

[1]Values are menas±SE for 9-10 rats. Means with different superscripts within a row are significantly different at $p<0.05$ determined by one-way ANOVA and Scheffe's tests.
[2]EDL, extensor digitorum longus. [3]The sum of forearm muscles.

Table 2 Relative tissue weights after 8 weeks of exercise[1]

	Control	Glucocorticoi	
		Sedentary	Exercise
Splanchnic tissues (g/kg body weight)			
Liver	26.4±0.6[b]	29.1±0.6[a]	29.0±0.7[a]
Heart	2.53±0.06[b]	2.71±0.09[ab]	2.81±0.05[a]
Kidney	6.65±0.23[b]	7.01±0.05[a]	7.25±0.09[a]
Spleen	1.28±0.33	1.38±0.31	1.28±0.29
Abdominal adipose tissues	34.9±1.0[b]	45.3±1.8[a]	40.6±3.2[ab]
Skeletal muscles (g/kg body weight)			
Gastrocnemius	10.4±0.2	10.2±0.2	9.8±0.2
Soleus	0.76±0.03	0.83±0.03	0.81±0.03
Plantaris	1.74±0.02	1.87±0.03	1.94±0.12
Tibialis anterior	4.13±0.18	3.99±0.07	4.12±0.08
EDL[2]	1.02±0.01	0.97±0.06	1.04±0.04
Quadriceps	15.6±0.3[ab]	15.9±0.2[a]	15.1±0.2[b]
Forearm muscles[3]	7.09±0.17[b]	7.43±0.09[ab]	7.71±0.11[a]
Total	40.7±0.7	41.2±0.3	40.5±0.4

[1]Values are menas±SE for 9-10 rats. Means with different superscripts within a row are significantly different at $p<0.05$ determined by one-way ANOVA and Scheffe's tests.
[2]EDL, extensor digitorum longus. [3]The sum of forearm muscles.

Table 3 Hematological status in rats after 8 weeks of exercise[1]

		Control	Glucocorticoi	
			Sedentary	Exercise
Hb concentration	(g/100mL)	15.4±0.2	15.6±0.4	16.3±0.2
Total Hb	(g/rat)	3.25±0.08[a]	2.99±0.09[b]	3.04±0.04[ab]
Hematocrit	(%)	47.1±0.7	47.3±1.2	49.5±0.6
Red blood cells	($x10^4$/mL)	718±83	807±25	836±13
White blood cells	(/mL)	4133±962	3240±803	3290±641
MCV	(fL)	60.4±0.9	58.7±0.8	59.4±0.8
MCH	(pg)	19.8±0.2	19.3±0.3	19.3±0.3
MCHC	(%)	32.8±0.4	32.9±0.2	32.8±0.2
Reticulocyte	(%)	2.89±0.47	2.97±0.56	2.85±0.44
G6PD activity	(mmol/min/mL erythrocyte)	272±29	263±17	260±24
GSHPx activity	(mmol/min/mL erythrocyte)	155±6	154±14	178±8

[1]Values are menas±SE for 9-10 rats. Means with different superscripts within a row are significantly different at $p<0.05$ determined by one-way ANOVA and Scheffe's tests.
Hb, hemoglobin; MCV, mean red blood cell volume; MCH, mean red blood cell hemoglobin
MCHC, mean red blood cell hemoglobin concentration; G6PD, glucose 6-phosphate dehydrogenase
GSHPx, glutathione peroxidase

glucocorticoid administration attenuates the subsequent muscle atrophy but does not prevent it.[4,9,10] To simulate resistance exercise training in animals, researchers surgically remove a skeletal muscle and examine the effects of overload on the synergistic muscle. This is an ablation model of functional overload.[4,9,10] Using this ablation model, both Goldberg et al.[9] and Kurowski et al.[10] demonstrated significantly less atrophy (58% and 90%, respectively) in the rat plantaris muscle with simultaneous cortisone administration. On the other hand, weight-lifting training in rats induced by electric stimulation has been shown to reduce glucocorticoid-induced atrophy in the gastrocnemius muscle by 46%.[9] In a clinical investigation, Horber et al.[22,23] evaluated the effects of an isokinetic training program for quadriceps in patients with renal transplants receiving prednisone therapy. Patients demonstrated improved isokinetic muscle strength and increased thigh area (9-44%) as measured by computed tomography. Our present findings do not support these animal and clinical studies. The discrepancies between our results and others might be due to the magnitude of work load on skeletal muscles or the length of the experimental period. The maximal load in our climbing exercise was rat body weight and the daily climbing distances in the GE group after 2, 4, 6, and 8 weeks were 141±2, 148 ±3, 151±4, and 137± 2 m/day, respectively. The work levels (calculated by body weight×g×distance) required by our climbing exercise in the GE groups were 421 ±7, 457±10, 481±11 and 443±9 J/day, respectively. This level of exercise may be too light to prevent glucocorticoid-induced atrophy. Fimbel et al.[24] suggested that sprint-training is not sufficient to counteract the muscular effects of glucocorticoids.

In the hematological variables, total Hb level was higher in the C group than the GS and GE groups (Table 3), while Hb concentration, hematocrit value, red and white blood cell counts and the percentage of reticulocytes did not differ among the three groups (Table 3). The difference in total Hb level was due to the difference in final body weight. Mean red blood cell volume (MCV), mean red cell hemoglobin (MCH) and mean red blood cell hemoglobin (MCHC) were calculated from Hb concentration, hematocrit and red blood cell counts. MCV, MCH and MCHC were the same among the three groups (Table 3). The activities of G6PD and GSHPx, anti-peroxidative enzymes, were influenced by neither the glucocorticoid administration nor the climbing exercise (Table 3). It was shown that glucocorticoid administration influenced hematological status.[14,25] Ortoft et al.[14] reported that glucocorticoid administration decreased spleen and thymus weight as well as the white blood cell count, mainly due to a decrease in lymphocytes. Ganong[25] demonstrated that the red blood cell count was increased by glucocorticoid administration. Since we previously demonstrated that voluntary climbing exercise stimulated bone marrow heme biosynthesis in severly iron deficient rats[26], we examined the effects of resistance exercise on hematological status in glucocorticoid-injected rats. The present findings suggest that daily 2 mg/kg prednisolone does not affect hematological status with or without climbing exercise. Glucocorticoid-induced atrophy would be tissue specific and show markedly in skeletal muscle.

In conclusion, 8 weeks of climbing exercise did not attenuate glucocorticoid-induced muscle atrophy. Since it is suggested that this exercise may be too light to prevent loss of muscle, further studies of voluntary climbing exercise using additional weights will be required to clarify whether or not this exercise model can improve glucocorticoid-induced muscle atropy.

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タワークライミング運動がグルココルチコイド投与ラットの筋肉量および血液状態に及ぼす影響

松尾達博

要　　約

タワークライミング運動がグルココルチコイド投与ラットの筋肉量と血液状態に及ぼす影響を検討した．29匹の10週齢Sprague-Dawley系雄ラットを3群に分けそれぞれ，コントロール群（C群，n=9），グルココルチコイド投与－非運動群（GS群，n=10）およびグルココルチコイド投与－運動群（GE群，n=10）とした．GSおよびGE群には体重1kgあたり2mgのプレドニゾロンを，C群には体重1kgあたり2mlの生理食塩水を毎日皮下投与した．各群のラットに等エネルギーの市販飼料と水を与え8週間飼育した．GE群のラットについては，頂上に水入れを取り付けた200cmのタワー内を自発運動させた．実験期間中の体重増加量はGSおよびGE群に比べてC群で有意に大きかった．肝臓，心臓，腎臓，脾臓および腹腔内脂肪組織重量には3群間で差を認めなかった．下腿筋肉量および総下肢筋肉量はC群が他の群に比べて大きかった．体重に対する相対筋肉重量には，大腿筋および前肢筋重量をのぞいて，3群間で差は見られなかった．血液状態にはクライミング運動の有無にかかわらず，グルココルチコイド投与の影響は見られなかった．これらの結果から，タワークライミング運動はグルココルチコイド投与ラットの筋減弱を抑制しないことが示唆された．筋減弱を抑制するにはタワークライミング運動の強度が低かったのかもしれない．