Tech. Bull. Fac. Agr. Kagawa Univ., Vol. 56, 15～22, 2004

TOWER CLIMBING EXERCISE WITH HIGH-PROTEIN SNACK IMPROVES BONE MASS IN GLUCOCORTICOID-INJECTED OSTEOPOROTIC RATS

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Abstract

We examined the effects of a voluntary resistance exercise (climbing) together with high-protein snacks (60% protein) on bone mass in rats given glucocorticoid-injections (2 mg/kg/day) as a model of age-related osteopenia. Fifty-two male Sprague-Dawley rats, 8 weeks age, were assigned to exercise or sedentary groups. These groups were further divided into groups that received no snack, snack during activity or a snack during rest. All groups were meal-fed 7 : 30-8 : 30 h and 19 : 30-20 : 30 h and the snack was fed 23 : 30-0 : 30 h (active) or 11 : 30-12 : 30 h (resting). Energy and protein intake were approximately equal in all groups. The exercise groups were allowed to climb a wire-mesh tower cage (ϕ 20 cm × 200 cm) to drink water from a bottle set at the top. Weight gain during the 8-week experimental period was inhibited by a glucocorticoid-injection. Bone mass and strength were increased by climbing exercise with a high-protein snack, while no synergistic effect of exercise and snack nor any effect of snack timing was observed. Bone weight, calcium content and protein content were positively correlated to maximum load or structural stiffness. These results suggest that resistance exercise and high-protein supplementation may be a preventive therapy for osteoporosis associated with aging.

Key words : resistance exercise, high-protein snack, bone mass, bone strength, glucocorticoid

Introduction

Aging is a process that all living organisms experience over time, resulting in a general decline in various biological and physiological functions.[1] Osteoporosis, a serious problem in elderly people, is characterized by bone loss leading to fractures and high bone turnover.[2] In the elderly, more amino acids are absorbed from the digestive tracts and extracted by splanchnic tissues, which can result in a lower availability of dietary amino acids to the peripheral tissues.[3] It would be reasonable to hypothesize that in cases of low protein intake or increased protein requirement, this limited systemic availability of dietary amino acids could contribute to decreased bone protein synthesis, which could result in osteoporosis in elderly persons. Recently, in studies of bone protein synthesis and osteoporosis, glucocorticoid-injected rats are commonly used as a model of aging because glucocorticoid hormones are involved in the aging process. Adrenalectomy attenuates the development of age-specific effects, such as lower amino acid availability in the peripheral tissues.[4,5]

Protein supplementation with a high insulinogenic carbohydrate after meals should increase amino acid supply to peripheral tissues. We previously reported that high protein snack feeding 3 h after regular meals increased total blood amino acid flow calculated by the area under the curve of diurnal amino acid concentration in glucocorticoid-injected rats.[6] In addition, high protein snack together with resistance exercise showed significant preventive effect on glucocorticoid-induced sarcopenia and osteopenia.[6] However, these studies did not investigate all the effects of exercise on bone mass and strength in detail.

Many studies suggest that exercise has a beneficial effect on bone in humans[7,8] and animals.[9,10] Because exercise is effective in maintaining bone mineral density in early postmenopausal women, it has been proposed for a long- term prevention of osteoporosis.[7] Animal exercise models are used to examine the preventive or recovery effect of exercise on bone mass and strength as endpoints of an experiment. Animal studies using voluntary wheel running,[11] jumping,[12] treadmill running,[13] or voluntary climbing[9,14,15] have demonstrated the beneficial effect of increased load on bone mass and mechanical properties. Voluntary tower climbing is a light resistance exercise that creates little stress and strain and has been used in several studies.[5,9,14-17] We previously demonstrated that voluntary tower climbing exer

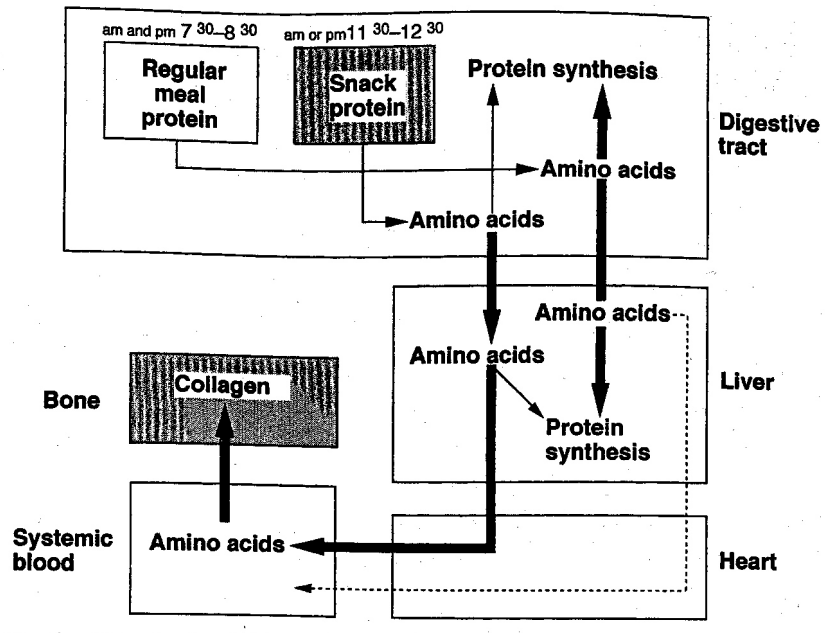

Fig. 1 Hypothesis of this study. In rats given glucocorticoid-injections, the peripheral availability of dietary amino acids is low due to high amino acid extraction by splanchnic tissues, which decreases protein synthesis and results in low protein content in peripheral tissues including bone. A high-protein snack fed a few hours after a meal added amino acids to peripheral tissues because the amino acid pool in splanchic tissues may be saturated by the preceding meal. This could increase protein synthesis in the bone. Exercise will enhance the effect of the snack.

cise increased bone mass and strength mainly by increasing bone formation in growing,[10] orchidectomized,[15] or ovariectomized osteopenic rats.[16] The purpose of this study was to examine the preventive effects of voluntary climbing exercise together with a high-protein snack on bone mass and strength in glucocorticoid-induced aging model rats. The hypothesis of this study is shown in Fig. 1.

Materials and Methods

Animals and experimental design.

Fifty-two male Sprague-Dawley rats (3 weeks old) were purchased from Japan SLC, Inc. (Shizuoka) and were acclimatized for a week under standard laboratory conditions (22±2℃, 60% humidity). The light/dark cycle was 12 h with lights on from 7 : 00 h to 19 : 00 h. Rats were housed in metal cages with a wire mesh tower (ϕ 20 cm × 200 cm) that had two water bottles set at the top to adjust climbing exercise[5,9,14-17] from 20 : 30 h to 7 : 30 h for 3 weeks. There were no bottles in the bottoms of the tower cages. At the beginning, the bottles were set at a height of 20 cm. The set drink bottles were gradually elevated to 200 cm over 1 week. At the age of 8 weeks, all rats were randomized by body weight to seven groups. One group was a saline control (C, n=7) and the other groups were glucocorticoid-injected sedentary group (GS, n=7), glucocorticoid-injected climbing exercise group (GC, n=7), glucocorticoid-injected sedentary with snack feeding groups (GSA, n=8 ; GSB, n=8), and glucocorticoid-injected climbing exercise with snack feeding groups (GCA, n=8 ; GCB, n=7). Group C was given 2 ml/kg/day of saline and the other groups were given 2 mg/kg/day of prednisolon (Wako Pur Chemical Industries, Ltd., Osaka) intraperitoneally at 8 : 30 h. The C, GS and GC groups were fed with a mixture of 5 g of commercial rat chow (CE-2, Japan CLEA, Inc., Tokyo), 1.5 g of a high protein snack (60% casein and 40% sucrose) twice a day (7 : 30-8 : 30, 19 : 30-20 : 30 h) for 8 weeks (from 8 to 16 weeks old). The GSA and GCA groups were fed with 5 g of CE-2 twice a day (7 : 30-8 : 30, 19 : 30-20 : 30 h) and 3 g of a high protein snack at 23 : 00-0 : 30 h. The GSB and GCB groups were fed with 5 g of CE-2 twice a day with 3 g of a high protein snack at 11 : 00-12 : 30 h. All rats were fed the experimental diets

isoenergetically during the 8 weeks of experimental period. The GC, GCA and GCB groups exercised continuously in tower climbing cages for 8 weeks. The C, GS, GSA and GSB groups were sedentary. At the end of the experiment (16 weeks old), the rats were killed by decapitation at 10 : 00 h after overnight fasting. Blood was collected to obtain serum and tibias were quickly removed and freed from connective tissues and measured for length, mid shaft width and wet weight.

Bone protein and calcium measurement.

Bone protein content was determined by Kjeldahl technique using automatic nitrogen/protein measurement system (Model VS-FA-1, Mitamura Industries, Ltd., Tokyo). Bone calcium was determined by flame atomic absorption spectrophotometry (AAS Z-5000, Hitachi, Tokyo) after dry-ashing at 550℃ and oxidizing at 100℃ with a mixture of 4 ml of 0.5 M H_2SO_4, 2 ml of 0.1 M HNO_3, 3 drops of concentrated $HClO_4$ (60%) and an excess (c.a. 0.3 ml) of 30 g/l $KMnO_4$. Samples were then diluted with 0.1 M HNO_3 and the concentration of calcium was determined by atomic absorption spectrophotometry.

Mechanical testing

A three-point bending test was performed as previously described [10,18] using a load tester (Rheoner, Model RE-33005, Yamaden, Co. Ltd., Tokyo). Each specimen of left femur and tibia was placed on a holding device with supports located at a distance of 12 mm, with the lesser trochanter proximal to, and in contact with, the proximal transverse bar. The mid point served as the anterior (upper) loading point. A bending force was applied by the crosshead at a speed of 0.1 mm/sec until fracture occurred. The breaking load (N) and structural stiffness (N/mm) were obtained directly from the load-deformation curves that were recorded continually in a computerized monitor linked to the load tester.

Serum analysis.

Serum protein concentration, albumin concentration, albumin/globulin ratio and ALP activity were determined using kits (A/G B-test and K-test, Wako pure chemical Industries, Ltd., Osaka).

Statistical analysis.

All values are expressed as mean ± SE. Except for group C, data were assessed by two-way ANOVA for the exercise and snack feeding. When the treatment was found to have a significant overall effect, the difference between the experimental groups was assessed by Fisher's PLSD test. Student's t test was used to examine the differences between group C and the others. Statistical significance was set at less than 0.05. All analyses were performed with a commercially available statistical package (StatView J-5.0, SAS Institute Inc., Cary, NC).

Results

Body weight, muscle weight and bone measurements

Body weight gain and final body weight in group C were significantly greater than those in the other groups (Fig. 2). Gastrocunemius muscle in group C was also significantly greater than those in the other groups (C, 3.19±0.12; GS, 2.87±0.07; GC, 2.91±0.05; GSA, 2.85±0.06; GSB, 2.88±0.07; GCA, 2.80±0.06; GCB, 2.88 ±0.07 g). Bone wet and dry weights were significantly heavier in group C than in the GS, GSA and GSB groups (Table 1). Tibial length were decreased but midshaft width and MW/L were not influenced by glucocorticoid injections (Table 1). Chronic climbing exercise significantly enhanced bone weight and midshaft width but did not alter tibial length in the glucocorticoid injected groups (Table 1). High protein snack feeding, whether fed in active or resting phases, influenced no structural parameters (Table 1). There was no interaction between climbing and the high-protein snack for tibial structural measurements (Table 1).

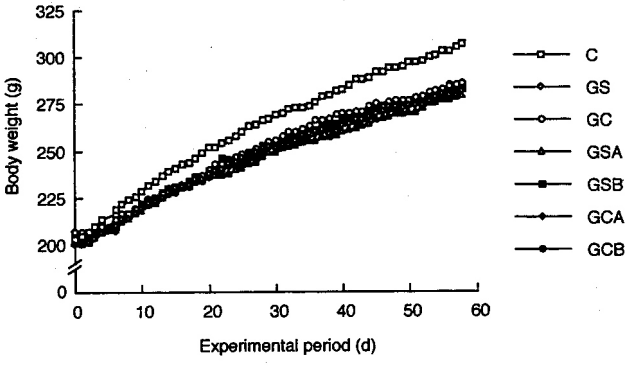

Fig. 2 Change in body weight of each group of rats over 8 weeks. Data represent average values (n=7-8). Range of SE was 3.2-6.9 g for all points (data not shown).

Table 1 Structual measurements of bone from each group of rats

Group	Rat number	Wet weight (mg)	Dry weight (mg)	Length (L) (mm)	Midshaft width (MW) (mm)	MW/L x 100
C	7	432±9	380±8	40.0±0.3	2.76±0.05	6.91±0.13
GS	7	396±14*^{d}	351±12*^{d}	39.0±0.3*	2.77±0.09$^{a\,b}$	7.09±0.21$^{b\,c}$
GC	7	464±8*^{a}	410±7*^{a}	39.1±0.3*	2.85±0.03$^{a\,b}$	7.29±0.12$^{a\,b\,c}$
GSA	8	385±8*^{d}	340±7*^{d}	38.8±0.3*	2.72±0.03^{b}	7.01±0.09^{c}
GSB	8	407±10$^{c\,d}$	360±9$^{c\,d}$	39.1±0.2*	2.78±0.07$^{a\,b}$	7.11±0.15$^{b\,c}$
GCA	8	459±10$^{a\,b}$	405±8$^{a\,b}$	38.8±0.1*	2.88±0.06^{a}	7.42±0.15*$^{a\,b}$
GCB	7	451±7$^{a\,b}$	399±6$^{a\,b}$	38.6±0.2*	2.92±0.02^{a}	7.56±0.07*^{a}
ANOVA	Exercise (E)	$p<0.05$	$p<0.05$	ns	$p<0.05$	$p<0.05$
	Snack (S)	ns	ns	ns	ns	ns
	E × S	ns	ns	ns	ns	ns

Values are means±SE for 7-8 rats in each group. ns, not significant.
Means with different superscripts within a column are significantly different at $p<0.05$ calculated bytwo-way ANOVA and Fisher's PLSD tests (except for C group). *Significantly difference ($p<0.05$) versus C group (Student's t test).

Table 2 Bone protein and calcium content and mechanical parameters of bone from each group of rats

Group	Rat number	Protein (mg)	Calcium (mg)	Calcium/Protein	Maximum load (N)	Structural stiffness (N/mm)
C	7	100±3	106±3	1.06±0.02	83±2	115±2
GS	7	90±5*^{c}	109±4^{c}	1.23±0.05*^{d}	81±4^{b}	108±5^{c}
GC	7	105±2^{a}	140±3*^{a}	1.34±0.03*$^{b\,c}$	93±4*^{a}	122±2^{a}
GSA	8	89±1*^{c}	121±2*^{b}	1.37±0.02*$^{b\,c}$	75±3^{b}	101±4*^{c}
GSB	8	96±2$^{b\,c}$	128±3*^{b}	1.33±0.02*^{c}	82±4^{b}	110±4$^{b\,c}$
GCA	8	105±2^{a}	148±3*^{a}	1.41±0.02*$^{a\,b}$	93±3*^{a}	122±3^{a}
GCB	7	101±2$^{a\,b}$	147±2*^{a}	1.45±0.01*^{a}	94±3*^{a}	120±4$^{a\,b}$
ANOVA	Exercise (E)	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$
	Snack (S)	ns	$p<0.05$	$p<0.05$	ns	ns
	E × S	ns	ns	ns	ns	ns

Values are means±SE for 7-8 rats in each group. ns, not significant.
Means with different superscripts within a column are significantly different at $p<0.05$ calculated by two-way ANOVA and Fisher's PLSD tests (except for C group). *Significantly difference ($p<0.05$) versus C group (Student's t test).

Bone protein and calcium content

Bone protein content was significantly higher in group C than in group GS, whereas bone calcium did not differ between groups C and GS (Table 2). Tibial calcium/ protein ratio was significantly lower in group C than in the other groups, whereas femoral calcium/protein ratio did not differ among all groups (Table 2). Climbing prevented the loss of bone protein due to glucocorticoid injections (Table 2). Tibal calcium content were significantly increased by both climbing and high protein snacks (Table 2). No differences in bone calcium content were found between groups GSA and GSB, or groups GCA and GCB (Table 2). There was no interaction between climbing and high- protein snacka for bone protein and calcium content (Table 2).

Maximum load and structural stiffness

The results of mechanical tests are shown in Table 2. Chronic climbing significantly enhanced bone maximum load and structural stiffness in the glucocorticoid injected groups. High-protein snacks, whether in active phase or resting phase, had no influence on any mechanical parameters. There was no interaction between climbing and high-protein snacks for the bone mechanical test results.

Serum protein and enzyme activity

Serum albumin concentration was lower in groups C than in the other groups (C, 3.80±0.04 ; GS, 3.88±0.05 ; GC, 3.86±0.07 ; GSA, 3.88±0.07 ; GSB, 3.81±0.03 ; GCA, 4.03±0.07 ; GCB, 4.06±0.09 mg/100 ml). Serum total protein concentration and albumin/globulin ratio did not differ for any groups (serum protein concentration : C, 6.46±0.11 ; GS, 6.45±0.14 ; GC, 6.38±0.14 ; GSA, 6.33±0.21 ; GSB, 6.12±0.08 ; GCA, 6.54±0.23 ; GCB, 6.43±0.21 mg/100 ml, albumin/globulin ratio : C, 1.45±0.06 ; GS, 1.52±0.05 ; GC, 1.54±0.04 ; GSA, 1.54±0.08 ; GSB, 1.54±0.08 ; GCA,

1.66±0.11 ; GCB, 1.73±0.06). Serum ALP activity did not differ among any groups (C, 41.5±4.3 ; GS, 34.5 ±4.3 ; GC, 35.1±2.8 ; GSA, 40.4±3.6 ; GSB, 46.7 ±5.0 ; GCA, 39.5±2.5 ; GCB, 40.5±6.4 K-A units)

Relationship between structural parameters and mechanical test results

Dry weight, midshaft width, calcium content and protein content of tibia were positively correlated to maximum load or structural stiffness (Fig. 3). Tibial length was not correlated to any mechanical test results (data not shown).

Discussion

This study demonstrated that climbing prevented tibial bone loss and loss of mechanical strength in glucocorticoid-injected rats. High-protein snacks increased bone calcium content and calcium/protein ratio. There was no interaction between climbing and high-protein snacks for any bone structural or mechanical parameters in glucocorticoid- injected rats. These results suggest that chronic voluntary climbing is more effective than dietary protein supplementation in rats given glucocorticoid-injection. We previously demonstrated that climbing or high-protein snacks alone could not suppress glucocorticoid effects, but climbing together with snacks showed significant preventive effects on glucocorticoid-induced osteopenia.[6] These results support, at least in part, our previous findings, though the differences remain between two studies. In this study, the high-protein snack contained just 60% casein and 40% sucrose. These were no trace elements such as vitamins and minerals. The high-protein snack we used previously conteined the following ingredients, in grams per kilogram : casein, 591.8 ; L-methionine, 9.0 ; mineral mixture, 70.0 ; choline bitartrate, 3.0 ; vitamin mixture 7.0 ; fiber, 50.0 ; corn starch 201.5 ; soybean oil, 67.7.[6] This snack provided 60, 20 and 20% of energy as protein, fat and carbohydrate, respectively. The discrepancy between our studies might be due to the differences between the high-protein snacks. We offered the high-protein snack 3 h after regular meals everyday because trace nutrients could be present in blood or stages of metabolism in the rat body. Some studies[19,20] report the importance of the time element for nutrients, with delayed supplementation of deficient nutrients failing to improve suppression of animal growth. A detailed study will be required to clarify this discrepancy.

We have demonstrated that glucocorticoid in rats decreases body weight gain, which is caused by skeletal muscle atrophy. On the other hand, bone weights, protein and calcium contents in the GC, GCA and GCB groups did not differ from those in group C. These results suggest that 8 weeks of climbing prevented glucocorticoid-induced osteopenia but did not avert glucocorticoid-induced muscle atrophy. Some researchers indicated that resistance exercise initiated with or before glucocorticoid administration attenuates the subsequent muscle atrophy but dose not prevent it.[21-23] To stimulate resistance exercise in animals, skeletal muscles were surgically removed and the effects of overload on the synergistic muscles were examined.[21-23] Using this ablation model of functional overload, Goldberg and Goodman[22] and Kurowski *et al.*[23] demonstrated significantly less atrophy in the rat skeletal muscle with simultaneous exercise and glucocorticoide. In addition, weight-lifting in rats induced by electric stimulation reduces glucocorticoid-induced muscle atrophy in the gastrocunemius muscle.[24] The discrepancies between our results and others could be due to the magnitude of the load on skeletal muscles or the length of the experimental period. The maximal load in our climbing exercise was rat body weight. This level of exercise may be too light to prevent glucocorticoid-induced muscle atrophy.

Many studies have been performed on the role of glucocorticoid in osteopenia and osteoporosis. Glucocorticoid induced osteoporosis is the results of a number of factors that adversely affect calcium homeostasis.[25-28] Systemic effects resulting in abnormalities in gonadal hormone secretion, calcium absorption, and renal handling of calcium and specific effects of glucocorticoids on bone all contribute to bone loss.[29,30] In this study, bone calcium content and serum ALP activity were not reduced by glucocorticoid injections, although bone weight and protein contents were markedly reduced. These results may be due to amount of glucocorticoid injected. Because the effect of glucocorticoid hormone increases dose-dependently,[31] we previously examined rats given 1-10 mg/kg/day prednisolon (data not shown), leading to a dose of 2 mg/kg being selected. However, the amount of glucocorticoid injected should be reconsidered.

In conclusion, we show in this study that voluntary resistance exercise together with high-protein snacks increases bone mass and strength in rats given glucocorticoid-injection, while no synergistic effect of exercise and snack

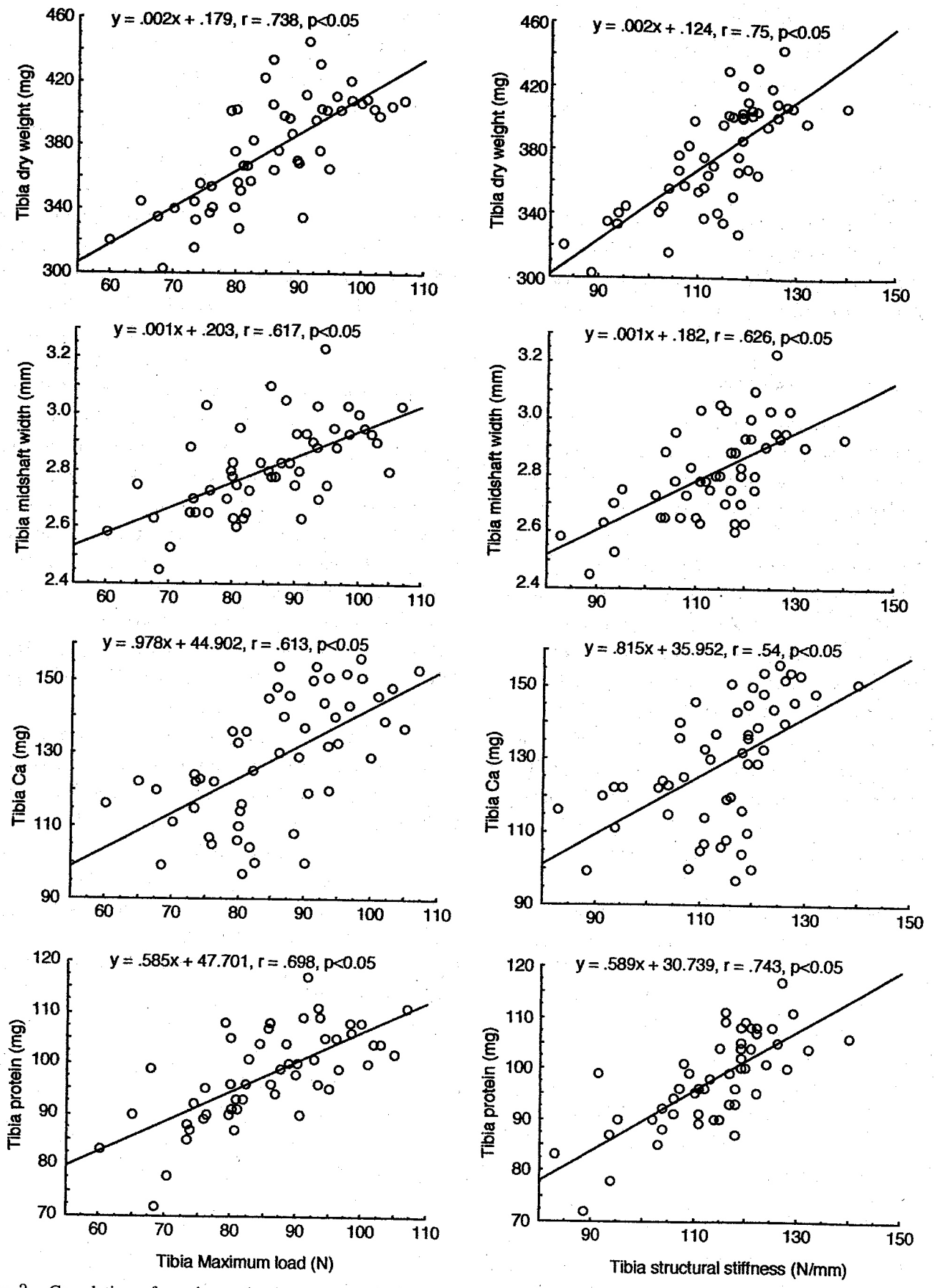

Fig. 3 Correlation of maximum load or structural stiffness of tibia versus tibia dry weight, midshaft width, calcium content and protein content for each group of rats.

nor any effect of snack timing was observed. These results suggest that resistance exercise and high protein supplementation may be an effective preventive therapy for osteoporosis associated with aging. However, further studies will be required to address several unsolved problems.

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(Received September 30, 2003)

タワークライミング運動と高蛋白質間食はグルココルチコイド投与による骨減弱ラットの骨量を改善する

松尾達博

要　　旨

自発的抵抗性であるクライミング運動と高蛋白質間食が，グルココルチコイド投与ラットの骨重量および骨組成に及ぼす影響について検討した．8週齢のSprague-Dawley系雄ラット52匹を運動群と非運動群に分け，さらにそれらを間食なし，休息期間食，活動期間食のサブグループに分類した．全てのラットに配合試料を7：30～8：30および19：30～20：30に与え，休息期間食群には11：30～12：30に，活動期間食群には23：30～0：30に，それぞれ高蛋白質間食を与え，摂取エネルギー量とタンパク質量が等しくなるように調節した．運動群のラットにはクライミング運動用タワー内を昇降させた．体重増加量はグルココルチコイド投与によって抑制された．骨重量および強度はクライミング運動と高タンパク質間食によって増加したが，高蛋白質間食単独での有意な効果は見られなかった．骨重量，カルシウム含量および蛋白質含量と最大骨強度および骨剛性とは有意に相関した．以上の結果から抵抗性運動と高蛋白質間食が老化に伴う骨減弱症の予防に有効である可能性が示唆された．