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Effects of a Calcium-Deficient Diet and Resistance Exercise on Bone in Male and Female Rats of Different Biological Maturity

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ABSTRACT

We investigated the effects of resistance exercise, sex, and biological maturity on bone in rats fed a calcium (Ca)-deficient diet. Growing (age: 4-7 weeks) and mature (age: 12-15 weeks) rats were divided into four groups: sedentary and climbing exercise groups on a Ca-sufficient diet (5 g Ca/kg), and sedentary and climbing exercise groups on a Ca-deficient diet (1 g Ca/kg). Exercise increased the muscle weight in both sexes of the growing rats, but not in both male and female mature rats. In rats fed a Ca-deficient diet, the weight, central axis width, breaking energy, and stiffness of the femur were significantly lower in both sexes of growing rats and were not affected by exercise. In mature rats, neither a Ca-deficient diet nor exercise had any effect on these measurements in either sex. The rupture energy and stiffness of the femur were decreased by the Ca-deficient diet in growing rats of both sexes, but exercise did not suppress this weakening. In mature rats of both sexes, the Ca-deficient diet did not induce fragility of the femur.

Abbreviations: Ca: Calcium; BMD: Bone Mineral Density; SD: Sprague-Dawley Rats; ANOVA: Analysis of Variance

Introduction

Calcium (Ca) is the most abundant mineral in the body, accounting for approximately 2% of an adult›s body weight, and approximately 99% of it is found in the bones. Factors that determine bone mass include body size, growth rate, and genetics [1]. It has been suggested that there is a correlation between the bone mass and muscle mass, and also between bone density and back muscle strength [2], and that the higher the muscle mass, the higher the bone mineral density [3]. It has been reported that bone mass is also related to exercise and hormonal status [4,5]. Jumping exercise training increases bone mass to a greater extent than aerobic exercise training in growing rats [6]. It has been shown that the mineral density and amount in the tibia, as well as the weight of the femur, were higher in rats given food immediately after squat exercise than in rats given food 4 h later [7]. The effect of resistance exercise on increasing bone mass has also been observed in adult rats [8]. On the other hand, Rubin et al. showed that the effect of physical stimuli on stimulating new bone formation was greater in young adult turkeys (age: 1 year) than in older turkeys (age: 3 years) [9], suggesting that the effect of Ca deficiency on bone differs between young and mature turkeys.

A breastfeeding mother loses approximately 200 mg of Ca per day from her bones to produce milk [10], reducing her bone mineral density (BMD) by 3-9% over a 6-month period [11]. Lovelady et al. reported that 16 weeks of resistance and aerobic exercise from 4 to 20 weeks postpartum reduced BMD loss in the lumbar spine of breastfeeding mothers [10]. Fujii et al. reported that resistance exercise, which induced muscle hypertrophy, increased hemoglobin

synthesis and promoted iron recycling in the body, mitigating anemia in rats fed an iron-deficient diet [12]. Collagen, a protein synthesized in the body, is one of the main components of bones. Thus, if resistance exercise stimulates bone collagen synthesis, accumulation of Ca in the bone may increase through Ca recycling in rats fed a Ca-deficient diet.

When rats were subjected to running exercise, the diameter of the tibia increased in males, while the weight and diameter of the tibia increased in females, and the effect of increasing bone weight and bone diameter was more pronounced in females than in males [13]. Muscle hypertrophy occurs in both male and female rats that perform spontaneous exercise; however, it has been reported that although females exercise more than males, muscle hypertrophy is greater in males than in females [14]. Thus, there are sex differences in the effects of exercise on bones and muscles. Therefore, the effects of a Ca-deficient diet and exercise on bones would differ between the growth and maturation stages and between males and females. Accordingly, we investigated the effects of a Ca-deficient diet and resistance exercise on rat bones in growing and mature male and female rats. We hypothesized that resistance exercise would alleviate the bone fragility that occurs due to a Ca-deficient state.

Materials and Method

Diet

Both Ca-sufficient and Ca-deficient diets were prepared according to AIN-93G [15]. The Ca content in the Ca-sufficient diet was 5 g/kg and that in the Ca-deficient diet was 1 g/kg.

Animals

Growing Stage: Twenty-two 4-week-old male Sprague-Dawley rats (SD) rats and 22 female rats (CLEA Japan, Osaka, Japan) were used. Room temperature was 23±1°C, the dark period was from 8:00 to 20:00, and the light period was from 20:00 to 8:00. Rats of both sexes were divided into a sedentary group (n = 5 for males and n = 5 for females) and an exercise group (n = 6 for males and n = 6 for females) fed a Ca-sufficient diet, a sedentary group (n = 5 for males and n = 5 for females), and an exercise group (n = 6 for males and n = 6 for females) fed a Ca-deficient diet. The study period was four weeks. The exercise group performed climbing exercises three times a week, every other day, from 17:00 to 18:00. The exercise protocol has previously been reported [16]. The rats were fed from 18:00 to 10:00 the following day. Food was given from 18:00 because ingesting a meal immediately after exercise has been regarded as effective for muscle hypertrophy due to exercise [17], and the rats were hungry at the end of the exercise session because they fasted from 10:00 am. Deionized water was provided to prevent the rats from ingesting Ca from their drinking water. Body weight, food intake, and water consumption were recorded daily. This study was approved by the Experimental Animals Subcommittee of the Research Integrity Committee of the Osaka University of Health and Sport Sciences (Approval numbers 22-1 and 22-2).

Mature Stage: Twelve-week-old sexually mature male (n =21) and female (n= 22) SD rats (CLEA Japan) were used in this study. All rats performed the climbing exercise described below from seven weeks of age to acclimatize themselves to exercise. All rats received a Ca-sufficient diet from 10 to 11 weeks of age. At 12 weeks of age, rats of both sexes were divided into a sedentary group (n = 5 for males and n = 5 for females), and an exercise group (n = 5 for males and n = 6 for females) fed a Ca-sufficient diet, a sedentary group (n = 5 for males and n = 5 for females), and an exercise group (n = 6 for males and n = 6 for females) fed a Ca-deficient diet. The study period was four weeks. The protocol was the same as that used in the growing stage experiment. This study was approved by the Experimental Animals Subcommittee of the Research Integrity of the Osaka University of Health and Sport Sciences (Approval numbers: 21-3 and 21-5).

Dissection

On the last day of the experiment, the rats were sacrificed under isoflurane anesthesia. Organs (liver, heart, kidneys, adrenals, spleen, digestive tract), skeletal muscle (flexor hallucis longus, soleus, gastrocnemius, plantaris), and adipose tissue (perirenal, parametrial, retroperitoneal, mesenteric) were excised and weighed.

The weight, length, and central axis width of the femurs on both sides were measured.

Analysis of Femur and Whole-Body Bones

The length and width of the central axis of the femur on the left and right sides were measured using a digital caliper (220-150-S; As One, Tokyo, Japan), and the rupture energy and rigidity (stiffness) were measured using a bone strength tester (TK-252C; Muromachi Kikai, Tokyo, Japan). The mean values of the left and right sides were used for the statistical analysis. For a whole-body bone analysis, after the removal of epidermal tissues, the bones of the whole body were incinerated using an oven (HTO-300S, AS ONE) at 550°C and weighed. Femora were also incinerated.

Statistics

A two-way analysis of variance (ANOVA) with diet and exercise as factors was performed. $P < 0.05$ were considered statistically significant (IBM SPSS Statistics Version 27.0.1.0).

Results

The Ca-deficient diet only decreased body weight and food intake in growing males (Table 1). The effect of exercise was observed only in mature male rats, which decreased both body weight and food intake. Exercise increased the weight of the FHL in growing males (Table 2). The weight of the FHL per 100 g of body weight in growing males and females and the weight of the gastrocnemius per 100 g of body weight in growing males were higher in the exercise group than in the sedentary group (Table 3). Exercise did not affect the other muscles. In growing rats, the Ca-deficient diet decreased weight, central width, rupture energy, and stiffness of the femora, whereas the diet did

not affect length in either sex (Table 4). Exercise did not affect the measurements, except for the rupture energy in males, which was decreased by exercise. In mature rats, males were not affected by diet or exercise. In females, no effects of diet or exercise were observed, except for stiffness, which was higher in the exercise group than in the sedentary group.

Table 1: Body weight and Food intake (g).

			Normal		Deficiency		2-way ANOVA		
			Sedentary	Exercise	Sedentary	Exercise	Diet	Exercise	Interaction
Growing rats	Male	Body weight	325.2 (27.5)	320.3 (17.5)	306.8 (24.6)	289.2 (25.9)	0.027	0.287	0.541
		Food intake (g/28 days)	563.0 (3.2)	544.3 (3.0)	544.4 (2.7)	500.0 (1.9)	0.040	0.908	0.192
	Female	Body weight	222.8 (19.2)	221.0 (14.6)	223.0 (8.4)	202.3 (12.6)	0.146	0.081	0.138
		Food intake (g/28 days)	480.2 (1.6)	467.5 (1.8)	468.2 (1.3)	454.2 (1.3)	0.378	0.353	0.963
Mature rats	Male	Body weight	471.0 (27.2)	454.0 (17.6)	486.0 (35.7)	445.8 (16.3)	0.759	0.018	0.304
		Food intake (g/28 days)	619.6 (1.3)	586.6 (1.2)	650.0 (1.5)	591.2 (1.2)	0.336	0.019	0.475
	Female	Body weight	291.6 (13.8)	288.5 (13.6)	296.4 (15.7)	289.8 (28.0)	0.679	0.516	0.814
		Food intake (g/28 days)	490.6 (1.3)	473.5 (1.1)	502.2 (1.0)	487.0 (1.0)	0.468	0.353	0.956

Note: Mean (SD)

Table 2: Skeletal muscle weight (g).

			Normal		Deficiency		2-way ANOVA		
			Sedentary	Exercise	Sedentary	Exercise	Diet	Exercise	Interaction
Growing rats	Male	FHL	0.85 (0.11)	1.05 (0.05)	0.87 (0.05)	0.93 (0.11)	0.162	0.002	0.088
		Soleus	0.21 (0.01)	0.21 (0.02)	0.20 (0.01)	0.19 (0.02)	0.048	0.818	0.644
		Gastrocnemius	3.14 (0.18)	3.45 (0.28)	3.15 (0.32)	3.04 (0.19)	0.077	0.373	0.062
		Plantar	0.60 (0.05)	0.59 (0.06)	0.59 (0.07)	0.55 (0.05)	0.333	0.410	0.546
	Female	FHL	0.61 (0.05)	0.63 (0.09)	0.61 (0.06)	0.65 (0.10)	0.839	0.422	0.760
		Soleus	0.17 (0.03)	0.17 (0.02)	0.16 (0.02)	0.15 (0.02)	0.072	0.348	0.776
		Gastrocnemius	2.36 (0.29)	2.33 (0.23)	2.45 (0.13)	2.25 (0.24)	0.961	0.282	0.404
		Plantar	0.45 (0.04)	0.44 (0.04)	0.45 (0.05)	0.41 (0.05)	0.555	0.163	0.426
Mature rats	Male	FHL	1.38 (0.07)	1.43 (0.15)	1.42 (0.08)	1.30 (0.18)	0.472	0.575	0.167
		Soleus	0.36 (0.01)	0.33 (0.01)	0.33 (0.03)	0.29 (0.02)	0.001	0.001	0.336
		Gastrocnemius	4.78 (0.34)	4.63 (0.28)	4.77 (0.28)	4.41 (0.33)	0.419	0.076	0.437
		Plantar	0.87 (0.08)	0.88 (0.07)	0.86 (0.07)	0.81 (0.09)	0.264	0.495	0.357
	Female	FHL	0.90 (0.03)	0.96 (0.13)	0.88 (0.07)	0.94 (0.08)	0.677	0.206	0.956
		Soleus	0.21 (0.02)	0.21 (0.03)	0.21 (0.02)	0.20 (0.02)	0.521	0.526	0.799
		Gastrocnemius	3.15 (0.15)	3.20 (0.23)	3.13 (0.33)	3.18 (0.23)	0.841	0.649	0.987
		Plantar	0.59 (0.05)	0.62 (0.08)	0.58 (0.08)	0.60 (0.04)	0.629	0.415	0.743

Note: Mean (SD).

FHL; flexor hallucis longus.

Table 3: Skeletal muscle weight (g/100g body weight).

			Normal		Deficiency		2-way ANOVA		
			Sedentary	Exercise	Sedentary	Exercise	Diet	Exercise	Interaction
Growing rats	Male	FHL	0.27 (0.02)	0.33 (0.01)	0.29 (0.02)	0.33 (0.02)	0.258	0.000	0.069
		Soleus	0.07 (0.00)	0.07 (0.01)	0.07 (0.00)	0.07 (0.00)	0.828	0.555	0.947
		Gastrocnemius	0.99 (0.07)	1.10 (0.05)	1.06 (0.07)	1.08 (0.05)	0.362	0.029	0.117
		Plantar	0.19 (0.01)	0.19 (0.01)	0.20 (0.01)	0.20 (0.01)	0.117	0.811	0.877
	Female	FHL	0.28 (0.02)	0.29 (0.05)	0.28 (0.02)	0.33 (0.03)	0.211	0.049	0.206
		Soleus	0.08 (0.01)	0.08 (0.01)	0.07 (0.01)	0.07 (0.00)	0.216	0.947	0.537
		Gastrocnemius	1.08 (0.06)	1.08 (0.07)	1.14 (0.05)	1.15 (0.05)	0.031	0.831	0.804
		Plantar	0.21 (0.01)	0.20 (0.01)	0.21 (0.02)	0.21 (0.01)	0.438	0.648	0.828
Mature rats	Male	FHL	0.30 (0.03)	0.32 (0.03)	0.30 (0.02)	0.30 (0.04)	0.416	0.493	0.444
		Soleus	0.08 (0.00)	0.07 (0.00)	0.07 (0.00)	0.07 (0.00)	0.000	0.062	0.880
		Gastrocnemius	1.03 (0.06)	1.04 (0.08)	1.01 (0.10)	1.01 (0.09)	0.424	0.907	0.977
		Plantar	0.19 (0.01)	0.20 (0.02)	0.18 (0.02)	0.18 (0.02)	0.231	0.439	0.705
	Female	FHL	0.32 (0.01)	0.34 (0.04)	0.31 (0.03)	0.33 (0.03)	0.585	0.109	0.953
		Soleus	0.08 (0.01)	0.07 (0.01)	0.07 (0.01)	0.07 (0.01)	0.494	0.697	0.848
		Gastrocnemius	1.10 (0.07)	1.13 (0.05)	1.09 (0.15)	1.12 (0.07)	0.740	0.460	0.928
		Plantar	0.21 (0.02)	0.22 (0.02)	0.20 (0.03)	0.21 (0.02)	0.590	0.280	0.810

Note: Mean (SD).

FHL; flexor hallucis longus.

Table 4: Femur.

			Normal		Deficiency		2-way ANOVA		
			Sedentary	Exercise	Sedentary	Exercise	Diet	Exercise	Interaction
Growing rats	Male	Weight (g)	0.97 (0.07)	0.96 (0.05)	0.75 (0.06)	0.72 (0.05)	0.000	0.461	0.586
		Length (mm)	34.92 (0.87)	34.68 (0.51)	34.03 (1.03)	34.07 (1.07)	0.066	0.796	0.713
		Center width (mm)	4.78 (0.23)	4.59 (0.24)	4.30 (0.38)	4.24 (0.33)	0.004	0.341	0.606
		Rupture energy (mJ)	83.2 (12.8)	63.1 (15.3)	32.0 (5.0)	26.7 (4.6)	0.000	0.012	0.808
		Stiffness (N/mm)	115.3 (9.5)	120.1 (11.0)	30.4 (4.01)	36.1 (15.1)	0.000	0.282	0.928
	Female	Weight (g)	0.71 (0.04)	0.73 (0.06)	0.62 (0.05)	0.60 (0.05)	0.000	0.954	0.463
		Length (mm)	32.18 (0.90)	32.26 (0.31)	32.11 (0.37)	31.77 (0.73)	0.302	0.631	0.441
		Center width (mm)	4.21 (0.19)	4.18 (0.19)	4.01 (0.17)	3.84 (0.21)	0.004	0.248	0.375
		Rupture energy (mJ)	69.7 (15.7)	84.2 (14.4)	28.5 (13.4)	24.5 (5.1)	0.000	0.343	0.104
		Stiffness (N/mm)	117.5 (7.6)	111.4 (8.0)	38.7 (10.5)	36.5 (11.5)	0.000	0.322	0.643

Mature rats	Meal	Weight (g)	1.23 (0.11)	1.25 (0.06)	1.18 (0.05)	1.19 (0.07)	0.097	0.701	0.952
		Length (mm)	40.95 (0.80)	40.32 (0.39)	40.49 (0.35)	40.16 (0.61)	0.227	0.073	0.570
		Center width (mm)	4.86 (0.21)	4.91 (0.23)	4.74 (0.16)	4.89 (0.08)	0.386	0.199	0.527
		Rupture energy (mJ)	57.0 (5.8)	53.6 (3.5)	55.7 (6.18)	61.1 (7.2)	0.250	0.704	0.108
		Stiffness (N/mm)	168.8 (5.1)	167.2 (3.5)	167.6 (8.5)	166.6 (8.0)	0.754	0.672	0.929
	Female	Weight (g)	0.95 (0.02)	0.93 (0.06)	0.89 (0.07)	0.91 (0.06)	0.167	0.996	0.527
		Length (mm)	36.05 (0.60)	35.95 (0.82)	35.46 (0.57)	35.87 (0.74)	0.313	0.646	0.452
		Center width (mm)	4.33 (0.13)	4.26 (0.15)	4.27 (0.22)	4.27 (0.14)	0.765	0.646	0.697
		Rupture energy (mJ)	58.8 (12.5)	55.1 (5.5)	59.5 (4.3)	57.1 (4.6)	0.672	0.344	0.830
		Stiffness (N/mm)	157.3 (7.9)	167.9 (4.3)	164.4 (9.4)	167.6 (3.8)	0.233	0.024	0.200

Note: Mean (SD).

The weight of the femur ash decreased with the Ca-deficient diet, except for the weight per 100 g of body weight in mature males. Although exercise increased the femur ash weight per 100 g of body weight in mature males, no other effects of exercise were observed (Table 5). The weight of femur ash in the Ca-deficient diet group was approximately 50% of that in the normal diet group of growing rats, while that of mature rats was approximately 90%, indicating that the decrease in mature rats was smaller than that in growing rats. (Table 6) shows that the whole-body ash weight was lower in the Ca-deficient diet group than in the normal diet group, except for the mature females. The effect of exercise was not consistent. The whole-body ash weight of the Ca deficient group was approximately 50% of that of the normal diet group in growing rats, while it was 88-102% in mature rats, which was similar to that observed in the femur.

Table 5: Femur ash weight.

			Normal		Deficiency		2-way ANOVA		
			Sedentary	Exercise	Sedentary	Exercise	Diet	Exercise	Interaction
Growing rats	Male	g	0.60 (0.04)	0.59 (0.05)	0.28 (0.03)	0.27 (0.02)	0.000	0.684	0.964
		g/ 100g body weight	0.19 (0.01)	0.19 (0.01)	0.09 (0.01)	0.10 (0.00)	0.000	0.727	0.644
	Female	g	0.53 (0.02)	0.52 (0.04)	0.25 (0.01)	0.24 (0.01)	0.000	0.305	0.920
		g/ 100g body weight	0.24 (0.01)	0.24 (0.01)	0.12 (0.01)	0.12 (0.00)	0.000	0.746	0.326
Mature rats	Male	g	1.00 (0.08)	1.02 (0.05)	0.88 (0.04)	0.91 (0.06)	0.097	0.455	0.852
		g/ 100g body weight	0.22 (0.01)	0.23 (0.01)	0.19 (0.02)	0.21 (0.01)	0.001	0.024	0.464
	Female	g	0.86 (0.02)	0.85 (0.07)	0.79 (0.06)	0.82 (0.06)	0.049	0.796	0.358
		g/ 100g body weight	0.30 (0.01)	0.30 (0.02)	0.27 (0.02)	0.29 (0.03)	0.041	0.453	0.280

Note: Mean (SD).

Table 6: Whole-body ash weight.

			Normal		Deficiency		2-way ANOVA		
			Sedentary	Exercise	Sedentary	Exercise	Diet	Exercise	Interaction
Growing rats	Male	g	7.03 (0.46)	7.25 (0.46)	3.41 (0.20)	3.40 (0.24)	0.000	0.506	0.478
		g/ 100g body weight	2.22 (0.13)	2.31 (0.07)	1.15 (0.06)	1.21 (0.04)	0.000	0.048	0.680
	Female	g	6.28 (0.43)	5.63 (0.23)	2.88 (0.14)	2.80 (0.16)	0.000	0.004	0.020
		g/ 100g body weight	2.90 (0.10)	2.62 (0.14)	1.34 (0.04)	1.43 (0.06)	0.000	0.035	0.000
Mature rats	Male	g	11.71 (0.53)	11.66 (0.51)	10.16 (0.25)	10.24 (0.29)	0.000	0.936	0.732
		g/ 100g body weight	2.53 (0.13)	2.61 (0.07)	2.14 (0.14)	2.34 (0.10)	0.000	0.013	0.228
	Female	g	9.60 (0.28)	9.49 (0.67)	8.98 (0.58)	9.40 (0.63)	0.172	0.529	0.294
		g/ 100g body weight	3.36 (0.12)	3.36 (0.16)	3.11 (0.21)	3.32 (0.27)	0.114	0.230	0.207

Note: Mean (SD).

Discussion

It has been reported that Ca deficiency reduces bone mineral density [18,19] and bone strength [20] in growing rats. The results of the present study are consistent with these previous reports. On the other hand, in mature male and female rats, the Ca-deficient diet decreased the femoral weight and width of the central axis and reduced bone strength, as assessed by the rupture energy and stiffness. These results suggest that Ca deficiency weakens bones during the growing stage, but not during the mature stage. Bone mineral density, bone rupture energy, and stiffness are believed to be correlated. The ash weights of the femur and whole body were reduced by the Ca-deficient diet in both growing and mature rats. However, during the growth stage, the weight of ash in the deficient diet group was approximately 50% of that in the sufficient diet group, whereas in the mature stage, it was approximately 90% or more. Therefore, during the mature stage, the decrease in bone Ca due to the Ca-deficient diet was not large; therefore, the rupture energy and stiffness did not decrease.

The reason for the lack of a large decrease in bone Ca due to the Ca-deficient diet in mature rats is that, in growing rats, Ca was deficient during the active period of bone formation. In contrast, in mature rats, Ca had accumulated in the bones by 12 weeks of age; therefore, the Ca-deficient diet did not cause a significant decrease in bone Ca. Saville et al. reported that applying a weight load to the hindlimb increased the bone Ca concentration in the hindlimb of rats [20]. Welten et al. showed that both Ca intake and exercise load contribute to maximum bone mass acquisition [21]. Lovelady et al. reported that exercise during breastfeeding reduced bone density loss without increasing Ca intake [10]. Frost suggested that bones adapt to loads, such as body weight and muscle forces, and increase their mineral content and strength [22]. Welch et al. stated that mechanical stimulation caused by exercise increases bone mass, and that mechanical stimulation above a certain threshold (minimum effective strain) is necessary during the bone mass acquisition phase [23].

In the present study, no effects of exercise were observed in the femur, except for a lower rupture energy in growing males and increased stiffness in mature females. Dalsky et al. observed that the endocrine system is involved in the effect of exercise load on bone metabolism [24]. Estrogen has the function of transmitting the stimulation caused by exercise to osteoblasts, and it has been suggested that when ovarian function declines, even with exercise, sufficient effects on the bones cannot be obtained [24]. The effect of exercise on stiffness observed only in adult female rats in the present study could be attributed to an estrogen-mediated effect. The exercise protocol used in this study has been reported to increase FHL in growing male rats [16]. In this study, an increase in the skeletal muscle weight due to exercise was observed in the FHL and the FHL per 100 g of body weight and gastrocnemius muscle in growing males, whereas it was only observed in the FHL per 100 g of body weight in growing females.

Therefore, muscle hypertrophy due to exercise was thus suggested to be greater in males than in females. On the other hand, in mature rats, no increase in skeletal muscle weight due to exercise was observed in rats of either sex. In the exercise regimen employed in this study, the heavier the weight, the higher the load on the body. However, even in males whose muscle weight increased with exercise during the growth stage, no increase in muscle weight due to exercise was observed in the mature stage. These results suggest that exercise-induced muscle hypertrophy is less likely to occur after maturation. Okano et al. [7] suggested that exercise-induced muscle hypertrophy may be partially responsible for the increase in bone mineral density

and content observed in rats subjected to squat training. Fujii et al. [16] reported that the exercise used in the present study increased muscle mass, increased aminolevulinic acid dehydratase activity (the rate-limiting enzyme in hemoglobin synthesis), and mitigated anemia in rats fed an iron-deficient diet. In the present study, we assumed that resistance exercise promoted the synthesis of bone collagen, which is synthesized in the body in the same way as hemoglobin, resulting in the accumulation of Ca in the bones and a reduction in mineral content and bone fragility in rats fed a Ca-deficient diet. In the present study, the collagen content of the bones was not measured to prioritize their ash weight. However, the difference between the weight of the femur shown in (Table 4) and the weight of the ash of the femur shown in (Table 5) can be considered to represent the weight of the organic matter in the femur. The main components of organic matter are collagen. Thus, this can be an indicator of collagen weight. No effect of exercise was observed when weight was calculated. Therefore, bone collagen was unlikely to increase with exercise in the present study.

In this study, we investigated the effects of a Ca-deficient diet and resistance exercise on rat bones in growing and mature male and female rats. As a result, the bones of both male and female rats were weakened by a Ca-deficient diet in growing rats but not in mature rats. Furthermore, no obvious effects of exercise on bones were observed in male or female rats, in either growing or mature animals. A possible reason why exercise did not reduce bone fragility due to the Ca-deficient diet in growing rats could be that the Ca level in the diet was too low or that the effect of exercise was insufficient. In conclusion, bone weakness caused by a Ca-deficient diet was observed in growing rats; however, exercise did not alleviate this weakness.

Authors Contributions

Kawata and Kitaguchi performed experiments and analysis. Fujii performed autopsies, analyzed data, and summarized. Okamura reviewed and wrote the paper.

Competing Interests

The authors declare that they have no competing interests.

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