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Calorie restriction aggravated cortical and trabecular bone architecture in ovariectomy-induced estrogen-deficient rats

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ABSTRACT

We hypothesized that calorie restriction (CR) and estrogen deficiency (ovariectomy [OVX]) would aggravate bone biomarkers and structural parameters in rats. Seven-week-old female Sprague-Dawley rats were randomized to sham-operated groups and fed either an ad libitum diet (SHAM-AL) or a CR diet (SHAM-CR); ovariectomy-operated groups were fed an ad libitum diet (OVX-AL) or a CR diet (OVX-CR). For 8 weeks, the OVX-AL and SHAM-AL groups were fed the same diet, whereas CR groups were fed a diet containing 50% fewer calories. Bone-related biomarkers and structural parameters (OC; deoxypyridinoline [DPD]; N-terminal telopeptide, NTx; architecture and mineralization; and microcomputed tomography images) were analyzed at the end of the experiment. The serum OC levels of calorie-restricted groups (SHAM-CR and OVX-CR) were significantly lower than those of the AL groups (SHAM-AL and OVX-AL) ($P < .05$). Urinary DPD levels of calorie-restricted and ovariectomized groups were higher than those of their counterparts ($P < .05$), whereas urinary NTx levels of calorie-restricted groups were higher than those of AL groups ($P < .05$). In regard to trabecular bone, the calorie-restricted and ovariectomized groups had lower values of bone volume to total volume, trabecular number, and bone mineral density, but higher values of trabecular separation than those of their counterparts ($P < .05$). Regarding cortical bone, the calorie-restricted groups had reduced values of bone volume, mean polar moment of inertia, and cortical thickness compared to the AL groups ($P < .05$). In conclusion, severe CR with or without OVX during the growth period in rats is equally detrimental to bone; CR has detrimental effects on trabecular and cortical bone; and estrogen deficiency only had an effect on trabecular bone.

Abbreviations: AL, ad libitum; ALP, alkaline phosphatase; ANOVA, analysis of variance; BMD, bone mineral density; BS/BV, bone surface to volume; BV, bone volume; BV/TV, bone volume to total volume; CR, calorie restriction; Cs.Th, cortical thickness; DPD, deoxypyridinoline; FER, food efficiency ratio; MMI, mean polar moment of inertia; NTx, N-terminal telopeptide; OC, osteocalcin; OVX, ovariectomy; Tb.N, trabecular number; Tb.Pf, trabecular pattern factor; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness; SMI, structure model index; μ-CT, microcomputed tomography; 3D, three-dimensional.

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1. Introduction

Development and maintenance of bone are controlled by the dynamic balance between bone formation (via osteoblasts) and bone resorption (via osteoclasts). The activities of these cells are regulated by various genetic and environmental factors (diet, physical activity, and lifestyle), and imbalance among these factors is responsible for bone loss. Recently, calorie restriction (CR) has emerged as a serious risk factor for bone loss [1–5].

Calorie restriction has been shown to improve health, extend lifespan, and delay aging-associated diseases in humans and animal models [6,7]. In addition, CR is known to have beneficial cardioprotective effects [8,9]. Recently, several studies have suggested that CR can result in bone loss, mainly through suppression of bone formation and the rate of bone turnover [2,4]. Talbott et al [1,4] reported that energy restriction had a negative effect on bone mass and on the maintenance of bone health in mature and older rats, but not growing rats. Under CR, an altered hormonal balance with reduced bone constituents, including matrix proteins and minerals, presents a major problem. However, the impact of CR is controversial when considering age at onset, duration, and degree of CR.

Estrogen deficiency is the most influential factor for osteoporosis in women. Previous studies [9,10] suggested that ovarian estrogen preserves bone mass by restraining the local production of cytokines that promote bone resorption. Thereby, deficient estrogen production at the time of menopause promotes osteoclastic bone resorption [9]. In fact, once women reach menopause, the risk for both obesity and osteoporosis increases substantially. Furthermore, obesity and postmenopausal osteoporosis are often observed in the same individual [9]. Therefore, CR for obesity care might be prudent in postmenopausal women. Although CR in estrogen deficiency can aggravate bone loss, the impact of CR on bone biomarkers, bone mineral density (BMD), and structural parameters in estrogen deficiency is limited. A greater understanding of these factors will necessitate different guidelines for weight control in postmenopausal women.

We hypothesized that CR and estrogen deficiency (ovariectomy [OVX]) would aggravate bone biomarkers and structural parameters in rats. To demonstrate the important impact that adequate calorie intake has on bone health, the effects of CR on bone biomarkers, BMD, and structural parameters were investigated in growing ovariectomized rats.

2. Methods and materials

2.1. Experimental design and diet

The experimental protocol was approved by the Animal Care and Use Review Committee of Kyung Hee University (protocol number: KHUASP (SU)-12-084). Forty-seven-week-old, female, 150- to 170-g Sprague-Dawley rats were purchased from SLC, Inc (Shizuoka, Japan). The rats were individually housed in polycarbonate cages, in temperature-controlled rooms (22°C ± 2°C), and maintained at a relative humidity of 55% ± 5% with a 12-hour light/dark cycle. The rats were fed a pellet chow diet and provided water ad libitum (AL), over a 1-week adaptation period.

After adaptation, individual animals were randomized into 4 groups: the control group fed the American Institute of Nutrition diet (AIN-93G, D10012G, Research Diets, Inc, USA) AL (SHAM-AL) (n = 10); the sham group fed a 50% calorie-restricted diet based on the AIN-93G diet (SHAM-CR) (n = 10); the ovariectomized group fed AIN-93G AL (OVX-AL) (n = 10); and the ovariectomized group fed a 50% calorie-restricted diet (OVX-CR) (n = 10). Daily intake of vitamins and minerals was equal in both diets. The experimental diet ingredient compositions are shown in Table 1.

The OVX groups (n = 20) were ovariectomized by ligating and excising the ovaries, whereas control groups (n = 20) underwent a sham operation in which the ovaries were exposed without excision. The rats were anesthetized using isoflurane inhalation (2%-4% dissolved in oxygen). After a 1-week recovery period, each group was assigned its respective diet for the remaining 8 weeks.

2.2. Body weight and food consumption

Food consumption and body weight were measured daily and weekly, respectively. The food efficiency ratio (FER) was

Table 1 – Ingredient compositions of the diets fed to rats

Ingredients	AIN-93G	50% CR
Casein (g)	200	100
L-Cystein (g)	3	1.5
Corn starch (g)	398	199
Maltodextrin (g)	132	66
Sucrose (g)	100	45
Cellulose (g)	50	50
Soybean oil (g)	70	35
t-Butylhydroquinone (g)	0.014	0.014
Mineral mix [a] (g)	35	35
Vitamin mix [b] (g)	10	10
Choline bitartrate (g)	2.5	2.5
% kcal		
Protein	20	20
Carbohydrate	64	64
Fat	16	16
kcal/g	4.0	3.7

[a] Mineral mix composition (AIN-93G MIX): calcium carbonate 357.00 g/kg, potassium phosphate monobasic 196.00 g/kg, potassium citrate H_2O 70.78 g/kg, sodium chloride 74.00 g/kg, potassium sulfate 46.60 g/kg, magnesium oxide 24.00 g/kg, ferric citrate 6.06 g/kg, zinc carbonate 1.65 g/kg, manganouscarbonate 0.63 g/kg, cupric carbonate 0.30 g/kg, potassium iodate 0.01 g/kg, sodium selenate 0.01025 g/kg, ammonium paramolybdate H_2O 0.00795 g/kg, sodium metasilicate $9H_2O$ 1.4500 g/kg, chromium potassium sulfate $12H_2O$ 0.2750 g/kg, lithium chloride 0.0174 g/kg, boric acid 0.0815 g/kg, sodium fluoride 0.0635 g/kg, nickel carbonate 0.0318 g/kg, ammoniumvanadate 0.0066 g/kg, sucrose finely powdered 221.026 g/kg.

[b] Vitamin mix (10 g/kg): nicotinic acid 3.00 g/kg, calcium pantothenate 1.60 g/kg, pyridoxine-HCl 0.70 g/kg, thiamine-HCl 0.60 g/kg, riboflavin 0.60 g/kg, folic acid 0.20 g/kg, biotin 0.02 g/kg, vitamin E acetate (500 IU/g) 15.00 g/kg, vitamin B12 (cyanocobalamine) (0.1% in mannitol) 2.50 g/kg, vitamin A palmitate (500 000 IU/gm) 0.008 g/kg, vitamin D3 (cholecalciferol) (400 000 IU/g) 0.250 g/kg, vitamin K1 (phylloquinone) 7.50 g/kg.