Long-term supplementation with young coconut juice does not prevent bone loss but rather alleviates body weight gain in ovariectomized rats

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Abstract. Young coconut (*Cocos nucifera* Linn.) juice (YCJ) has traditionally been consumed to alleviate symptoms associated with the menopause. Recently, the authors demonstrated that short-term (6-week) YCJ supplementation to ovariectomized rats resulted in increased bone mass and bone formation parameter, suggesting that YCJ consumption has a positive effect on bone metabolism and may represent an intervention to help slow the bone loss during menopause transition. The present study sought to determine how long-term (12-week) YCJ supplementation affects bone metabolism in ovariectomized rats, to investigate whether such supplementation may be helpful to in osteoporosis treatment. Ten-week-old female Wistar rats were subjected to either a sham operation (Sham) or bilateral ovariectomy (Ovx). The Ovx+YCJ group received 5X-concentrated YCJ at a dose of 15 ml/kg/day for 12 weeks. Rats in the Ovx group had significantly lower femur bone mineral density than those in the Sham group. YCJ supplementation did not significantly affect this difference. However, YCJ prevented the increase in bone area of the mid third of the femur, a site high in cortical bone, and body weight gain observed following Ovx. Our findings indicate that long-term YCJ intake does not alter bone loss, but rather alleviates body weight gain following menopause.

Introduction

Osteoporosis is a skeletal disorder characterized by compromised bone strength that predisposes affected individuals to an increased risk of fracture. Osteoporosis is more common in women than in men and greatly reduces the healthy life years expectancy, defined as the number of years that a person is expected to live in a healthy condition (1,2). Although osteoporosis is a multifactorial disorder, estrogen deficiency following menopause is strongly associated with rapid bone resorption and loss of bone density (3). Thus, hormone replacement therapy (HRT) has been regarded as one treatment option for the prevention of postmenopausal bone loss (4,5), and some studies have indeed reported that HRT reduces the risk of fractures (6). However, findings from Women's Health Initiative trials revealed that the risks associated with HRT outweighed the benefits, despite the role of HRT in preventing osteoporotic fracture (7-10). Although related societies recommend HRT as a first-line option for the prevention and treatment of osteoporosis in postmenopausal women <60 years, there are still many concerns about HRT and the potential risks to various aspects of women's health (11,12).

Alternatives to HRT for the treatment of postmenopausal osteoporosis include bisphosphonates, selective estrogen receptor modulators (SERMs), teriparatide and denosumab (13). However, bisphosphonate alendronate is only effective in osteoporotic women (T-score <-2.5) without previous fractures (14), and the SERM raloxifene fails to show any non-vertebral fracture protection (15). In addition, there is concern about the side effects of gastroesophageal reflux disease, osteonecrosis of the jaw and atypical femoral fractures for alendronate and venous thromboembolism for raloxifene (13). These unfavorable findings associated with pharmaceutical alternatives to HRT have heightened interest in diet and lifestyle changes that can minimize postmenopausal bone loss, and thereby decrease the necessity for drugs in the treatment of osteoporosis.

Cocos nucifera Linn. (Arecaceae), also known as coconut, is widely used in traditional medicine (16,17). Young coconut juice (YCJ), defined as the juice extracted from the fruit of a 6-month-old coconut, is consumed by women in Thailand to alleviate symptoms associated with menopause (18-20), which suggests that YCJ may have phytoestrogenic effects and may

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prevent postmenopausal osteoporosis. The authors demonstrated that short-term (6-week) supplementation with YCJ in ovariectomized rats significantly reduced bone loss, which suggests that YCJ supplementation may mitigate the rapid bone loss observed in women during early menopause (21). In the present study, the effects of long-term (12-week) YCJ supplementation on bone metabolism were examined in ovariectomized rats to investigate whether such supplementation of YCJ could help to prevent postmenopausal osteoporosis.

Materials and methods

Preparation of YCJ. The composition of YCJ has been reported by Pungmatharith (22) previously. Dried powder of YCJ was prepared as previously described (18,21). Briefly, YCJ obtained from the fruit of ~6-month-old coconuts was freeze dried and then reconstituted in distilled water, which resulted in 5X-concentrated YCJ. The final solutions were stored at -20°C until use.

Animals and diets. Female Wistar rats (10-weeks-old) were purchased from Japan SLC, Inc. (Hamamatsu, Japan). Upon arrival, they were housed in a temperature (23±1°C)- and humidity (55±5%)-controlled room under a 12 h light-dark cycle and fed standard rodent chow and water *ad libitum*. Following acclimatization for 2 weeks, the rats were randomly divided into four groups (Baseline, n=5 rats; Sham, Ovx, and Ovx+YCJ groups, n=10 rats per group). The rats in the baseline group were anesthetized by intraperitoneal injection of 10% chloral hydrate (400 mg/kg body weight), and immediately sacrificed by cardiac puncture. This group provided reference values for skeletal measurements, which enabled the determination of changes in skeletal tissue resulting from surgery and aging (23). The rats in the remaining three groups were anesthetized and subjected either to sham operation (Sham group) or bilateral ovariectomy (Ovx and Ovx+YCJ groups) using the dorsal approach. At 2 days following surgery, 5X YCJ was administered by gavage to rats in the Ovx+YCJ group at a daily dose of 15 ml/kg body weight (equivalent to 75 ml/kg body weight of 1X YCJ) for 12 weeks. The dose of YCJ was determined as previously described (18,21). The rats in the other groups were administered tap water by gavage daily for 12 weeks. At 12 weeks following surgery, the rats in all three groups were anesthetized and sacrificed by cardiac puncture. The rats were given intraperitoneal injections of the fluorochrome markers calcein (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany; 10 mg/kg body weight) and tetracycline-HCl (Sigma-Aldrich; Merck KGaA; 25 mg/kg body weight) 5 and 2 days prior to necropsy, respectively, to evaluate the bone dynamics by histomorphometry. At necropsy, the uterus was resected and weighed to determine whether the Ovx was successful. Blood was collected by cardiac puncture; serum was obtained and stored at -80°C until analysis by ELISA. The right femurs were wrapped in a saline-soaked gauze and stored at -20°C for subsequent densitometry. The left femurs were cleaned of soft tissue and fixed in 70% ethanol for bone histomorphometry. The present study was conducted in parallel with a previous study of the authors (24) in order to minimize the number of control rats that were sacrificed (Baseline, Sham and Ovx groups). The

experimental protocol was approved by the Animal Ethics Committee of the University of Shizuoka (Shizuoka, Japan).

Bone densitometry. The right femurs were completely thawed at room temperature, and the bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry (DXA) and analyzed using small animal software (version 12.5; QDR-4500A; Hologic, Inc., Bedford, MA, USA). The BMD was calculated as the bone mineral content (BMC) divided by the bone area (BA). In addition, the BA, BMC and BMD in the proximal, mid diaphyseal and distal parts of the femur were determined by dividing the femur into three equal parts according to length. The coefficients of variation for the scans and standards were <1.0%.

Peripheral quantitative computed tomography (pQCT). The right femurs were placed in a polypropylene tube filled with saline solution and scanned using pQCT (XCT Research SA+; Stratec Medizintechnik GmbH, Pforzheim, Germany) at a voxel size of 0.12 mm. The scan line was adjusted using scout view, and transverse sections were recorded at the distal femoral metaphysis (3 mm proximal to the distal growth plate) and the midshaft (50% bone length). Images were analyzed using XCT software (version 6.20; Stratec Medizintechnik GmbH) in contour mode 2 and peel mode 2 (threshold 395 mg/cm^3) to calculate the trabecular and total bone parameters at the metaphysis and in cortical mode 1 (threshold 690 mg/cm^3) to calculate the cortical bone parameters at the diaphysis. The following parameters were measured at the femoral metaphysis: Total BMC (T.BMC; mg), total BMD (T.BMD; mg/cm^3), total cross-sectional area (CSA) (T.CSA; mm^2), trabecular BMC (Tb.BMC; mg), trabecular BMD (Tb. BMD; mg/cm^3), and trabecular CSA (Tb.CSA; mm^2). At the midshaft, the cortical BMC (Ct.BMC; mg), cortical BMD (Ct BMD; mg/cm^3), cortical CSA (Ct.CSA; mm^2), cortical thickness (Ct.Th; mm), periosteal circumference (Peri.C; mm) and endosteal circumference (Endo.C; mm) were evaluated.

Bone histomorphometry. The left femurs were stained with Villanueva bone stain (Maruto Instrument Co., Ltd., Tokyo, Japan) for 7 days prior to dehydration in a graded series of ethanol and embedded without decalcification in methyl methacrylate (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Frontal sections (5 μm) of the distal femur were cut using a microtome (RM2255; Leica Microsystems GmbH, Wetzlar, Germany) and mounted on slides. The specimens were examined under a fluorescence microscope (BX53; Nikon Corporation, Tokyo, Japan). The structural and dynamic histomorphometric indices were measured in the cancellous bone at the secondary spongiosa located 500 μm from the epiphyseal growth plate and 250 μm from the endocortical surface using a semi-automatic image analysis system (Histometry RT CAMERA; System Supply Co., Ltd., Ina, Japan) at a magnification of x320. The histomorphometric nomenclature used in the present study is in accordance with the report of the American Society for Bone and Mineral Research Histomorphometry Nomenclature Committee (25). Bone section preparations and histomorphometric measurements were performed at the Niigata Bone Science Institute (Niigata, Japan).

Serum bone turnover markers. Osteocalcin (OC) and RatLaps (bone-related degeneration from C-terminal telopeptides of type I collagen) were determined in order to evaluate bone formation and resorption status, respectively. OC (Rat-MID Osteocalcin EIA; Immunodiagnostics Systems Holdings PLC, Tyne & Wear, UK; cat no. AC-12F1) and RatLaps (RatLaps EIA; Immunodiagnostics Systems Holdings PLC; cat. no. AC-06F1) was measured using an ELISA following protocols provided by the manufacturer.

Statistical analyses. The data are presented as the mean ± standard error of the mean, and data management and statistical analyses were performed with JMP 9.0.2 (SAS Institute Inc., Cary, NC, USA). The specific effects of Ovx and YCJ supplementation were examined by comparing values of the Sham, Ovx and Ovx+YCJ groups using analysis of variance and analysis of covariance with body weight as the covariate (26,27), with Tukey's honest significant difference (HSD) test. P<0.05 was considered to indicate a statistically significant difference.

Results

Effects of YCJ on body and uterine weights. There were early increases in the body weight of rats in the Ovx group, with significant differences between the Ovx and Ovx+YCJ groups during the experimental period. At the end of the study, the Ovx group had a significantly greater body weight than the Ovx+YCJ group and tended to have a greater body weight than the Sham group. There was no significant difference in body weight between the Sham and Ovx+YCJ groups (Fig. 1). The rats in the Ovx group had a significantly lower mean uterine weight than those in the Sham group, suggesting that the Ovx procedure was successful. There were no significant differences in uterine weight between the Ovx and Ovx+YCJ groups (data not shown).

DXA of the femur. The right femoral BMD of the rats in the Ovx group was significantly lower than that of the rats in the Sham group. The BMD of the rats in the Ovx+YCJ group was not significantly different from that of the rats in the Ovx group. When the femur was divided into three segments of equal length, there were significant differences in the BMD and BMC between the Sham and Ovx groups at the proximal and distal ends of the femur, sites that are high in cancellous bone, but not at the mid diaphysis, a site that is high in cortical bone. The rats supplemented with YCJ (Ovx+YCJ group) presented no significant differences in the BMC or BMD of the right femur compared with the rats in the Ovx group, regardless of the femur site (i.e., proximal, diaphyseal and distal sites; Table I). The BA at the mid diaphysis was significantly greater in the Ovx group than in the Sham group, whereas there was no difference in the mid diaphyseal BA between the Sham and Ovx+YCJ groups.

pQCT of the femur metaphysis and diaphysis. The Tb.BMC of the distal metaphysis was significantly higher in the Ovx group than in the Sham group. However, due to a significant increase in the Tb.CSA, the Tb.BMD in the Ovx group was significantly lower than that in the Sham group. There were no significant differences in the parameters between the
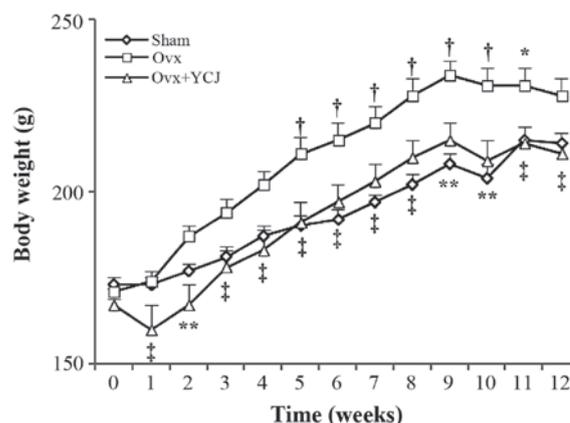
Figure 1. Mean body weight change of sham-operated (Sham) and ovariectomized rats supplemented with (Ovx+YCJ) or without (Ovx) YCJ for 12 weeks (n=10/group). The values represent the mean ± standard error of the mean. *P<0.05, †P<0.01 vs. Sham; ‡P<0.05, **P<0.01 vs. Ovx (Sham vs. Ovx vs. Ovx+YCJ, analysis of variance with Tukey's honest significant difference test). YCJ, young coconut juice; Ovx, bilateral ovariectomy.

Sham and Ovx groups at the diaphysis. YCJ supplementation induced no significant differences in any parameter evaluated by pQCT at the diaphyseal and distal sites (Table II).

Bone histomorphometry. The histomorphometric indices for distal femoral metaphyseal cancellous bone are summarized in Table III. The bone volume and trabecular number were significantly lower in the Ovx group than in the Sham group, whereas the osteoid volume and osteoid surface were higher in the Ovx group than in the Sham group. Regarding the dynamic indices, a larger mineralized surface was found in the Ovx group than in the Sham group. However, these differences were not significantly affected by long-term YCJ supplementation.

Serum bone turnover markers. The serum OC level was significantly lower in rats in the Ovx+YCJ group than in those in the Sham and Ovx groups, which suggests that YCJ supplementation suppressed bone formation. On the other hand, the serum bone resorption marker RatLaps was not affected by YCJ supplementation (Fig. 2).

Discussion

It was recently reported that ovariectomized rats supplemented with YCJ for a short term (6 weeks) demonstrated a significantly higher femoral BMD (as measured by DXA) and higher bone formation rates (as measured by trabecular bone histomorphometry) than rats that did not receive YCJ (21), which suggests that YCJ may mitigate the rapid bone loss observed in women during early menopause. In the present study, the effects of long-term (12-week) YCJ supplementation on bone metabolism was examined in ovariectomized rats to investigate whether YCJ could help prevent the development of postmenopausal osteoporosis. However, the present study demonstrated that ovariectomized rats with longer-term (12 weeks) YCJ supplementation (Ovx+YCJ group) had a BMD similar to that of rats without YCJ supplementation (Ovx group).

Table I. Femur BMC and BMD in sham-operated (Sham) and ovariectomized rats supplemented with (Ovx+YCJ) or without (Ovx) YCJ for 12 weeks.

	0 weeks	12 weeks		
Parameters	Baseline (n=5)	Sham (n =8)	Ovx (n=8)	Ovx+YCJ (n=8)
Femur BMC (g)				
Whole	0.215±0.004	0.298±0.004	0.292±0.009	0.276±0.007[a]
Proximal	0.081±0.003	0.113±0.003	0.108±0.004[a]	0.103±0.003[a]
Mid	0.050±0.003	0.070±0.002	0.077±0.004	0.073±0.003
Distal	0.087±0.001	0.119±0.002	0.112±0.003[a]	0.104±0.003[b]
Femur BA (cm^2)				
Whole	1.220±0.010	1.457±0.017	1.535±0.032	1.450±0.017
Proximal	0.443±0.010	0.542±0.009	0.563±0.011	0.536±0.010
Mid	0.340±0.006	0.396±0.004	0.435±0.012[a]	0.409±0.005
Distal	0.458±0.004	0.542±0.009	0.563±0.011	0.527±0.011
Femur BMD (g/cm^2)				
Whole	0.176±0.002	0.204±0.002	0.190±0.003[b]	0.190±0.003[b]
Proximal	0.183±0.002	0.209±0.002	0.192±0.003[b]	0.192±0.004[b]
Mid	0.146±0.006	0.177±0.007	0.176±0.007	0.178±0.006
Distal	0.190±0.004	0.219±0.002	0.198±0.003[b]	0.198±0.004[b]

Data are presented as the mean ± standard error of the mean. [a]P<0.05, [b]P<0.01 vs. Sham. BMD, bone mineral content; BMD, bone mineral density.

Table II. Trabecular and cortical parameters determined by peripheral quantitative computed tomography in sham-operated (Sham) and ovariectomized rats supplemented with (Ovx+YCJ) or without (Ovx) YCJ for 12 weeks.

	0 weeks	12 weeks		
Parameters	Baseline (n=5)	Sham (n=8)	Ovx (n=8)	Ovx+YCJ (n=8)
Distal metaphysis				
T.BMC (mg)	8.72±0.32	9.85±0.29	8.72±0.22[b]	8.37±0.28[b]
T.BMD (mg/cm^3)	611±14	658±11	540±8[b]	538±11[b]
T.CSA (mm^2)	14.3±0.3	15.0±0.3	16.2±0.5	15.5±0.3
Tb.BMC (mg)	0.774±0.160	0.815±0.042	1.129±0.046[b]	1.050±0.072[a]
Tb.BMD (mg/cm^3)	304±16	253±8	177±5[b]	167±5[b]
Tb.CSA (mm^2)	2.65±0.58	3.26±0.25	6.40±0.27[b]	6.30±0.43[b]
Diaphysis				
Ct.BMC (mg)	4.55±0.04	5.91±0.06	6.05±0.13	5.82±0.10
Ct.BMD (mg/cm^3)	1230±5	1320±3	1310±4	1310±4
Ct.CSA (mm^2)	3.69±0.04	4.48±0.04	4.63±0.10	4.45±0.07
Ct.Th (mm)	0.494±0.010	0.598±0.004	0.594±0.006	0.586±0.009
Peri.C (mm)	9.04±0.08	9.38±0.07	9.66±0.15	9.44±0.08
Endo.C (mm)	5.94±0.13	5.63±0.08	5.93±0.15	5.76±0.10

Data are presented as the mean ± standard error of the mean. [a]P<0.05, [b]P<0.01 vs. Sham. Tb, trabecular; T, total; Ct, cortical; BMC, bone mineral content; BMD, bone mineral density; CSA, cross-sectional area; Th, thickness; Peri.C, periosteal circumference; Endo.C, endosteal circumference.

It is unknown why the results of the present investigation failed to corroborate the findings of the prior study of the authors, in which trabecular bone histomorphometry revealed that short-term (6-week) supplementation of YCJ accelerated the parameters of bone formation without affecting bone resorption parameters in ovariectomized rats. In addition to its use in

Table III. Dynamic cancellous bone indices in the distal femoral metaphysis in sham-operated (Sham) and ovariectomized rats supplemented with (Ovx+YCJ) or without (Ovx) YCJ for 12 weeks.

	0 weeks	12 weeks		
Parameters	Baseline (n=5)	Sham (n=8)	Ovx (n=8)	Ovx+YCJ (n=8)
Static indices				
Bone volume (%)	28.5±1.2	28.8±1.3	15.0±0.9[b]	14.6±2.5[b]
Trabecular thickness (μm)	55.4±1.0	62.0±1.5	64.2±2.2	64.9±3.5
Trabecular number (N/mm)	5.15±0.20	4.63±0.17	2.34±0.12[b]	2.18±0.23[b]
Osteoid volume (%)	4.09±0.44	2.03±0.23	4.36±0.61[a]	4.37±0.46[b]
Osteoid surface (%)	26.2±2.4	15.7±1.4	32.8±2.5[b]	33.6±2.4[b]
Osteoblast surface (%)	13.39±2.21	3.97±0.54	7.34±1.23	8.54±2.22
Dynamic indices				
Mineralizing surface (%)	33.1±1.5	17.7±2.1	31.9±2.4[b]	25.9±1.8[a]
Mineral apposition rate (μm/day)	2.50±0.17	1.27±0.05	1.45±0.10	1.42±0.06
Bone formation rate (mm^3/mm^2/year)	0.302±0.023	0.081±0.010	0.173±0.023	0.135±0.012[a]
Bone formation rate (%/year)	1,100±97	261±35	536±73	414±29

Data are presented as the mean ± standard error of the mean. [a]$P<0.05$, [b]$P<0.01$ vs. Sham.
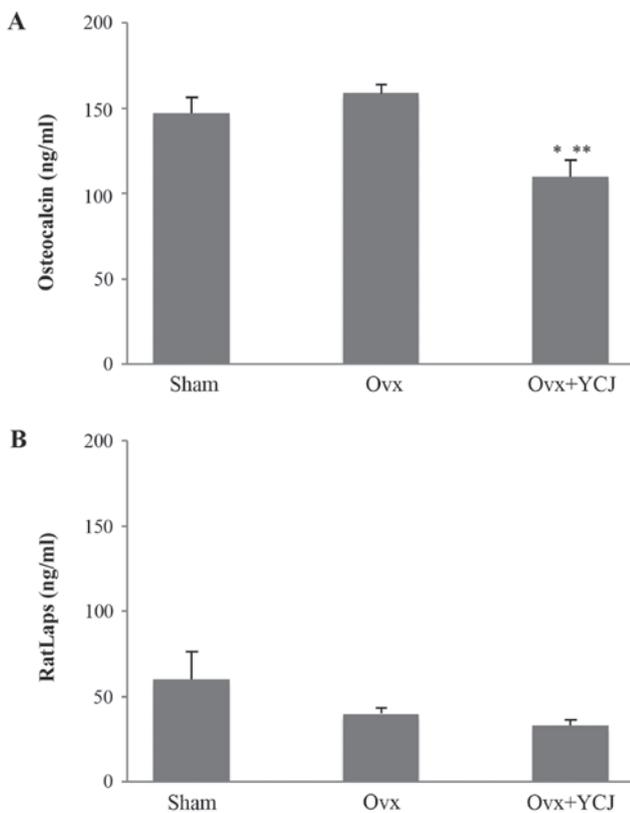
Figure 2. (A) Serum osteocalcin and (B) RatLaps in the sham-operated (Sham) and ovariectomized rats supplemented with (Ovx+YCJ) or without (Ovx) YCJ for 12 weeks. The values represent the mean ± standard error of the mean. *$P<0.05$ vs. Sham; **$P<0.01$ vs. Ovx (Sham vs. Ovx vs. Ovx+YCJ, analysis of covariance adjusted for body weight with Tukey's honest significant difference test). YCJ, young coconut juice; Ovx, bilateral ovariectomy.

alleviating menopausal symptoms, coconut juice has also been used as a temporary contraceptive in Thailand and Indonesia.

In contrast, women in these areas are prohibited from drinking coconut juice because it disrupts their menstrual cycle, and individuals in Java are afraid to drink coconut milk because it may diminish their fertility (18-20). It therefore seems that YCJ may exert its bone protective effects only in the presence of endogenous sex steroids, suggesting that the positive effect on bone observed 6 weeks following ovariectomy (21) were no longer apparent at 12 weeks following ovariectomy.

Although we have still not identified the exact constituents in YCJ that prevented bone loss following Ovx in the previous study, YCJ contains estrogens (e.g., 17β-estradiol, estrone and estrone-3-glucuronide), testosterone and various phytoestrogens (22). All of these constituents may have positively affected bone parameters in the authors' previous study. It is important to note that 17β-estradiol exerts its bone-protective effects by suppressing both bone formation and resorption, with a greater emphasis on the latter (28,29). It has been speculated that the effect of YCJ supplementation on bone may differ from that of estradiol. Ovariectomy in young, growing rodents decreases BMD of the trabecular bone and increases radial growth of the cortical bone, and both of these effects can be reversed by estrogen (30). Estrogen exerts many of its effects via two nuclear estrogen receptors (ERs), ERα and ERβ. Although both receptors have been detected in all skeletal cell types, for example chondrocytes, bone marrow stromal cells, osteoblasts, osteocytes and osteoclasts and their progenitor cells, the vast majority of the estrogen bone-sparing effects are mediated by ERα in both the cortical and cancellous bone compartments (31,32). By contrast, ERβ may not serve a major role in bone, with the possible exception that it may exert an inhibitory effect on periosteal apposition (30,31). In the present study, DXA measurements demonstrated that the BA at the mid diaphysis, a site that is high in cortical bone, was significantly greater in the Ovx group than in the Sham group, whereas there was no difference in the mid diaphyseal BA

between the Sham and Ovx+YCJ groups. YCJ contains various phytoestrogens, such as β-sitosterol (58% of the total composition of 1 ml YCJ), stigmastatrienol, stigmasterol, fucosterol and α-spinasterol (22). Phytoestrogens are plant derivatives that behave differently from estrogen but similarly to SERMs with a higher affinity for ERβ (33). The serum bone formation marker OC was significantly lower in rats in the Ovx+YCJ group than in those in the Ovx group. However, bone histomorphometry indicated that trabecular bone formation was not affected by YCJ supplementation. Thus, the authors speculate that long-term supplementation with YCJ may prevent the periosteal apposition of cortical bone, possibly via ERβ.

Unexpectedly, long-term supplementation with YCJ (Ovx+YCJ) resulted in a significantly lower body weight compared to no supplementation. Ovariectomized animals present body weight gain and excessive feeding. The treatment of ovariectomized animals with estrogen prevents these changes, and these effects are mediated, in large part, through ERα (34,35). The present study demonstrated that the uterine weight of the rats that were administered YCJ at a dose equivalent to 75 ml/kg/day was not significantly different from that of the rats that were not administered YCJ. Punghmatharith (22) reported that 17β-estradiol was present in YCJ at 2.45 pg/ml and that the subcutaneous injection of an ethereal extract of YCJ at a dose equivalent to 7,500 ml/kg/day for 3 consecutive days significantly increased the uterine wet weight of immature rats; however, this effect was not seen when YCJ was injected at 4,000 ml/kg/day (22). Thus, the dose of 17β-estradiol in YCJ is small, suggesting that YCJ may alleviate body weight gain following Ovx in rats through mechanisms different from those of ERα. However, some recent transgenic studies have indicated that ERβ may serve a key role in preventing obesity and obesity-related metabolic disease (36,37). Radenahmad *et al* (20) demonstrated that both ERα and ERβ were highly expressed in a cutaneous wound of ovariectomized rats supplemented with YCJ, although ERα is predominantly expressed in normal skin. These findings may endorse the authors' speculation that the effects of YCJ may be exerted through ERβ.

In conclusion, long-term supplementation with YCJ did not improve indices of bone mass or bone histomorphometry in ovariectomized rats, arguing against the benefits of YCJ in preventing osteoporosis in postmenopausal women. Although accumulated evidence suggests that consuming a phytoestrogen-rich diet alleviates symptoms associated with menopause, studies into the direct effects of dietary phytoestrogens on bone in postmenopausal women are extremely limited (38). Thus, physicians should not hesitate to initiate drug treatment in women at risk of osteoporosis and fracture, even if patients prefer diet and lifestyle alterations. Nevertheless, considering the results of our previous study (21), YCJ may be beneficial in mitigating bone loss in women during early menopause, which may delay the start and reduce the total duration of anti-osteoporotic drug therapy. In addition, YCJ consumption may help to prevent body weight gain following menopause.

Acknowledgements

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