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Influence of Soy Aglycon Isoflavones on Bone-Related Traits and Lens Protein Characteristics of Ovariectomized Rats and Bioactivity Performance of Osteoprogenitor Cells

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Health benefits of soy isoflavones have attracted the concern of the public and the interest of health-care professionals. In this study, two trials were conducted in characterizing bone-related traits and lens proteins as affected by supplementation of soy aglycon isoflavones (SAI). In trial 1, an in vivo study, 20 Sprague−Dawley rats were ovariectomized (OVX) and randomly distributed into OVX and OVX+SAI (135 mg of SAI/kg of feed; 8.33 mg/kg body weight; 2.5 mg/day) groups. Another group containing 10 rats with a sham operation was control (Sham). The experiment period was 3 months, and the rats were subjected to bone-related traits and lens protein characterization. In trial 2, an in vitro study, osteoprogenitor cells (UMR-106) were divided into SAI-supplemented (0.5 mg of SAI/mL of medium) and unsupplemented groups. Results of the in vivo study indicated that daily BW gains in the OVX and OVX+SAI groups were greater than that of the Sham group ($p < 0.05$). Bone ash and Ca contents of the Sham and OVX+SAI groups were higher than those of the OVX group ($p < 0.05$), while bone density, strength, and phosphorus contents among groups varied insignificantly ($p > 0.05$). When the lens proteins were extracted and analyzed with size-exclusion HPLC, the contents of β- and γ-crystallins were lowest in the OVX group and the protein solubility decrease could be recovered by dietary SAI supplementation (shown by OVX+SAI group). Based on Raman spectra of the isolated lens proteins, disulfide bonds were observed more in OVX lens than in the Sham and OVX+SAI lens. Results of in vitro study with osteoprogenitor cells revealed that cell viability, alkaline phosphatase activity, osteocalcin, and Ca contents of the SAI-supplemented group were higher than those of the unsupplemented group ($p < 0.05$). The likely potency to enhance bone and lens health by SAI supplementation is worth pointing out.

KEYWORDS: Soy aglycon isoflavones; bone-related traits; lens proteins; osteoprogenitor cells; osteocalcin; bone calcium

INTRODUCTION

It has been demonstrated that sex hormones play an important role in normal bone tissue metabolism and that their insufficiency or deficiency may lead to bone loss in both human genders (*1*, *2*). For postmenopausal women, estrogen deficiency may increase bone turnover and result in loss of bone mass and consequent osteoporosis (*1*). Ishimi et al. (*3*) reported that estrogen deficiency may facilitate bone marrow hemopoiesis and result in selective accumulation of pre-β-lymphocytes in the bone marrow. They also reported that increase of lymphopoieisis of mice is closely related to mass reabsorption of trabecular bones.

In comparison, age-related bone loss is generally more serious for women than for men. An initial phase of rapid bone loss usually develops in the initial 10 years when women step into the menopausal period after cessation of menses or when women have the ovaries surgically removed. The caused hormone deficiency may increase bone turnover rate, leading to an imbalance between reabsorption and bone formation and resultant bone loss and osteoporosis (*4*). To improve bone loss due to estrogen deficiency, hormone replacement therapy (HRT) is a popular treatment to slow bone loss progression and to decrease the risk of fractures, including hip fracture (*5*). However, prolonged estrogen therapy may result in some undesirable side effects and increase the risk of endometrial and breast cancer incidence (*6*, *7*).

Phytoestrogen is a natural plant origin chemical with structural similarity to estrogen and functions as a competitor to bind estrogen receptor (*8*, *9*). The binding affinity of phytoestrogen

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to the estrogen receptor is about 1/100 to 1/1000 of estradiol, and thus, phytoestrogen is usually recognized as a weak estrogen (*10*) or a selective estrogen receptor modulator (*11*). Based on the fact that estrogen administration for HRT may increase the incidence risk of uterus endometrial cancer (*12*), it is promising to use phytoestrogenic isoflavones in substitution of estrogen for chemoprevention of postmenopausal women syndrome. Soybeans are a rich source of phytoestrogenic isoflavones. Daidzein and genistein are the predominant components of soy isoflavones (*13*, *14*). In comparison, aglycon isoflavones are more easily absorbed by gut tract than are glycosidic isoflavones (*15*, *16*).

As equally important as osteoporosis, conformational change and solubility decrease of the lens proteins is also an aging-related syndrome. The lens is a lifelong growing organ with no shedding of cellular elements and continuous deposits of the posttranslationally altered proteins in the nucleus and newly synthesized proteins in the cortex. Lens proteins might progressively show conspicuous changes during aging and increase water-insoluble proteins as aggregates resulting from oxidation of sulfhydryl groups to disulfide bonds (*17*, *18*). Changes of the sulfhydryl groups could be monitored by Raman spectroscopy. In the literature, quality maintenance of lens proteins contributed by dietary supplementation of soy isoflavones has been meagerly investigated. In this study, ovariectomized rats were used as a model for postmenopausal investigation addressed to bone-related traits and lens protein characterization. Supplementation of soy aglycon isoflavones mainly including daidzein and genistein was conducted. The investigation was extended to an in vitro experiment with an osteoprogenitor cell line (UMR-106) addressed to determinations of various cellular activities as affected by supplementation of the soy aglycon isoflavones.

MATERIALS AND METHODS

Ovariectomization of Rats. Sprague−Dawley rats were ovariectomized (OVX) when they were sexually mature (11 weeks old). For each rat, the abdomenal area was sterilized with 75% ethanol and opened by a surgical operation. The uterus and ovary parts were taken out, and only the ovary was ligated and cut off. Then, the uterus and adipose tissue were put back into the abdomen and sewn up. For the sham rats, their abdomens were just opened by surgery and then sewn up. Rats were raised with normal practice for an additional 2 months for recovery prior to initiation of the following experiments. Handling and killing of the rats were in full accordance with the Institutional Animal Care and Use Committee (IACUC) guidelines for the care and management of laboratory animals.

Trial 1. In Vivo Study: Animal Treatment. Thirty Sprague−Dawley rats were randomly assigned to one of three groups, i.e., the positive control (OVX) group, the treatment group (OVX+SAI), and the negative control (Sham) group containing 10 sham rats. The OVX and Sham groups were fed a basal diet (**Table 1**). The rats for the OVX+SAI group were fed a basal diet supplemented with 135 mg of soy aglycon isoflavones (SAI)/kg of feed (equivalent to 8.33 mg/kg body weight (BW) and 2.5 mg/day per rat) (SAI, a popular dietary supplement, comprises 80% soybean flour base, 4.5% daidzein, 14.5% genistein, and 1.0% other isoflavones, w/w) (Glory Biotech Co., Chiayi, Taiwan). The supplemented equivalency was estimated mainly according to the dose of 80 mg of isoflavones per day for a 60-kg human recommended by Alekel et al. (*19*) [80 mg/60 kg × 6.25 (a conversion factor) = 8.33 mg/kg BW (dose for rats); 8.33 mg/kg × 0.3 kg (BW of rats) = 2.5 mg/day; 2.5 mg/0.0185 kg (average daily feed intake) = 135 mg/kg feed (dietary level)]. Water and feed were freely accessible, the ambient temperature was 25 °C, and light/dark was set at a 12 h cycle. The rats were housed in individual cages for 3 months.

Bone-Related Traits and Lens Protein Characterization. At the end of the experiment, rats were anesthetized by CO_2 and sacrificed.

Table 1. Composition of the Basal Diets Used in This Experiment

ingredients	content, g/kg
casein	200.0
sucrose	500.0
corn starch	150.0
fiber	50.0
corn oil	50.0
DL-methionine	3.0
AIN vitamin premix[a]	10.0
AIN mineral premix[b]	35.0
choline bitartarate	2.0
total weight	1000.0
calculated values	
crude protein, %	18.78
metabolizable energy, MJ/kg	12.72
lysine, %	1.49
methionine, %	0.59
Ca, %	0.50
P, %	0.39

[a] AIN vitamin mixture ingredient: vitamin A, 22.01 IU/g; vitamin D3, 4.5 IU/g; vitamin E, 49.01 IU/g; carotene, 4.5 ppm; vitamin K, 0.5 ppm; thiamin, 17.2 ppm; riboflavin, 8.0 ppm; niacin, 123.7 ppm; pantothenic acid, 24.0 ppm; folic acid, 5.9 ppm; pyridoxine, 6.0 ppm; biotin, 0.2 ppm; B12, 22.0 mcg/kg. [b] AIN mineral mixture ingredient: $CaHPO_4$, 50%; NaCl, 7.4%; $K_3C_6H_5O_7 \cdot H_2O$, 22%; K_2SO_4, 5.2%; MgO, 2.4%; $MnSO_4$, 0.35%; $FeSO_4$, 0.6%; ZnO, 0.16%; $CuSO_4$, 0.03%; KIO_3, 0.001%; $Na_2SeO_3 \cdot 5H_2O$, 0.001%; $CrK(SO_4)_2 \cdot 12H_2O$, 0.055%.

Legs were taken off and boiled in a water bath for 5 min, and muscle tissue was entirely removed. Then, leg bones (tibia) were air-dried and stored at −20 °C for the following analyses. Conducted concurrently, the rat eyes were removed for lens protein characterization.

Bone density was measured by dividing bone weight by bone volume. Bone volume was determined by deposition of the bone samples into a 10 mL measuring cylinder containing 6 mL of water to measure the volume increase after deposition. Bone strength was examined by a texture analyzer (TA-XT 2, Haslemere, England). Bone ash, Ca, and phosphorus contents were determined following AOAC methods (*20*).

Preparation of Lens Proteins. All treatments of the lens samples were conducted in a cold room (ca. 4 °C). For each analysis, four intact lenses were taken from two rats in the same group, weighed, frozen with liquid nitrogen, lyophilized, and ground into powder. Then, a precisely weighed 0.145 g of lens powder was homogenized with 2.0 mL of 0.1 M phosphate buffer solution (pH 6.8) and subjected to centrifugation (13500*g*) at 4 °C for 30 min. The transparent and soluble portion was collected and subjected to high-performance liquid chromatographic (HPLC) analysis.

Size-Exclusion HPLC of Lens Proteins. Analysis of lens proteins were carried out with a TSK G3000 SW column (30 cm × 8 mm) and a TSK gel SW guard column (4 cm × 8 mm) (Toyo Soda, Tokyo, Japan) and equipped with a Hitachi D-7000 HPLC system (Hitachi, Ltd., Tokyo, Japan) containing a dual pump (model L-7100), an ultrasonic degasser (model 2510, Branson Ultrasonic Co., Danbury, CT), a UV−vis detector (model L-7420), and a Rheodyne injector (model 7725). Peaks were detected at 280 nm. Acquisition and processing of data were completed by Hitachi B-7000 software with an analog/digital interface. An isocratic mobile phase was 0.1 M sodium phosphate buffer solution (pH 6.8) containing 0.05% sodium azide. Each sample was filtered using 0.45 μm sterile units (Millipore Co., Bedford, MA). Injection volume and flow rate were 15 μL and 0.6 mL/min, respectively. A typical analysis could be finished in 40 min.

Raman Spectrometry of Lens Proteins. Fourier transform (FT) Raman spectra of the lens proteins were obtained by using a Bruker RFS-100 FT spectrophotometer (Bruker Optik GmbH, Lubeck, Germany). Fresh lens was directly put into the reflected quartz cell with a stainless steel holder for Raman monitoring. Continuous near-infrared wave excitation at 1064 nm was provided by a diode laser pumped Nd:YAG laser (Coherent Lubeck GmbH, Lubeck, Germany). Laser light with a power of 150 mW was introduced and focused on the sample.

The scattered radiation was collected at 180° with an ellipsoidal mirror and was filtered, modulated, and reflected back into the highly sensitive GaAs detector that had been cooled by liquid nitrogen. Raman spectra were produced over the Raman shift 0−3500 cm^{-1}. Typically, 1000 interferograms were co-added at 4 cm^{-1} resolution with a sampling period of about 30 min. FT-Raman spectra reported in this study were all original, not smoothed, normalized, or baseline corrected through data manipulation.

Trial 2. In Vitro Study. An osteoprogenitor cell line UMR-106 (ATCC CRL-1661) was used. Aliquots of 1.56×10^4 cells/mL were distributed into a series of flasks and randomly assigned to SAI-supplemented (0.5 mg of SAI/mL of medium) and unsupplemented (control) groups. The basal medium was DMEM (Dulbecco's modified Eagle's medium/Ham's HEPES, 1:1, pH 7.2) supplemented with 2 mg/mL sodium bicarbonate, 100 U/mL penicillin, 100 μg/mL streptomycin, 2.5 μg/mL amphotericin, and 10% BCS (bovine calf serum). Cell proliferation rates were determined by direct counting of the cells after 3 days of incubation. When the cells were grown about 90% confluent, cell differentiation was induced for 3 days by addition of 250 μM L-ascorbic acid, 100 nM of dexamethasone, and 10% BCS. Then a prolonged incubation for cell differentiation was conducted for an additional 16 days. During incubation, the medium was changed every 3 days. After incubation, the medium was withdrawn and cells were harvested by a cell scraper. The cells were quickly frozen by immersion into liquid nitrogen for 10 min, thawed at room temperature, ultrasonicated for 10 min, and centrifuged at 3000*g* for 10 min. The supernatant was collected and stored at −20 °C for later analyses.

Biochemical Determinations. Cell survival ability was examined by using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method described by Fernandes et al. (*21*). Cell suspensions of the test groups were seeded into a 96-well plate and incubated for 2 days. Then, 0.5 mg/mL MTT was added. After withdrawing and discarding the medium, 150 μL of DMSO was added and absorbance at 490 nm was determined by an ELISA reader (Multiscan Ascent, Helsinki, Finland). The secreted osteocalcin contents were determined by an ELISA kit (DSL-D-7600, Active Inc., Dallas, TX). The protein levels of the media before and after incubation were determined by the Lowry method (*22*).

The differentiated cell number was viewed and examined with an invert microscope. Six fields containing ca. 500 cells were selected, examined, and counted. Cell calcium and phosphorus concentrations were determined by a kit (Roche Co., Basel, Switzerland). Alkaline phosphatase (ALP) activity was determined following the method described by Federici et al. (*23*). Briefly, cell samples were combined with 150 μL of *p*-nitrophenyl phosphate solution (containing 13 mM *p*-nitrophenyl, 0.1 M AMP, and 10 mM $MgCl_2$, pH 10) prior to absorbance determination at 405 nm with an ELISA reader. The reaction time was 30 min, and each activity unit was defined as release of 1 pmol of nitrophenol per min.

Statistical Analysis. Experimental data were subjected to statistical analyses by using SAS (statistical analysis system) software for variance analysis (*24*). Significant differences among groups were determined by Duncan's New Multiple Range Test in trial 1 and the *t* test in trial 2, respectively.

RESULTS AND DISCUSSION

The effects of SAI on bone traits are shown in **Table 2**. In comparison, averaged daily BW gains in the OVX and OVX+SAI groups were greater than that of the Sham group ($p < 0.05$). Apparently, BW gain was related to OVX (ovariectomy). Feed intake in the ovariectomized rats is regulated by the hypothalamic−pituitary−gonadal system (*25*). Stimulation of feed intake and resultant increase of BW gain, mainly due to shortage of estrogen secretion, have been observed in the ovariectomized rats (*26*, *27*). For rats, feed intake and BW gain decreased when diets were supplemented with estrogen (*28*). In this study, based on the fact that BW gain of the OVX+SAI group was greater than that of the Sham group (**Table 2**), the supplemented level of SAI to compensate ovarian estrogen to decrease BW gain of the OVX rats was not observed.

Table 2. Effect of Dietary Supplementation of Soy Aglycon Isoflavones on Bone Traits of Rats

item	treatment and determination[a]		
	Sham[b]	OVX[b]	OVX+SAI[b]
daily body weight gain, g	0.56 ± 0.16 B	0.83 ± 0.23 A	0.83 ± 0.20 A
bone density, g/mL	6.32 ± 0.53 A	6.66 ± 0.36 A	6.75 ± 0.81 A
bone strength, g	6213 ± 933 A	6147 ± 1377 A	6071 ± 1261 A
bone ash, %	48.01 ± 3.19 A	45.97 ± 1.01 B	48.82 ± 2.83 A
bone ash Ca, %	16.58 ± 2.25 A	13.45 ± 1.95 B	15.41 ± 2.13 A B
bone ash P, %	9.33 ± 0.41 A	9.61 ± 0.83 A	9.59 ± 0.84 A

[a] Means of determinations with standard deviation ($n = 10$) are significantly different when followed by different letters in the same row ($p < 0.05$). [b] Sham, rats with sham operation as a negative control; OVX, ovariectomized rats as a positive control; OVX+SAI, supplementation with soy aglycon isoflavones.

Bone density, strength, and bone ash P (phosphorus) contents among the Sham, OVX, and OVX+AVI groups were not significantly different ($p > 0.05$) (**Table 2**). In comparison, bone ash and bone ash Ca contents of the OVX group were lower than those of the Sham group ($p < 0.05$). This implies that the OVX rats might have had more bone loss due to ovariectomy. As noticed, bone ash and bone ash Ca contents of the OVX+SAI group were greater than those of the OVX group ($p < 0.05$). Estrogen has been demonstrated to be effective in protection from bone loss for postmenopausal women (*29*) and ovariectomized rats (*30*, *31*). It is of merit to point out the likelihood that diets supplemented with SAI have rendered estrogen effectiveness in protection of bones from Ca loss for the OVX rats. Uesugi et al. (*32*) reported that bone density and strength of rats are improved by diets supplemented with soy daidzein, genistein, and glycitin. Breitman et al. (*33*) further reported that the bone density and bone biomechanical strength of ovariectomized rats have been better protected by diets supplemented with both soy isoflavones and Ca than by diets supplemented with soy isoflavones alone.

Since bone metabolism is slow, it usually takes a long time for bone loss to become an apparent defect or syndrome of bone density and strength. In this study, based on the fact that the OVX+SAI group did not exhibit marked bone loss as shown by bone density and strength (**Table 2**), it is likely that the experimental period of 3 months may not be long enough to differentiate bone loss as affected by diets supplemented with SAI. Nevertheless, in the test period, bone ash and bone ash Ca contents in the OVX+SAI group are shown to be higher than those of the OVX group. This was in agreement with the results of Arjmandi and Smith (*34*), who reported that soy isoflavones increase intestinal absorption of Ca and, consequently, increase bone Ca and matter contents.

Lens is a lifelong growing organ with no shedding of cellular elements. Lens proteins may progressly show conspicuous changes during aging and increase water-insoluble protein aggregation as a result of continuous oxidation of the sulfhydryl groups to form disulfide bonds. Spectroscopic studies of eye lens proteins have proved that Raman spectroscopy is capable of detecting directly the formation of disulfide bonds and may serve as a noninvasive tool to monitor lens development (*17*, *18*). In this study, the HPLC proteins including high-molecular-weight (HMW) proteins and various crystallins (*35*) have been resolved and are shown in **Figure 1**. Apparently, in comparison to the Sham group, contents of β- and γ-crystallins were lower in the OVX group. Correlated with the fact that bone ash and ash Ca contents were observed to be lower in the OVX group

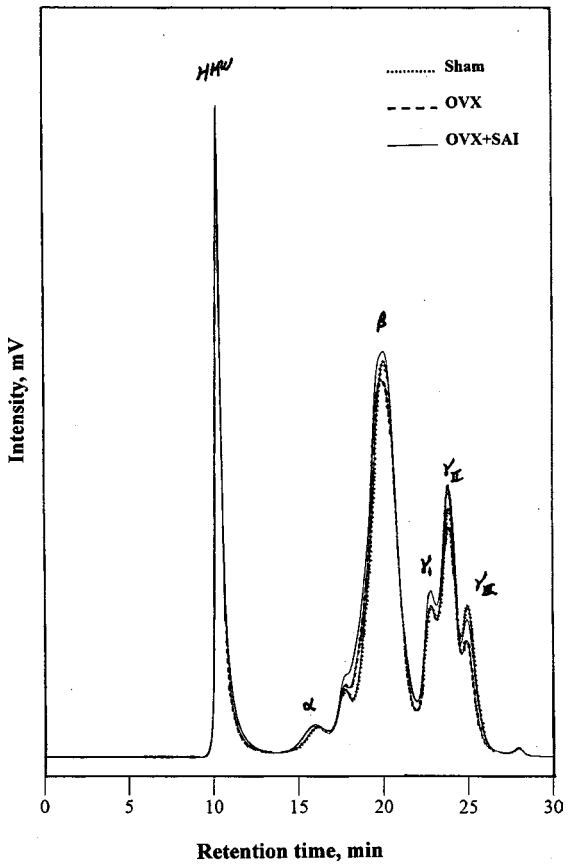

Figure 1. HPLC protein patterns of the lens proteins extracted from rats as affected by supplementation of soy aglycon isoflavones. HMW, high-molecular-weight proteins.

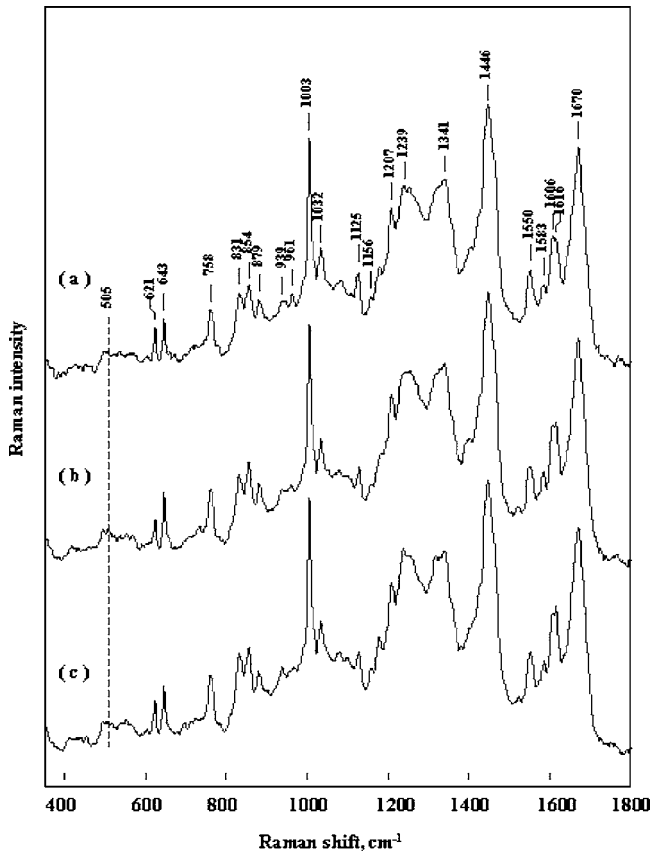

Figure 2. FT-Raman spectra of the lens proteins extracted from rats as affected by supplementation of soy aglycon isoflavones: (**a**) Sham, rats with sham operation as a negative control; (**b**) OVX, ovariectomized rats as a positive control; (**c**) OVX+SAI, supplementation with soy aglycon isoflavones.

than those in the Sham group (**Table 2**), the lower contents of β- and γ-crystallins imply that OVX treatment may accelerate rat aging. This was in agreement with the report of Uchiumi et al. (*35*), who have observed marked changes of lens crystallins after aging. In comparison of the OVX+SAI and SAI groups, β- and γ-crystallin contents except γ_{III}-crystallin were higher in the former than in the latter. It is worth pointing out the observation that loss of β-, γ_I-, and γ_{II}-crystallins in the OVX rats could be recovered by dietary SAI supplementation. The reason for the distinct behavior of γ_{III}-crystallin is not clear and needs to be elucidated.

FT-Raman spectra of rat lens proteins for the Sham, OVX+SAI, and OVX groups are shown in **Figure 2**. A distinct S−S stretching at 505 cm^{-1} was observed in the OVX group (**Figure 2b**), which might enhance protein aggregation, a resultant phenomenon of aging (*17*), as observed in the HPLC protein patterns (**Figure 1**). As a further comparison was made among the spectra, there remains no significant change in the secondary structure (antiparallel β-pleated sheet) of lens proteins as evidenced by the identical Raman vibrational stretching of amide I and amide III at 1672 and 1239 cm^{-1}, respectively.

Effects of SAI supplementation on proliferation of the osteoprogenitor cells are shown in **Table 3**. In general, cell proliferation was not affected by SAI supplementation. This was in agreement with Kanno et al. (*36*), who reported that soy aglycon isoflavones did not influence proliferation of the osteoprogenitor cells. In further comparison, cell survival ability and alkaline phosphatase (ALP) activity of the SAI-supplemented group were greater than those of the unsupplemented group ($p < 0.05$). This was in agreement with the report of Jia et al. (*37*), who used the MTT method to determine cellular

Table 3. Effect of Media Supplemented with Soy Aglycon Isoflavones on Proliferation of Osteoprogenitor Cells (UMR-106)

item	treatment and determination[a]	
	without SAI	with SAI
proliferated cells, 10^5/mL	4.07 ± 1.57 A	3.93 ± 1.21 A
relative cell viability,[b] %	100 B	111.26 ± 9.11 A
cells ALP, pmol/min/μg of protein	205.93 ± 24.25 B	374.50 ± 44.76 A

[a] Means of determinations with standard deviation ($n = 9$) are significantly different when followed by different letters in the same row ($p < 0.05$). SAI, soy aglycon isoflavones. [b] The values are expressed as proportional percentage of the unsupplemented (control) group as 100%.

survivability and reported that cellular survivability is enhanced by soy isoflavones. Enhanced survival activity and life span of the osteoprogenitor cells might subsequently render increase of bone life or slowing of aging. ALP is an enzyme related to bone mineralization; the increased activity might stimulate bone mineralization and slow down bone loss.

Effects of SAI supplementation on differentiation and cellular metabolite contents of the osteoprogenitor cells are shown in **Table 4**. The osteocalcin concentrations and cell Ca levels in the SAI-supplemented group were greater than those in the unsupplemented group ($p < 0.05$). Normally, during differentiation of the osteoprogenitor cells, secretion of specific proteins, such as collagen, is initiated to form a matrix structure and is followed by deposition of Ca and P for calcification (*38*). Eventually, the osteoprogenitor cells become mature to form osteo cells. As noticed in this study, it is likely that soy isoflavones have rendered effectiveness in the enhancement of

Table 4. Effect of Media Supplemented with Soy Aglycon Isoflavones on Differentiation of Osteoprogenitor Cells (UMR-106)

item	treatment and determination[a]	
	without SAI	with SAI
differentiated cells, %	39.07 ± 0.08 A	36.84 ± 0.05 A
osteocalcin, pg/mL	0.088 ± 0.015 B	0.108 ± 0.091 A
secreted protein, μg/mL	2115.35 ± 97.41 A	2023.56 ± 156.67 A
cell Ca, mg/dL	0.29 ± 0.09 B	1.51 ± 0.16 A
cell P, mg/dL	1.30 ± 0.06 A	1.23 ± 0.07 A
cell ALP,[b] pmol/min/μg of protein	526.75 ± 120.33 A	612.05 ± 90.45 A

[a] Means of determinations with standard deviation ($n = 9$) are significantly different when followed with different letters in the same row ($p < 0.05$). SAI, soy aglycon isoflavones. [b] The values are expressed as proportional percentage of the unsupplemented (control) group as 100%.

osteoprogenitor cell calcification and bone formation to slow down bone loss. This was also supported by the activity of ALP, an enzyme involved in mineralization of the osteoprogenitor cells which was observed bearing higher activity by the SAI-supplemented group than by the unsupplemented group ($p < 0.05$) (**Table 3**). The likelihood was also supported by Chen et al. (*39*), who reported that genistein is active to bind estrogen receptor β and stimulate bone formation.

In conclusion, as shown by an in vivo study conducted with the Sham and OVX rats, bone ash and bone ash Ca levels of the latter were lower than those of the former. Dietary supplementation of SAI for the OVX rats was effective in maintaining bone ash and Ca levels. As characterized by HPLC chromatography and Raman spectroscopy of the lens proteins, the aging-related soluble crystallins decreased and disulfide bonds increased for the OVX rats in comparison to the Sham rats. The analyzing patterns and spectra further supported the possibility that decrease of soluble crystallins and increase of disulfide bonds could be improved by dietary SAI supplementation. As studied in in vitro trials with an osteoprogenitor cell line, cell survivability, ALP activity, osteocalcin secretion, and Ca deposition were enhanced by supplementation of SAI in the media. It is of merit to demonstrate the effectiveness to enhance bone health by SAI supplementation and increase bioactivities of the osteoprogenitor cells.

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