Chinese Journal of Physiology 57(3): 121-127, 2014
DOI: 10.4077/CJP.2014.BAC199

Effects of Chronic Treatment with Diosgenin on Bone Loss in a D-Galactose-Induced Aging Rat Model

Ying-Tzu Hung[1, #], Maria A Tikhonova[2, #], Shinn-Jyh Ding[3, 4], Pan-Fu Kao[5, 6], Howard Haw-Chang Lan[7, 8, 9], Jiuan-Miaw Liao[10], Jian-Horng Chen[11], Tamara G Amstislavskaya[2], and Ying-Jui Ho[1, 12]

[1]*School of Psychology, Chung Shan Medical University, Taichung City 40201*
[2]*Laboratory of Biological Psychiatry, State Research Institute of Physiology and Fundamental Medicine SB RAMS, Novosibirsk 630117, Russia, and*
[3]*Institute of Oral Science, Chung Shan Medical University, Taichung City 40201*
[4]*Department of Dentistry, Chung Shan Medical University Hospital, Taichung City 40201*
[5]*Department of Nuclear Medicine, School of Medicine, Chung Shan Medical University Taichung City 40201*
[6]*Molecular Imaging Laboratory, Chung Shan Medical University Hospital Chung Shan Medical University, Taichung City 40201*
[7]*Department of Radiology, Taichung Veterans General Hospital, Taichung City 40201*
[8]*School of Radiological Technology, Central Taiwan University of Science and Technology Taichung City 40601*
[9]*School of Physical Therapy, Hungkuang University, Taichung City 403302*
[10]*Department of Physiology, Colleges of Medicine, Chung Shan Medical University, Taichung City 40201*
[11]*School of Physical Therapy, Chung Shan Medical University, Taichung City 40201 and*
[12]*Chun Shan Medical University Hospital, Chung Shan Medical University, Taichung City 40201 Taiwan, Republic of China*

Abstract

D-galactose is known to cause oxidative stress and induce aging-related diseases. Our previous study demonstrated that diosgenin can prevent osteoporosis in menopausal rats. The aim of the present study was to determine the effects of oral administration of diosgenin on bone loss in a D-galactose-induced aging rat model. Three groups of twelve-week-old male Wistar rats received a daily injection of D-galactose (150 mg/kg/day, i.p.) and orally administered diosgenin (0, 10, or 50 mg/kg/day) for eight weeks, while a control group received saline injection (1 ml/kg/day, i.p.), then the femurs were taken to measure mechanical and morphological properties. The results showed that frame volume and femur volume decreased and porosity and frame density increased in the D-galactose-induced aging rats compared to controls and that these effects were prevented by co-administration of diosgenin. This suggests that diosgenin might prevent bone loss during aging and provide beneficial effects in osteoporosis in the elderly.

Key Words: aging, bone loss, D-galactose, diosgenin, osteoporosis

Corresponding authors: [1] Ying-Jui Ho, Ph.D., School of Psychology, Chung Shan Medical University Hospital, Chung Shan Medical University, No. 110, Sec. 1, Jianguo N. Rd., Taichung City 40201, Taiwan, R.O.C. Tel: +886-4-24730022 ext. 11858; 11288, Fax: +886-4-23248191, E-mail: yjho@csmu.edu.tw; joshuayjho@yahoo.com.tw; joshuayjho@gmail.com; [2] Jian-Horng Chen, Ph.D., School of Physical Therapy, Chung Shan Medical University, No. 110, Sec. 1, Jianguo N. Rd., Taichung City 402, Taiwan, R.O.C. Tel: +886-4-24730022 ext. 12391, Fax: +886-4-23248176, E-mail: jhchen@csmu.edu.tw; and [3] Tamara G. Amstislavskaya, Ph.D., Sc.D., Laboratory of Biological Psychiatry, State Research Institute of Physiology and Fundamental Medicine SB RAMS, Novosibirsk, Russia. P.O. Box 237, Novosibirsk 630117, Russia. Tel: +7-383-335-98-01, Fax: +7-383-335-97-54, E-mail: amstislavskaya@yandex.ru
[#]Contributed to this paper equally.
Received: January 31, 2013; Revised: May 4, 2013; Accepted: August 12, 2013.
 ISSN : 0304-4920. http://www.cps.org.tw

Introduction

Aging is associated with a reduction in mineralization and increase in porosity in cortical and trabecular bones, which results in bone loss and increased risk of osteoporosis and fracture. Osteoporosis is one of the most prevalent and serious diseases in the elderly. Oxidative stress and reactive oxygen species (ROS) have been proposed as major causes of aging (38, 48) and are involved in inflammatory arthritis (30) and age-related bone loss (57). Thus, age-related bone loss might be alleviated by antioxidant treatment.

D-galactose, a reducing sugar, stimulates the production of free radicals, causes accumulation of ROS (56), and decreases the activity of antioxidant enzymes *in vivo* (49), resulting in oxidative stress and aging-related changes (54). Because of this, animals chronically injected with D-galactose have been used for pharmacological studies of aging (49).

Antioxidants can scavenge free radicals and protect cells and organs from oxidative damage. Among the natural antioxidants, dioscorea (wild yam) is worthy of note. Dioscorea, a common food and Chinese medicine (34) that contains phytosteroids, such as diosgenin and steroidal saponins (25), decreases anxiety levels and inflammatory cytokine levels in the brain of menopausal rats (27) and has anti-osteoporotic activity (10, 53). Diosgenin, the main steroidal saponin in dioscorea, has a similar chemical structure to sex hormones and is used as a precursor in the manufacture of steroid hormones, such as estrogen, progesterone, testosterone, and cortisol (17, 41). In addition, diosgenin has antioxidant and free radical scavenging activity in rats (44), prevents H_2O_2-induced apoptosis in human vein endothelium cells (20), shows anti-aging effects in menopausal animals (26) and in human chronic myelogenous leukemia K562 cells (33), and improves epidermal functions in aging mice (46). Our previous study showed that dioscorea increases bone strength in menopausal rats (10). Further studies are needed to determine the effect of diosgenin on bone loss in aging. Although rodents chronically injected with D-galactose have been used as an animal aging model for osteoporosis (39) and for anti-aging pharmacology research (49), to our knowledge, this is the first study reporting the anti-osteoporotic effects of diosgenin on morphological and mechanical properties of bone in D-galactose-induced aging rats.

Materials and Methods

Animals

Twelve-week-old male Wistar rats (387 ± 3.9 g; National Laboratory Animal Center, ROC) were housed in groups of five in acrylic cages (35 × 56 × 19 cm) in an animal room with a 12 h light-dark cycle (lights on at 07:00 h) with food and water available *ad libitum*. Each animal was handled for 5 min/day on 3 consecutive days, starting one day after arrival. All experimental procedures were performed according to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care Committee of Chung Shan Medical University (IACUC approval No.: 1018).

General Procedure

Experimental rats received peritoneally injection with D-galactose (150 mg/kg/day, i.p.), while control rats received injection with saline (1 mg/kg/day, i.p.), for 8 weeks. The experimental rats were co-administered with 10, or 50 mg/kg/day of diosgenin (Sigma, St. Louis, MO, USA) orally. The diosgenin was wrapped in a ball of raw dough (about 1 g, as vehicle) and fed daily to each rat. The control group was fed the same amount of raw dough without diosgenin. After the experiment, all the rats were sacrificed by exposure to CO_2 and transcardially perfused with phosphate-buffered saline.

Preparation of Femurs and Determination of Morphometric and Mechanical Properties

During removal of the muscle and the fibrous periosteum, the femurs were kept wet in distilled water. After defatting in chloroform (43) and drying, the right femur was used to sequentially measure the morphometric parameters of wet weight (Ww), total volume, and dry weight to calculate the porosity. The left femur was kept at –20°C until mechanical testing. A previous report has shown that drying, rewetting, and freezing have no effect on the mechanical properties of bone (15). We have previously reported the methods for measuring bone parameters (10), and briefly describe as the followings.

Measurement of the Ww. The right femur was placed in an unstoppered glass vial containing distilled water which was placed in a vacuum desiccator for 90 min to remove air diffusing out of the bone (28). After gently wiping off the water on the surface of the specimen, the femur was weighed on an analytical balance (AE240-S, Mettler, Zurich, Switzerland) to obtain the Ww.

Measurement of the total volume. According to the theory of porous media (6), the porous structure of the bone consists of the 'solid skeleton' (bone frame volume; V_B) and the 'interstitial fluid' (void volume; V_P) (50). Instead of using a conventional caliper or mathematical equations, we have, by combining

Table 1. Effect of diosgenin on mechanical and morphological properties of the femur in a D-galactose-induced aging rat model

	Control	D-galactose-treated		
	diosgenin 0 mg/kg/day (n = 10)	diosgenin 0 mg/kg/day (n = 11)	diosgenin 10 mg/kg/day (n = 11)	diosgenin 50 mg/kg/day (n = 12)
Mechanical Properties				
Stiffness (N/mm)	148.7 ± 7.3	158.0 ± 7.3	163.8 ± 4.0	142.5 ± 6.6
Toughness (N • mm)	97.4 ± 5.7	97.7 ± 5.8	107.4 ± 4.8	91.0 ± 4.2
Ultimate force (N)	167.9 ± 6.7	168.1 ± 5.7	166.3 ± 5.5	160.3 ± 6.5
Ultimate displacement (mm)	1.17 ± 0.03	1.16 ± 0.03	1.23 ± 0.03	1.16 ± 0.02
Morphological Properties				
Dry weight (g)	0.77 ± 0.02	0.76 ± 0.02	0.79 ± 0.02	0.75 ± 0.02
Wet weight (g)	0.96 ± 0.02	0.97 ± 0.02	0.97 ± 0.02	0.93 ± 0.03
Pore volume (cm^3)	0.19 ± 0.01	0.20 ± 0.01	0.18 ± 0.03	0.19 ± 0.01
Frame density (g/ml)	1.66 ± 0.04	1.81 ± 0.04**	1.60 ± 0.04$^{\#\#\#}$	1.65 ± 0.06$^{\#\#}$
Femur density (g/ml)	1.19 ± 0.01	1.21 ± 0.01	1.17 ± 0.02$^{\#\#}$	1.17 ± 0.01$^{\#\#}$

**$P < 0.01$ compared to the controls. $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ compared to the D-galactose/0 mg/kg/day diosgenin group. The data are expressed as the mean ± SEM.

Newton's third law and Archimedes' principle, developed a novel and accurate method to directly measure the total volume (V_T; $V_T = V_B + V_P$; cm^3) of the femur (32, 52). Briefly, each right femur was suspended by a thin silk yarn and fully immersed in water in a beaker on the analytical balance and the buoyant force ($\bar{B}$; g) was read directly from the balance display, then the total volume, V_T, of the femur was calculated as V_T = buoyant force/0.9971, where 0.9971 is the density (g/ml) of distilled water at 25°C and 1 atmosphere (58).

Measurement of the dry weight. After measuring the total volume, each right femur was placed in an incubator at 50°C for 72 h to remove the interstitial fluid and was weighed at 1 h intervals until a constant weight (< 0.05% change) was obtained and taken as the dry weight (W_D).

Calculation of the morphometric parameters. Bone porosity was calculated as follows. In the saturated right femur, the void space was filled with W_f g of distilled water. The pore volume, V_P, was calculated using the equations $V_P = W_f/0.9971$ and $W_f = W_W - W_D$ and the porosity, P, calculated using the equation $P = V_P/V_T$. The frame density was calculated using the equation W_D/V_B, and femur density calculated using the equation W_D/V_T.

Evaluation of the mechanical properties. Before mechanical testing, the left femur was soaked in saline at room temperature for 12 h. A material testing system, EZ Test (Shimadzu, Kyoto, Japan), was used to perform the "three-point bending test using a span width of 15.7 mm and a rate of compression of 0.5 mm/min, and the relationship between load (force; N) and displacement (mm) was recorded at a sampling rate of 10 Hz. The slope of the linear section of the load-displacement curve gives the stiffness (N/mm). The force required to fracture the bone is the ultimate force (N), which causes the ultimate displacement (mm), and the toughness (N • mm), the work or energy needed to fracture the bone, was calculated as the area under the curve from zero displacement to ultimate displacement.

Data Analysis

Analysis of variance (ANOVA) followed by the least-significant difference (LSD) *post-hoc* test was used to analyze the data. All results are expressed as the mean ± SEM. The level of significance was defined as $P < 0.05$ (two-tailed).

Results

As shown in Table 1, D-galactose treatment did not affect the dry weight, wet weight, or pore volume of the femur or its mechanical properties, such as stiffness, toughness, ultimate force, and displacement, measured using the three-point bending test.

ANOVA with the LSD *post-hoc* test revealed that chronic administration of D-galactose significantly decreased the frame volume [$F(3,43) = 8.584$, $P = 0.001$] and femur volume ($F(3,43) = 3.436$, $P = 0.027$) and significantly increased the porosity [$F(3,43) = 5.621$, $P = 0.003$, Fig. 1] and the frame density [$F(3,43) = 5.740$, $P = 0.002$, Table 1] compared to controls (all

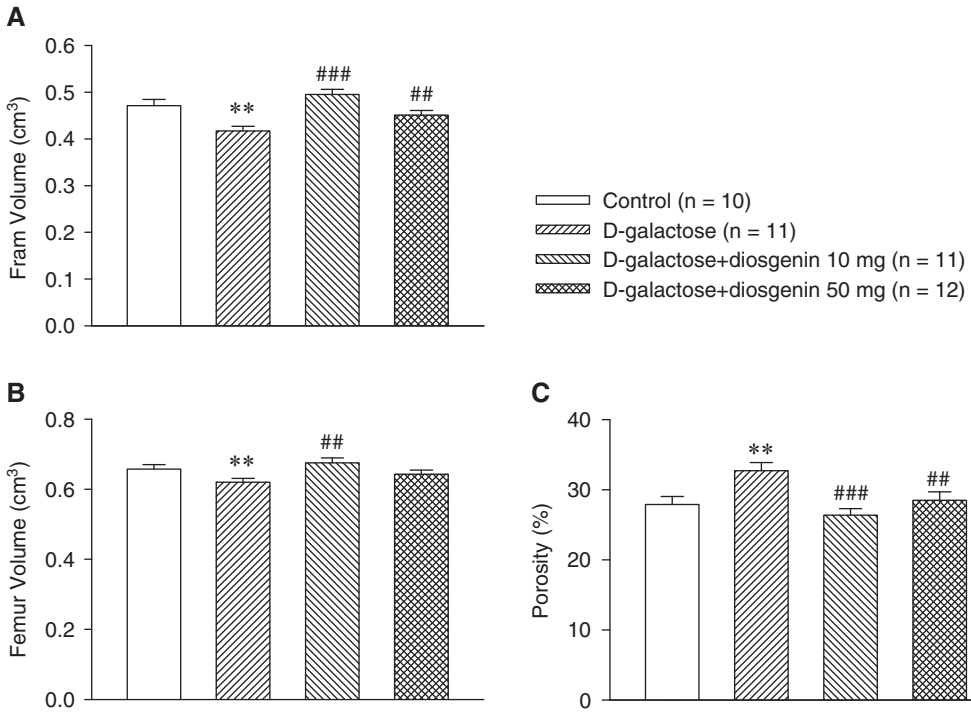

Fig. 1. Effect of diosgenin on the morphological properties of bone in a D-galactose-induced aging rat model. Diosgenin (10 or 50 mg/kg/day) was co-administered for 8 weeks. (A) frame volume, (B) femur volume, and (C) porosity. $^{**}P < 0.01$ compared to the controls. $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ compared to the D-galactose-treated group not treated with diosgenin. The data are expressed as the mean ± SEM.

P values < 0.05) and all effects were prevented by co-treatment with 10 or 50 mg/kg/day of diosgenin.

Discussion

Aging-related changes can be experimentally modeled by chronic administration of D-galactose in rodents (55). The D-galactose-induced aging model in rodents is established by daily injection of D-galactose at a dosage of 50-500 mg/kg/day for 6-10 weeks (9, 14, 29, 31, 45) and, to date, more than 100 papers have reported studies using this model. In the present study, we administered 150 mg/kg/day of D-galactose to Wistar rats for 8 weeks and noted a decrease in femur volume and frame volume and an increase in bone porosity and frame density, indicating induction of the aging animal model of bone loss. Co-administration of diosgenin at the dosages of 10 or 50 mg/kg/day prevented the D-galactose-induced bone loss, suggesting that diosgenin may have potential for preventing osteoporosis during aging. This is the first report of a change in frame density after D-galactose treatment and further studies are needed to elucidate the underlying mechanism.

It is difficult, by using the fluid-displacement method, to measure the volume of an irregular small object because the surface tension of the fluid causes difficulty of precisely observation of the displacement of fluid in a graduated cylinder. In order to conquer this problem, we developed the method in 2006 (52), which ingeniously combined Newton's third law and Archimedes' principle. The buoyant force of the fluid acting on the bone sample can be read directly from the balance display, so that the total volume of the femur can be calculated. The surface tension of the fluid will not interfere with the measurement of total volume. This method provides rapid, convenient, and accurate measure for the total volume of an irregular object. Based on this method (52), several researches have been published (10, 32, 51), indicating the reliability and validity of this method.

Bone quality is related to its mechanical and morphological properties, which determines fracture risk. In human long bone, aging causes trabecularization of cortical bone and leads to a reduction in cortical thickness and an increase in bone porosity. This process underlies osteoporosis. Baron (2) demonstrated a change in porosity with age, which is similar to the change in the risk of fracture reported in the literature (13, 16). Thus, frame volume and porosity are reliable indexes of bone quality.

Bone remodeling continues throughout life in response to hormonal and biochemical signals. In aging, increased bone resorption and/or decreased

bone formation lead to bone loss and increase the risk of osteoporosis and fractures, and prevention of age-related bone loss is therefore an important issue in the treatment of osteoporosis (4, 22). The progression of bone loss takes decades till fracture occurs. Clinically, age-related osteoporosis is usually diagnosed when the mechanical properties of limb bones change large enough and result in pathological fracture. Similarly, 8-week of D-galactose treatment in the present study caused no change in dry weight, wet way, pore volume, and bone mechanical properties, however, morphological compromise, decreasing of frame volume and increasing of porosity, was observed after the treatment. More profound changes of mechanical and morphological properties would occur after longer treatment of D-galactose, which will better model age-related osteoporosis. Although we have found beneficial effects of diosgenin in the 8-week-D-galactose model, effects of diosgenin in a longer D-galactose-treated model would provide further data for elucidating the potential of diosgenin in the clinic application. In the present study, the decreasing of femur volume and frame volume and increasing of porosity indicated bone loss and these changes may result from imbalance of bone resorption and formation. One may expect an increased excretion of Ca^{2+} in the urine, but no significant changes in Ca^{2+} level in the blood would be observed because of homeostasis of chemical levels in the blood. Detecting bone mineral content by dual-energy X-ray absorption (DEXA) scans and measurement of chemical levels in the urine will provide direct evidence for evaluating the mechanisms underlying the bone loss, which are needed in future investigations. Administration of D-galactose for 8 weeks resulted in morphological changes, namely a decrease in femur volume and frame volume and an increase in porosity and frame density, these being early bone changes during aging occurring before mechanical properties were affected. This observation is consistent with findings from animal models, in which activation of intracortical remodeling leads to an increase in intracortical porosity, but does not compromise bone strength (7). Further, it should be noted that, using regression analysis, a previous study showed that a 3% increase in porosity is equivalent to an increase in age of 14 years (35). The recovery of porosity and prevention of the decrease in femur volume and frame volume by diosgenin seen in our study suggest a therapeutic potential of this drug in osteoporosis.

Diosgenin, one of the important bio-active ingredients in dioscorea, has a similar chemical structure to sex hormones and has long been used as a precursor in the manufacture of steroid hormones, such as estrogen, progesterone, testosterone, and cortisol (41). In addition, diosgenin has anti-aging activity (33) and anti-oxidant activity and prevents H_2O_2-induced apoptosis (20). A previous study reported that co-administration of diosgenin in D-galactose-induced senescent mice increases the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) and decreases levels of malondialdehyde (MDA), suggesting that diosgenin has beneficial effects on aging and oxidative stress-related disorders (12). Similarly, our previous study demonstrated that dioscorea improves the morphometric and mechanical properties of bone in menopausal animals (10). Our recent study found an increase of alanin aminotransferase (GPT) level, an index of oxidative damage of liver cells, in the blood of rats receiving 8-week of D-galactose treatment, which was normalized by co-administration of diosgenin, at the dosage of 10 and 50 mg/kg/day (data not shown). We therefore propose that oxidative damage may play a role in bone loss in the aging rat model induced by D-galactose and that anti-oxidative action may be one of the mechanisms by which diosgenin decreases bone loss.

A decrease in blood levels of sex hormones is one of the causes of osteoporosis. Estrogen is known to be involved in prevention of bone loss, as postmenopausal women with estrogen deficits exhibit fast bone loss and osteoporosis (8). Androgens have anabolic effects on bone and increase the mineral density (19). Although nothing is known about the pathway by which diosgenin is converted to other hormones *in vivo*, sustained delivery of diosgenin for 45 days reverses menopause-induced hypertrophy of the adrenal gland, indicating an influence on the hormonal system (5). In addition, we have reported that chronic administration of diosgenin at the dosage of 10 mg/kg/day improves emotion and neuroimmune functions in menopausal arts (26). Thus, in addition to serving as a precursor of sex hormones *in vivo*, diosgenin may affect bone loss by regulation of the hormonal and/or immune systems.

Oxidative damage is involved in the pathophysiology of aging-related diseases (3). Oxidative stress and inadequate antioxidant defense mechanisms result in excessive ROS production (42). D-galactose, a reducing sugar, can be converted to advanced glycation end products (AGEs) which cannot be metabolized further and therefore accumulate in tissues. Deposits of AGEs suppress the proliferation and differentiation of osteoblasts, resulting in decreased bone formation. In addition, they cause release of cytokines and growth factors and facilitate the proliferation and differentiation of osteoclasts, leading to increased bone resorption (37). The generation of free radicals generated from oxidation of D-galactose exceeds the capacity of the cell to remove them and they cause injury to the cell membrane (24) and speed up the aging process (45, 47). The “free-radical theory of

aging" proposes that endogenous oxygen radicals are generated in cells and cause cumulative damage (23). Collagen is the most important component of bone, and free radicals disrupt its formation and reduce mineral deposits and bone formation, resulting in osteoporosis. Thus, oxidative stress and accumulation of free radicals may play an important role in aging-related bone changes (40, 57) and consumption of foods containing anti-oxidants might delay the aging process (1, 36). The defense mechanisms in the organism against oxidative stress include SOD, catalase, and GSH-Px (18, 21). Interestingly, diosgenin increases the activities of SOD and GSH-Px and has anti-oxidative and anti-aging activity (11). Thus, anti-oxidation may underlie the prevention of bone loss after diosgenin treatment in the D-galactose-induced aging rat model.

In conclusion, the present findings show that chronic administration of D-galactose in Wistar rats causes bone loss, a decrease in femur volume and frame volume, and an increase in bone porosity and frame density and that these effects are prevented by co-administration of diosgenin, which might have potential for preventing bone loss during aging.

Acknowledgments

This work was supported by grants from the National Science Council of the ROC (NSC 102-2410-H-040-004, NSC 100-2923-H-040-009-MY3, and NSC99-2221-E-040-006) and was partially supported by the budget project No. 053 of the State Research Institute of Physiology and Fundamental Medicine SB RAMS.

References

1. Angelopoulou, R., Lavranos, G. and Manolakou, P. ROS in the aging male: model diseases with ROS-related pathophysiology. *Reprod. Toxicol.* 28: 167-171, 2009.
2. Baron, C. Using the gradient of human cortical bone properties to determine age-related bone changes *via* ultrasonic guided waves. *Ultrasound Med. Biol.* 38: 972-981, 2012.
3. Beal, M.F. Mitochondria, oxidative damage, and inflammation in Parkinson's disease. *Ann. N.Y. Acad. Sci.* 991: 120-131, 2003.
4. Bellantuono, I., Aldahmash, A. and Kassem, M. Aging of marrow stromal (skeletal) stem cells and their contribution to age-related bone loss. *Biochim. Biophys. Acta* 1792: 364-370, 2009.
5. Benghuzzi, H., Tucci, M., Eckie, R. and Hughes, J. The effects of sustained delivery of diosgenin on the adrenal gland of female rats. *Biomed. Sci. Instrum.* 39: 335-340, 2003.
6. Biot, M.A. General theory of three-dimensional consolidation. *J. Appl. Phys.* 12: 155-164, 1941.
7. Burr, D.B., Hirano, T., Turner, C.H., Hotchkiss, C., Brommage, R. and Hock, J.M. Intermittently administered human parathyroid hormone(1-34) treatment increases intracortical bone turnover and porosity without reducing bone strength in the humerus of ovariectomized cynomolgus monkeys. *J. Bone Miner. Res.* 16: 157-165, 2001.
8. Checa, M.A., Del Rio, L., Rosales, J., Nogues, X., Vila, J. and Carreras, R. Timing of follow-up densitometry in hormone replacement therapy users for optimal osteoporosis prevention. *Osteoporos. Int.* 16: 937-942, 2005.
9. Chen, B., Zhong, Y., Peng, W., Sun, Y. and Kong, W.J. Age-related changes in the central auditory system: comparison of D-galactose-induced aging rats and naturally aging rats. *Brain Res.* 1344: 43-53, 2010.
10. Chen, J.H., Wu, J.S.S., Lin, H.C., Wu, S.L., Wang, W.F., Huang, S.K. and Ho, Y.J. Dioscorea improves the morphometric and mechanical properties of bone in ovariectomised rats. *J. Sci. Food Agric.* 88: 2700-2706, 2008.
11. Chiang, T.H., Lee, Y.C., Tu, C.H., Chiu, H.M. and Wu, M.S. Performance of the immunochemical fecal occult blood test in predicting lesions in the lower gastrointestinal tract. *Can. Med. Assoc. J.* 183: 1474-1481, 2011.
12. Chiu, C.S., Chiu, Y.J., Wu, L.Y., Lu, T.C., Huang, T.H., Hsieh, M.T., Lu, C.Y. and Peng, W.H. Diosgenin ameliorates cognition deficit and attenuates oxidative damage in senescent mice induced by D-galactose. *Am. J. Chin. Med.* 39: 551-563, 2011.
13. Cooper, C., Atkinson, E.J., O'Fallon, W.M. and Melton, L.J., 3rd. Incidence of clinically diagnosed vertebral fractures: a population-based study in Rochester, Minnesota, 1985-1989. *J. Bone Miner. Res.* 7: 221-227, 1992.
14. Cui, X., Zuo, P., Zhang, Q., Li, X., Hu, Y., Long, J., Packer, L. and Liu, J. Chronic systemic D-galactose exposure induces memory loss, neurodegeneration, and oxidative damage in mice: protective effects of R-α-lipoic acid. *J. Neurosci. Res.* 84: 647-654, 2006.
15. Currey, J.D. The effects of drying and re-wetting on some mechanical properties of cortical bone. *J. Biomech.* 21: 439-441, 1988.
16. De Laet, C.E., van Hout, B.A., Burger, H., Hofman, A. and Pols, H.A. Bone density and risk of hip fracture in men and women: cross sectional analysis. *Brit. Med. J.* 315: 221-225, 1997.
17. Djerassi, C. Drugs from Third World plants: the future. *Science* 258: 203-204, 1992.
18. Dukan, S., Farewell, A., Ballesteros, M., Taddei, F., Radman, M. and Nystrom, T. Protein oxidation in response to increased transcriptional or translational errors. *Proc. Natl. Acad. Sci. USA* 97: 5746-5749, 2000.
19. Gillberg, P., Johansson, A.G. and Ljunghall, S. Decreased estradiol levels and free androgen index and elevated sex hormone-binding globulin levels in male idiopathic osteoporosis. *Calcif. Tissue Int.* 64: 209-213, 1999.
20. Gong, G., Qin, Y., Huang, W., Zhou, S., Wu, X., Yang, X., Zhao, Y. and Li, D. Protective effects of diosgenin in the hyperlipidemic rat model and in human vascular endothelial cells against hydrogen peroxide-induced apoptosis. *Chem. Biol. Interact.* 184: 366-375, 2010.
21. Goto, S., Nakamura, A., Radak, Z., Nakamoto, H., Takahashi, R., Yasuda, K., Sakurai, Y. and Ishii, N. Carbonylated proteins in aging and exercise: immunoblot approaches. *Mech. Ageing Dev.* 107: 245-253, 1999.
22. Grzibovskis, M., Pilmane, M. and Urtane, I. Today's understanding about bone aging. *Stomatologija* 12: 99-104, 2010.
23. Harman, D. Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* 11: 298-300, 1956.
24. Hayakawa, M., Hattori, K., Sugiyama, S. and Ozawa, T. Age-associated oxygen damage and mutations in mitochondrial DNA in human hearts. *Biochem. Biophys. Res. Commun.* 189: 979-985, 1992.
25. Hidaka, S., Okamoto, Y. and Arita, M. A hot water extract of *Chlorella pyrenoidosa* reduces body weight and serum lipids in ovariectomized rats. *Phytother. Res.* 18: 164-168, 2004.
26. Ho, Y.J., Tai, S.Y., Pawlak, C.R., Wang, A.L., Cheng, C.W. and Hsieh, M.H. Behavioral and IL-2 responses to diosgenin in ovariectomized rats. *Chinese J. Physiol.* 55: 91-100, 2012.
27. Ho, Y.J., Wang, C.F., Hsu, W.Y., Tseng, T., Hsu, C.C., Kao, M.D. and Tsai, Y.F. Psychoimmunological effects of dioscorea in ova-

riectomized rats: role of anxiety level. *Ann. Gen. Psychi.* 6: 21-28, 2007.
28. Kalu, D.N., Masoro, E.J., Yu, B.P., Hardin, R.R. and Hollis, B.W. Modulation of age-related hyperparathyroidism and senile bone loss in Fischer rats by soy protein and food restriction. *Endocrinology* 122: 1847-1854, 1988.
29. Lei, M., Hua, X., Xiao, M., Ding, J., Han, Q. and Hu, G. Impairments of astrocytes are involved in the D-galactose-induced brain aging. *Biochem. Biophys. Res. Commun.* 369: 1082-1087, 2008.
30. Leung, T.K., Chen, C.H., Lai, C.H., Lee, C.M., Chen, C.C., Yang, J.C., Chen, K.C. and Chao, J.S. Bone and joint protection ability of ceramic material with biological effects. *Chinese J. Physiol.* 55: 47-54, 2012.
31. Li, F., Gong, Q.H., Wu, Q., Lu, Y.F. and Shi, J.S. Icariin isolated from *Epimedium brevicornum* Maxim attenuates learning and memory deficits induced by D-galactose in rats. *Pharmacol. Biochem. Behav.* 96: 301-305, 2010.
32. Lin, H.C., Wu, J.S.S., Hung, J.P., Yeh, W.C. and Chen, J.H. The study of positive correlation between bone frame mineral density and volume fraction in cortical bone. *J. Med. Biol. Eng.* 27: 136-142, 2007.
33. Liu, M.J., Wang, Z., Ju, Y., Wong, R.N. and Wu, Q.Y. Diosgenin induces cell cycle arrest and apoptosis in human leukemia K562 cells with the disruption of Ca^{2+} homeostasis. *Cancer Chemother. Pharmacol.* 55: 79-90, 2005.
34. Liu, S.Y., Wang, J.Y., Shyu, Y.T. and Song, L.M. Studies on yams (*Dioscorea spp.*) in Taiwan. *J. Chin. Med.* 6: 111-126, 1995.
35. McCalden, R.W., McGeough, J.A., Barker, M.B. and Court-Brown, C.M. Age-related changes in the tensile properties of cortical bone. The relative importance of changes in porosity, mineralization, and microstructure. *J. Bone Joint Surg.* 75: 1193-1205, 1993.
36. Meydani, S.N., Wu, D., Santos, M.S. and Hayek, M.G. Antioxidants and immune response in aged persons: overview of present evidence. *Am. J. Clin. Nutr.* 62: 1462S-1476S, 1995.
37. Most, W., Schot, L., Ederveen, A., van der Wee-Pals, L., Papapoulos, S. and Lowik, C. *In vitro* and *ex vivo* evidence that estrogens suppress increased bone resorption induced by ovariectomy or PTH stimulation through an effect on osteoclastogenesis. *J. Bone Miner. Res.* 10: 1523-1530, 1995.
38. Olanow, C.W. A radical hypothesis for neurodegeneration. *Trends Neurosci.* 16: 439-444, 1993.
39. Pei, L.P., Hui, B.D. and Dong, F.H. [Influence of canthaxanthin on D-galactose induced osseous changes of rat]. *Zhongguo Gu Shang* 21: 613-616, 2008.
40. Reynolds, A., Laurie, C., Mosley, R.L. and Gendelman, H.E. Oxidative stress and the pathogenesis of neurodegenerative disorders. *Int. Rev. Neurobiol.* 82: 297-325, 2007.
41. Rosenkranz, G., Djerassi, C., Yashin, R. and Pataki, J. Cortical hormones from *all*osteroids: synthesis of cortisone from Reichsteen's compound *D. Nature* 168: 28, 1951.
42. Sanchez-Rodriguez, M.A., Ruiz-Ramos, M., Correa-Munoz, E. and Mendoza-Nunez, V.M. Oxidative stress as a risk factor for osteoporosis in elderly Mexicans as characterized by antioxidant enzymes. *BMC Musculosket. Dis.* 8: 124, 2007.
43. Skedros, J.G., Bloebaum, R.D., Mason, M.W. and Bramble, D.M. Analysis of a tension/compression skeletal system: possible strain-specific differences in the hierarchical organization of bone. *Anat. Rec.* 239: 396-404, 1994.
44. Son, I.S., Kim, J.H., Sohn, H.Y., Son, K.H., Kim, J.S. and Kwon, C.S. Antioxidative and hypolipidemic effects of diosgenin, a steroidal saponin of yam (*Dioscorea spp.*), on high-cholesterol fed rats. *Biosci. Biotechnol. Biochem.* 71: 3063-3071, 2007.
45. Song, X., Bao, M., Li, D. and Li, Y.M. Advanced glycation in D-galactose induced mouse aging model. *Mech. Ageing Dev.* 108: 239-251, 1999.
46. Tada, Y., Kanda, N., Haratake, A., Tobiishi, M., Uchiwa, H. and Watanabe, S. Novel effects of diosgenin on skin aging. *Steroids* 74: 504-511, 2009.
47. Tian, J., Ishibashi, K., Reiser, K., Grebe, R., Biswal, S., Gehlbach, P. and Handa, J.T. Advanced glycation endproduct-induced aging of the retinal pigment epithelium and choroid: a comprehensive transcriptional response. *Proc. Natl. Acad. Sci. USA* 102: 11846-11851, 2005.
48. Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M. and Telser, J. Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.* 39: 44-84, 2007.
49. Wei, H., Li, L., Song, Q., Ai, H., Chu, J. and Li, W. Behavioural study of the D-galactose induced aging model in C57BL/6J mice. *Behav. Brain Res.* 157: 245-251, 2005.
50. Wu, J.S. and Chen, J.H. Clarification of the mechanical behaviour of spinal motion segments through a three-dimensional poroelastic mixed finite element model. *Med. Eng. Phys.* 18: 215-224, 1996.
51. Wu, J.S., Hsu, S.L. and Chen, J.H. Evaluating the accuracy of wear formulae for acetabular cup liners. *Med. Biol. Eng. Comput.* 48: 157-165, 2010.
52. Wu, J.S.S., Lin, H.C., Hung, J.P. and Chen, J.H. Effects of bone mineral fraction and volume fraction on mechanical properties of cortical bone. *J. Med. Biol. Eng.* 26: 1-7, 2006.
53. Yin, J., Kouda, K., Tezuka, Y., Tran, Q.L., Miyahara, T., Chen, Y. and Kadota, S. Steroidal glycosides from the rhizomes of dioscorea spongiosa. *J. Nat. Prod.* 66: 646-650, 2003.
54. Zhang, Q., Li, X., Cui, X. and Zuo, P. D-galactose injured neurogenesis in the hippocampus of adult mice. *Neurol. Res.* 27: 552-556, 2005.
55. Zhang, X., Li, W.B. and Zhang, B.L. Biochemical changes in D-galactose induced subacute toxicity and mimetic aging in mice. *Chinese J. Pharm. Toxicol.* 4: 309-310, 1990.
56. Zhang, X.L., Jiang, B., Li, Z.B., Hao, S. and An, L.J. Catalpol ameliorates cognition deficits and attenuates oxidative damage in the brain of senescent mice induced by D-galactose. *Pharmacol. Biochem. Behav.* 88: 64-72, 2007.
57. Zhang, Y.B., Zhong, Z.M., Hou, G., Jiang, H. and Chen, J.T. Involvement of oxidative stress in age-related bone loss. *J. Surg. Res.* 169: e37-e42, 2011.
58. Zou, L., Bloebaum, R.D. and Bachus, K.N. Reproducibility of techniques using Archimedes' principle in measuring cancellous bone volume. *Med. Eng. Phys.* 19: 63-68, 1997.