Original Article

Prune extract prevents disuse osteoporosis by inhibiting the decrease in osteoblast-related gene expression in sciatic-denervated rats

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In sedentary modern society, disuse osteoporosis is a health issue. Here, we investigate whether prune extract prevents disuse osteoporosis in rats. After feeding a control diet or 10% (wt/wt) prune extract-containing diet for 14 days, we performed sham operation in the left leg and sciatic denervation in the right leg to induce disuse osteoporosis in rats. The rats were fed the same diet prior to surgery for 7 days. The rats fed a control diet before sham operation on both legs were set as the control group, and those with sciatic denervation in the right leg fed a control diet or prune extract containing diet were set as the denervation with control diet and denervation with prune extract diet groups, respectively. Femoral bone volume/tissue volume, trabecular number, and trabecular thickness were reduced in the right leg of denervation with control diet group; however, this reduction was not observed in the denervation with prune extract diet group. Similar results were obtained for mRNA levels of osteoblast-related genes, such as *osteocalcin*. Overall, prune extract inhibited bone loss by preventing the decrease in osteoblast-related gene expression in disuse osteoporosis, thus showing to improve the bone metabolism and quality of life.

***Key Words*: prune extract, disuse osteoporosis, bone morphology, osteoblast, osteoblast-related gene**

Osteoporosis is induced when there is an imbalance between the bone formation by osteoblasts and the bone resorption by osteoclasts. Aging is a major risk factor for osteoporosis owing to the predominance of bone resorption by osteoclasts.[1] In modern society, the aging population is growing due to the prolonged life span. The World Health Organization estimates that one in six people worldwide will be 60 years old by 2030.[2] In Japan, 33.84 million people are aged over 65 years, accounting for 26.7% of the Japanese population.[3] Up to 49 million people meet the World Health Organization criteria for osteoporosis in several industrialized countries.[4,5] In the elderly, osteoporosis is related to decreased quality of life and death.[6] Osteoporosis is a global public health issue, mainly affecting the aging population.[7]

The World Health Organization reports that physical inactivity is the fourth leading risk factor for global mortality.[8] In modern society, disuse osteoporosis and osteoporosis due to aging are public health issues. As mechanical stimuli, such as physical exercise, stimulate bone formation,[9] disuse osteoporosis is often caused by physical inactivity, such as bed rest and spaceflight.[10,11] In modern society, many people of working age are sedentary due to the nature of their work. Many desk-based workplaces in western countries require long periods of sitting and limited movement during working hours.[12] Sedentary behavior or sitting time of desk-based employees is more than 360 min at work.[13] Several studies have revealed a relationship between sedentary behavior and bone loss in young and adult individuals. Sedentary time is negatively associated with limber spine bone mineral density (BMD) in Americans aged 20–59 years.[14] Sedentary time is related to femoral neck BMD, but not to lumbar spine BMD, in 12 year old boys.[15] Physical inactivity due to sedentary behavior is also a risk factor for disuse osteoporosis.[16] Therefore, individuals of all ages should pay attention to prevent disuse osteoporosis and prolong their life span.

Prune (*Prunus domestica* L.), which is rich in pectin and chlorogenic acid, exhibits antioxidant activity and various health benefits. Prune juice intake for 8 weeks improves the fecal quality in subjects with chronic constipation.[17] Dietary prune intake for 12 months alters the microbiota in postmenopausal women.[18] Intake of prune extract-containing diet for five weeks lowers the blood pressure in a rat model of hypertension.[19] Intake of dried prunes, which is a common form of prunes, exerts beneficial effects on bone health in humans and animals. Intake of dried prunes (100 g/day) with 500 mg Ca and 400 IU vitamin D for 12 months improves BMD in postmenopausal women.[20] Intake of dried prune (50 g/day) for 6 months prevents total hip BMD loss in postmenopausal women.[21] Intake of a 25% dried prune-containing diet for 6 months increases the bone volume in the distal femoral metaphysis of old male mice[22] and for 45 day prevents bone loss in ovariectomized rats, a model of postmenopausal osteoporosis.[23] Prune intake contributes to the improvement of health.

Although dried prunes have some health benefits,[17–23] their dryness and portion size make them difficult to consume, especially by people with impaired chewing and swallowing abilities. Therefore, in this study, we used a prune extract, which is easy to consume as it is in a concentrated form. Moreover, prune extract can be mixed with other foods, making it easier to consume for people who do not like its taste or smell. Here, we investigated whether prune extract prevents disuse osteoporosis in sciatic-denervated rats.[24]

Materials and Methods

Animals. All animal experiments were approved by the Ethics Committee of the University of Hyogo, School of Human Science and Environment (approval no. 013). For surgical treatment, rats were anesthetized with isoflurane and all possible measures were taken to minimize their suffering.

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Table 1. Compositions of the control diet and a 10% prune extract containing diet

	Control diet	10% prune extract containing diet
	g/kg diet	
α-Starch	132	105
β-Starch	397.5	341.6
Casein[a]	200	190
Sucrose	100	50
Soybean oil	50	50
Cellulose	70	70
Vitamin mixture[b]	10	10
Mineral mixture[c]	35	35
L-Cystine	3	2.9
Choline bitartrate	2.5	2.5
Tert-butylhydroquinone	0.14	0.14
Prune extract[d]	—	143
Crude nutrients[e]		
Water	90	90
Protein	181	181
Fat	73	75
Ash	31	34
NFE	576	569

[a]Crude protein: 84.3%. [b]AIN93-VX. [c]AIN93-MX. [d]Prune extract contained 30% water, and it was adjusted to contain 10% as a solid components. [e]Values were obtained from Oriental Yeast Co., Ltd.

Nineteen five-week-old male Wistar rats were purchased from Japan SLC (Shizuoka, Japan). The rats were housed individually in wire-bottomed cages under a 12-h light/dark cycle (lights on 9:00–21:00). The rats were provided *ad libitum* access to food and water during the experimental period. The rats were fed a control diet (AIN-93G; Oriental Yeast Co., Ltd., Tokyo, Japan) for three days to adapt to the animal room conditions. Subsequently, the rats were divided into the C ($n = 7$), SC ($n = 6$), and SP groups ($n = 6$), and matched for body weight. After the C and SC groups were fed a control diet and the SP group was fed a 10% (w/w) prune extract-containing diet (PD) for 14 d, the SC and SP groups were subjected to sciatic denervation of the right leg and the left leg was sham-operated. In contrast, both legs in group C were sham-operated. After feeding the same diet for seven days prior to the operation, the rats were sacrificed at 13:00. Blood, liver, and gastrocnemius, plantaris, and soleus muscles were collected from all rats. Additionally, the tibia–fibula and femur were collected from the SC and SP groups, respectively. Blood and tibia–fibula were frozen immediately in liquid nitrogen and stored at −80°C until analysis. The femur was stored in 70% ethanol.

Compositions of all diets are presented in Table 1. All diets were in a pellet form and provided by Oriental Yeast Co., Ltd. MIKI prune extract, a concentrated prune juice, was supplied by MIKI Corporation (Osaka, Japan). Prune extract was added to the control diet to a level of 10% in solids after the removal of casein, L-cysteine, starch, and sucrose, because we confirmed that the prune extract contains protein and sucrose. The amount of crude protein nutrients in 10% prune extract-containing diet was adjusted to be the same as that in the control diet, and the amounts of crude fat, ash, and nitrogen-free extract (NFE) were adjusted to be as consistent as possible (Table 1).

Biochemical analysis. Serum Ca, alkaline phosphatase (ALP), and thiobarbituric acid-reactive substance (TBARS) levels were determined using Accuras Auto CaII (Shino-Test Corporation, Tokyo, Japan), L-Type Wako ALP IFCC (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan), and TBARS Assay Kit (Cayman Chemical Company, Ann Arbor, MI), respectively. Serum glutamic acid (Gla)-osteocalcin levels were measured using the rat-specific enzyme immunoassay kit (Rat Gla-Osteocalcin High-Sensitive EIA Kit; Takara Bio Inc., Shiga, Japan).

Bone morphological analysis. Femurs of both legs in the SC and SP groups were preserved in 70% ethanol for micro-computed tomography (CT) analysis. Bone morphology, including bone volume (BV), tissue volume (TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp), in 2D images of the distal femur were analyzed using the TRI/3D-BON software (Ratoc System Engineering Corporation, Tokyo, Japan). Micro-CT analysis was performed at Kureha Special Laboratories (Fukushima, Japan).

Total RNA extraction and real-time quantitative polymerase chain reaction (PCR). Total RNA was extracted from tibia–fibula using the ISOSPIN Cell & Tissue RNA (Nippon Gene, Tokyo, Japan) and reverse-transcribed into cDNA using the PrimeScript2 First Strand cDNA Synthesis Kit (Takara Bio Inc.). RT-qPCR was performed using the StepOnePlus Real-Time PCR system (Thermo Fisher Scientific, Waltham, MA) with the Fast SYBR Green Master Mix (Thermo Fisher Scientific). Moreover, 18S rRNA was used as an internal control to normalize the target gene expression. All primer sequences used for real-time PCR are listed in Table 2.

Statistical analyses. Results are expressed as the mean ± SEM. Body weight, total food intake, tissue weight, and serum parameters were analyzed using one-way analysis of variance, followed by Tukey's post-hoc test when $p<0.05$. Bone morphology and tibia–fibula gene expression were analyzed using two-way analysis of variance, followed by Tukey's post-hoc test when the interaction showed $p<0.05$. All statistical analyses were conducted using IBM SPSS Statistics ver. 28 (IBM Inc., Tokyo, Japan).

Results

Prune extract does not change the body weight, total food intake, and tissue weight in rats. Table 3 shows the body weight, total food intake, and tissue weight of rats. No significant differences were observed in the initial body weight, final body weight, total food intake, and liver weight of the rats (Table 3). Weights of the gastrocnemius, soleus, and plantaris muscles in the left legs were similar in all groups, whereas those in the right legs were lower in the SC and SP groups than in the C group due to sciatic denervation (Table 3).

Prune extract prevents the increase in serum TBARS levels after sciatic denervation in rats. Figure 1 shows the serum parameters, including Ca, ALP, Gla-osteocalcin, and TBARS levels. Serum Ca levels, which are maintained via bone formation and resorption, were lower in the SC group than in the C group (Fig. 1A). Serum ALP and Gla-osteocalcin levels, which is the active forms of osteocalcin, did not change in any of the groups (Fig. 1B and C). Serum TBARS levels were higher in the SC group than in the SP group (Fig. 1D), suggesting that the prune extract suppresses oxidative stress in sciatic denervated rats.

Prune extract prevents bone loss induced by sciatic denervation in rats. Figure 2 shows a representative image of the distal femur of both legs and bone morphology in SC and SP groups analyzed by micro-CT. In the SC group, trabecular bone of the denervated side exhibited lower density than that of the sham-operated side; however, this difference was not observed in the SP group (Fig. 2A and B). Notably, surgery significantly affected the bone morphology indices, BV/TV, Tb.Th, Tb.N, and Tb.Sp (Fig. 2C–F), confirming that sciatic denervation induced disuse osteoporosis in rats. Additionally, diet also significantly

Table 2. Primer sequences for real-time PCR

Gene	Forward primer (5'→3')	Reverse primer (5'→3')
18S	CGCCGCTAGAGGTGAAATTC	TTGGCAAATGCTTTCGCTC
Alp	CAAGAGCCCACAATGGACAG	AAGTGAGGCAGGTAGCAAAC
Cat	ATCAGGGATGCCATGTTGTT	GGGTCCTTCAGGTGAGTTTG
Col1a1	ACCAGACGCAGAAGTCATAG	AAGTTTCCTCCAAGACCAAG
Ctsk	GGAAGCAGTACAACAGCAAG	GGCCTCAAGATTATGGACAG
Gpx1	CGACATCGAACCCGATATAGA	ATGCCTTAGGGGTTGCTAGG
Hmox1	CAGAGTTTCTTCGCCAGAGG	TGCTGATCTGGGATTTTCCT
Nfatc1	ACGATGTGGAGGTGGAAGAC	GGACGCCTCAGAGTTACAGC
Nrf2	AGTCACTCGATAGCTCTCTG	TTTGAACGAGGTACAGGACG
Osteocalcin	TCTCTCTGCTCACTCTGCTG	TTCACCACCTTACTGCCCTC
Osteonectin	GGATCTTCTTTCTCCTTTGC	AACTCTCCCATTTCCACCTG
Osteopontin	GATTCTGTGAACTCGGATG	AAACGTCTGCTTGTGTGCTG
Postn	AGCAAACCACTTTCACGGAC	GAGAAGACGCCAACCCTAAC
Rankl	CTATGATGGAAGGTTCGTGG	GAGGACAGACTGACTTTATG
Runx2	GCCGGGAATGATGAGAACTA	GGACCGTCCACTGTCACTTT
Sod1	AGAAACATGGCGGTCCAG	ATGGACACATTGGCCACAC
Trap	GGCCAATGCTAAAGAAATCG	CAGAGAACACATCCTCAAAG

Table 3. Body weight, total food intake, and tissue weight

	C group	SC group	SP group	*p* value
Initial body weight (g)	103.7 ± 2.16	103.7 ± 1.90	103.7 ± 1.51	1.000
Final body weight (g/21 day)	209.1 ± 4.67	211.5 ± 3.99	208.8 ± 2.77	0.881
Total food intake (g/21 day)	301.8 ± 3.75	306.7 ± 4.29	295.6 ± 3.75	0.328
Liver weight (g/100 g BW)	4.35 ± 0.150	4.42 ± 0.097	4.29 ± 0.079	0.747
Gastrocnemius muscle weight (g/100 g BW)				
Left leg	0.466 ± 0.006	0.472 ± 0.009	0.481 ± 0.005	0.286
Right leg	0.459 ± 0.005[a]	0.277 ± 0.004[b]	0.280 ± 0.004[b]	<0.001
Plantaris muscle weight (g/100 g BW)				
Left leg	0.089 ± 0.004	0.086 ± 0.004	0.081 ± 0.004	0.393
Right leg	0.085 ± 0.003[a]	0.055 ± 0.002[b]	0.056 ± 0.002[b]	<0.001
Soleus muscle weight (g/100 g BW)				
Left leg	0.034 ± 0.001	0.034 ± 0.001	0.034 ± 0.001	0.997
Right leg	0.034 ± 0.001[a]	0.021 ± 0.001[b]	0.020 ± 0.001[b]	<0.001

Values are represented as the mean ± SEM. $n = 6$ or 7. Statistical analyses were conducted via one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test. Values in rows with different letters indicate the significant differences.

affected the bone morphology indices, with the SP group showing high BV/TV, Tb.Th, and Tb.N values and low Tb.Sp values (Fig. 2C–F). These results suggest that the prune extract prevents disuse osteoporosis induced by sciatic denervation in rats.

Prune extract prevents the decrease in osteoblast-related gene levels in the tibia–fibula of sciatic-denervated rats. To explore the mechanism of disuse osteoporosis prevention via prune extract intake, mRNA expression levels in the tibia–fibula were analyzed. Figure 3 shows the mRNA levels of osteoclast- and osteoblast-related genes in the tibia–fibula of both legs in the SC and SP groups. The mRNA levels of the osteoclast-related genes, *receptor activator of NF-κB ligand* (*Rankl*), *nuclear factor of activated T cells 1* (*Nftac1*), *cathepsin K* (*Ctsk*), and *tartrate-resistant acid phosphatase* (*Trap*), were not significantly affect by diet, surgery, or their interaction (Fig. 3A–D). In contrast, levels of *runt-related transcription factor 2* (*Runx2*), a transcription factor essential for osteoblast differentiation, showed significant interaction effects (Fig. 3E). Although the mRNA levels of osteoblast-related *periostin* showed no significant effect of diet, surgery, or their interaction (Fig. 3F), that of *collagen type I alpha 1 chain* (*Col1a1*) and *osteocalcin*, which are marker genes of osteoblasts, showed significant interaction effects (Fig. 3G and H) and were significantly higher in the SP group than in the SC group in the right leg of tibia–fibula of sciatic-denervated rats (Fig. 3G and H). In contrast, levels of the osteoblast-related genes, *osteonectin*, *osteopontin*, and *Alpl*, showed no significant diet effect, surgery effect, or interaction effect (Fig. 3I–K). These results suggest that the prune extract prevents disuse osteoporosis by inhibiting the decrease in osteoblast-related gene expression.

Prune extract does not affect the antioxidant-related gene levels in the tibia–fibula of sciatic-denervated rats. Serum TBARS levels were higher in the SC group than in the SP group (Fig. 1D). We further measured the mRNA levels of antioxidant-related genes in the tibia–fibula of SC and SP rats. Notably, mRNA levels of the antioxidant-related genes, *NF-E2-related factor 2* (*Nrf2*), *heme oxygenase 1* (*Hmox1*), *superoxide dismutase 1* (*Sod1*), *catalase* (*Cat*), and *glutathione peroxidase 1* (*Gpx1*), showed no significant diet effect, surgery effect, or interaction effect (Fig. 4). Therefore, prune extract did not affect the antioxidant-related genes in the tibia–fibula of sciatic-denervated rats.

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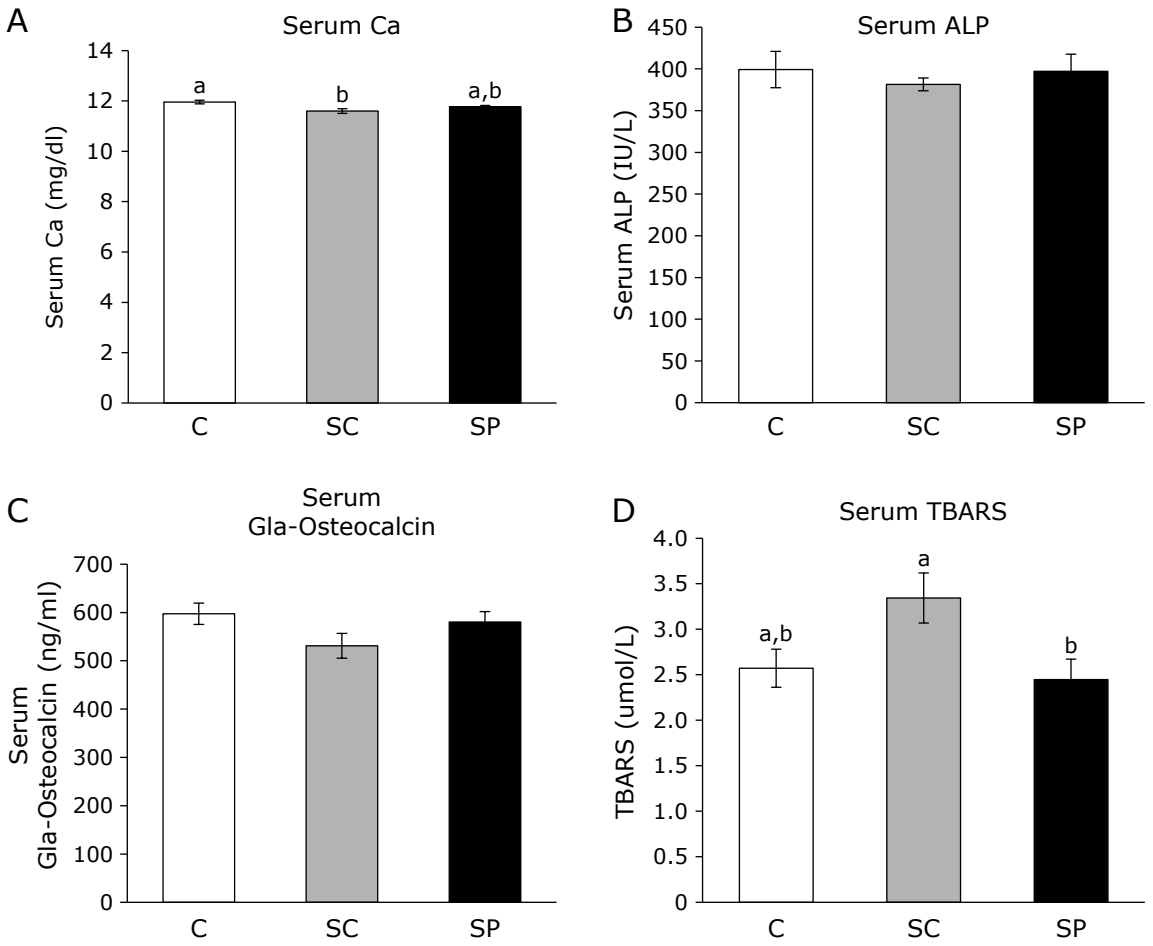

Fig. 1. Prune extract prevents the increase in serum thiobarbituric acid-reactive substance (TBARS) levels after sciatic denervation in rats. Serum levels of (A) Ca, (B) alkaline phosphatase (ALP), (C) glutamic acid (Gla)-osteocalcin, and (D) TBARS. Serum was collected on day 7 after the rats were sacrificed. Serum parameters are expressed as the mean ± SEM ($n = 7$ in C group; $n = 6$ in SC group; $n = 6$ in SP group). Statistical significance was assessed via one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test when $p<0.05$. Values in a row with different letters indicate the statistically significant differences.

Discussion

In sedentary modern society, prevention of disuse osteoporosis is essential to prolong the healthy life span. Dried prunes are widely used to prevent osteoporosis in animals and humans.[20–23] However, dried prunes are not always easy to eat due to their dryness and portion size, especially by people who impaired their chewing and swallowing abilities. Here, we investigated the effects of prune extract, which is easy to eat owing to its concentrated extract form, on bone metabolism using a rat model of disuse osteoporosis. Body weight, food intake, and liver weight were similar among all groups, indicating that the diet containing 10% prune extract had no adverse effects on the rat growth and health. However. dietary intake of prune extract prevented the worsen in bone morphology indices, BV/TV, Tb.Th, Tb.N, and Tb.Sp. Additionally, prune extract protected against decreased mRNA levels of osteoblast-related genes, *Col1a1* and *osteocalcin*, induced by sciatic denervation. Therefore, prune extract intake prevented disuse osteoporosis induced by sciatic denervation in male rats.

Hind limb unloading, a model of disuse, increases oxidative stress in skeletal muscles.[25] Similar to the hindlimb unloading model, sciatic-denervated model is also an inactive model, which probably increases oxidative stress in the bone. Oxidative stress further causes bone loss by enhancing the differentiation of pre-osteoclasts into osteoclasts.[26] In this study, SC group exhibited elevated serum levels of TBARS, an indicator of oxidative stress, compared to the SP group. Additionally, serum Ca levels were higher in the SC group than in the control group, indicating that bone resorption was enhanced by oxidative stress due to physically inactive by sciatic denervation in SC rats. Chlorogenic acid, which is abundant in prunes, improves BMD in ovariectomized rats.[27] Antioxidant chlorogenic acid in the prune extract possibly prevents bone loss similar to the dried prunes.[23] Serum levels of TBARS were significantly lower in the SP group than in the SC group. In addition, although no significant differences were observed, it appears that sciatic denervation decreased the tibia–fibula mRNA levels of antioxidant-related genes in the SC group, but not in the SP group. These data indicate that prune extract enhanced antioxidant activity and showed a beneficial effect on bone metabolism.

Dried prunes contain high levels of dietary fiber, vitamin K, and chlorogenic acid, which positively affect bone metabolism.[23,27–29] Here, prune extract also contained high levels of dietary fiber and chlorogenic acid, but small amount of vitamin K. On the other hand, it has been pointed out that other polyphenols and dietary fibers, but not chlorogenic acid, in dried prunes positively affect bone metabolism.[30] These components exhibit beneficial effects on bone metabolism; however, the prune component most beneficial for bone health remains unknown. Taken together,

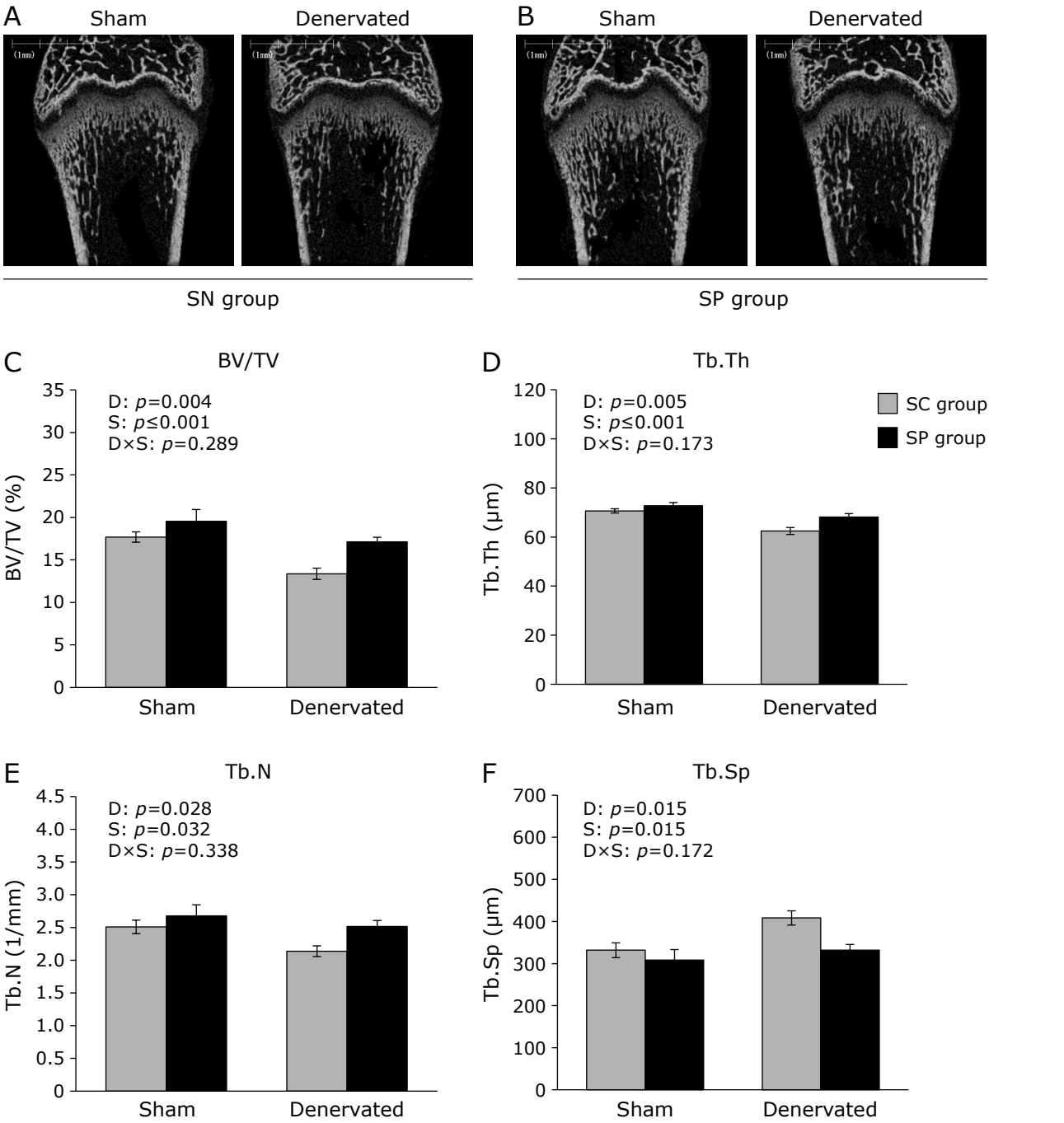

Fig. 2. Prune extract prevents the decrease in bone morphology indices. Representative images of the distal femurs of both legs in (A) SC group and (B) SP group. Bone morphology indices: (C) bone volume/tissue volume (BV/TV), (D) trabecular thickness (Tb.Th), (E) trabecular number (Tb.N), (F) trabecular separation (Tb.Sp). Bone morphology indices are expressed as the mean ± SEM (n = 6 in SC group; n = 6 in SP group). Statistical significance was assessed via two-way ANOVA, followed by Tukey's post-hoc test when interaction effect showed $p<0.05$. Values in a row with different letters indicate the statistically significant differences. D, diet effect; S, surgery effect; D × S, interaction effect.

prune extract contains some components that exert beneficial effects on bones, thereby preventing disuse osteoporosis.

Dried prunes protect against postmenopausal osteoporosis by increasing the rate of bone formation, without inhibiting bone resorption, and increase the mRNA levels of *Nftac1* and *Col1a1*, but not *Alpl* and *osteocalcin*, in ovariectomized rats or mice.[23,29] In this study, prune extract significantly suppressed the decrease in the mRNA levels of osteoblast-related genes, *Col1a1* and *osteocalcin*, but not osteoclast-related genes, *Rankl*, *Ctsk*, and *Trap*. These results are consistent with those of previous reports, which researched relationship between dried prune intake and bone metabolism.[23,29] Although not significantly different, mRNA levels of the osteoblast-related genes, *Runx2*, *periostin*, and *Alpl*, were decreased by sciatic denervation. These findings indicate that the prune extract prevents disuse osteoporosis induced by sciatic denervation by increasing the rate of bone formation.

Osteocalcin, the most abundant non-collagenous bone matrix

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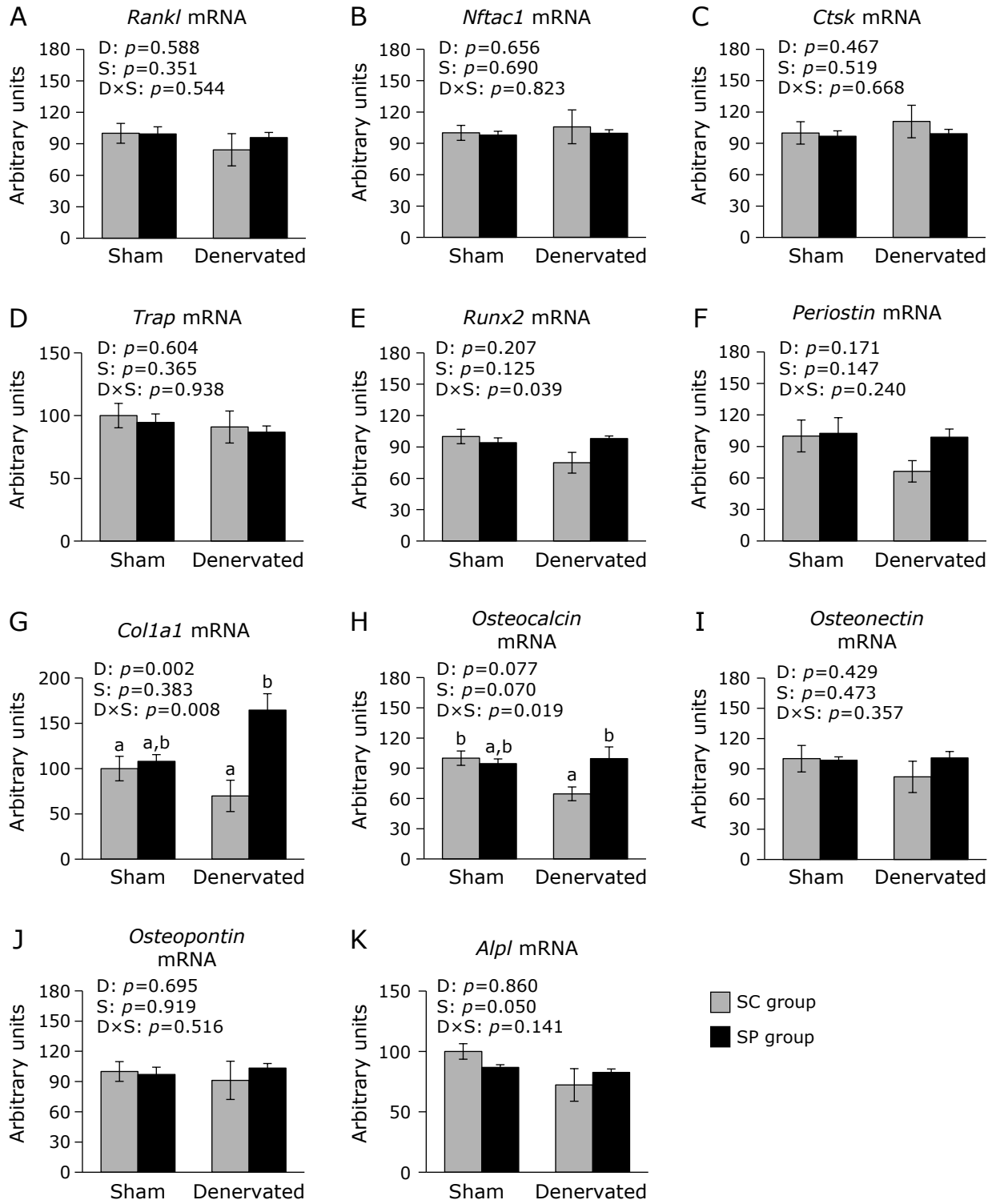

Fig. 3. Prune extract prevents the decrease in the mRNA levels of osteoblast-related genes in the tibia–fibula of sciatic-denervated rats. mRNA levels of (A) *receptor activator of NF-κB ligand* (*Rankl*), (B) *nuclear factor of activated T cells 1* (*Nftac1*), (C) *cathepsin K* (*Ctsk*), (D) *tartrate-resistant acid phosphatase* (*Trap*), (E) *runt-related transcription factor 2* (*Runx2*), (F) *periostin*, (G) *collagen type I alpha 1 chain* (*Col1a1*), (H) *osteocalcin*, (I) *osteonectin*, (J) *osteopontin*, and (K) *Alpl* in the tibia–fibula. The mRNA levels are expressed as the mean ± SEM ($n = 6$ in SC group; $n = 6$ in SP group). Statistical significance was assessed via two-way ANOVA, followed by Tukey's post-hoc test when interaction effect showed $p<0.05$. Values in a row with different letters indicate the statistically significant differences. D, diet effect; S, surgery effect; D × S, interaction effect.

protein, is activated when its glutamine (Glu) residue is converted to Gla by a vitamin K-dependent carboxylase.[31] Gla-osteocalcin has a high affinity for Ca. Vitamin K is also important for bone metabolism. On the other hand, vitamin K is essential for blood coagulation. It is therefore imperative that patients undergoing anticoagulant therapy with warfarin exercise

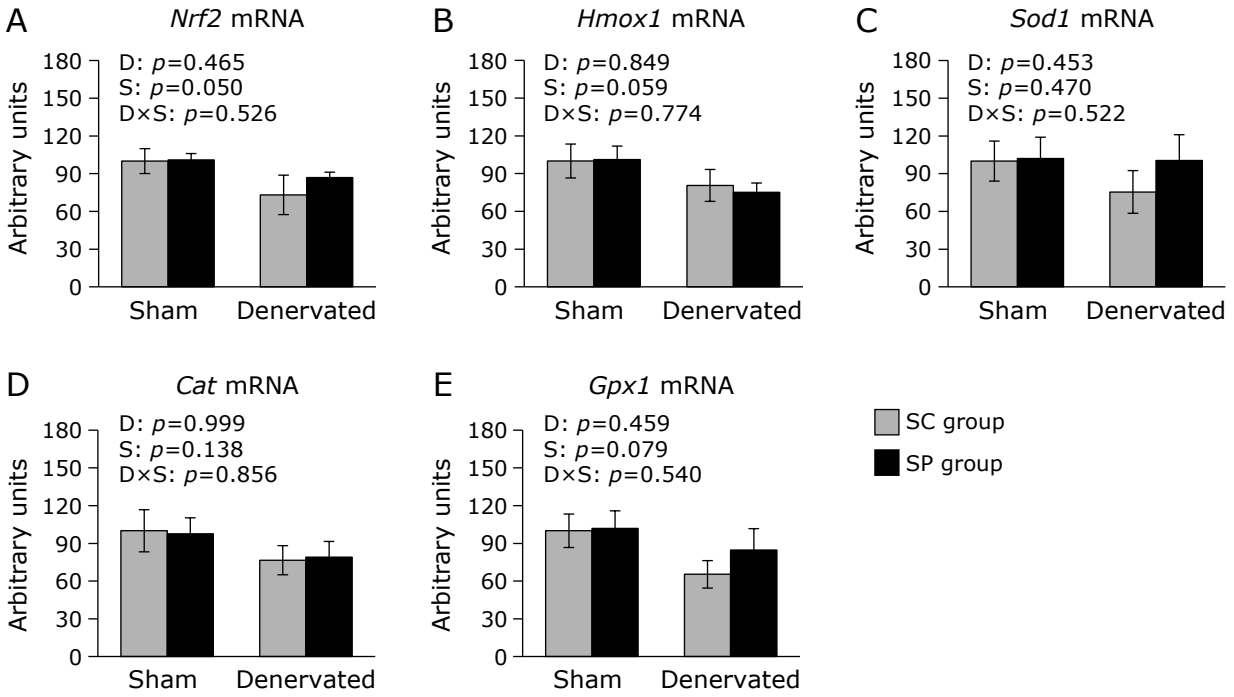

Fig. 4. Prune extract does not affect the mRNA levels of antioxidant-related genes in the tibia–fibula of sciatic-denervated rats. mRNA levels of (A) *NF-E2-related factor 2* (*Nrf2*), (B) *heme oxygenase 1* (*Hmox1*), (C) *superoxide dismutase 1* (*Sod1*), (D) *catalase* (*Cat*), and (E) *glutathione peroxidase 1* (*Gpx1*) in the tibia–fibula. The mRNA levels are expressed as the mean ± SEM ($n = 6$ in SC group; $n = 6$ in SP group). Statistical significance was assessed via two-way ANOVA, followed by Tukey's post-hoc test when interaction effect showed $p<0.05$. Values in a row with different letters indicate the statistically significant differences. D, diet effect; S, surgery effect; D × S, interaction effect.

caution in their dietary intake of vitamin K. In 2020, the number of warfarin users was estimated to be 2.2 million in the USA.[32] In Japan, more than one million people are estimated to use anticoagulants by 2050.[33] Long-term intake of warfarin induces bone loss in children and elderly people.[34,35] Although dried prunes exert beneficial effects on bone metabolism, they are also abundant in vitamin K.[28] In contrast, prune extract contains only small amounts of vitamin K and is expected to improve bone metabolism without mediating the beneficial effects of vitamin K on bone health. Furthermore, it well known that vitamin D plays a pivotal role in bone metabolism. Since vitamin D increases the intestinal calcium absorption, its deficiency is a risk factor for osteoporosis.[36] However, raw plum and dried prune did not contain vitamin D.[28,37] Combination of vitamin D and prune extract may lead to further improvement of bone metabolism.

To the best of our knowledge, this study is the first to show that prune extract intake prevents disuse osteoporosis in sciatic-denervated rats. Here, dietary prune extract prevented the decrease in bone morphology indices by inhibiting osteoblast-related gene expression. Overall, our results highlight the potential of prune extract to enhance bone metabolism, thereby extending the healthy quality of life in the current aging and inactive society.

Author Contributions

FH wrote the original manuscript. FH and KK designed the study. FH, KK, and HY conceptualized the study, curated the data, reviewed and edited the final manuscript. AN and AI curated the data. KK and HY supervised the study. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

KK and HY are employees of MIKI Corporation. The other authors declare no conflicts of interest.

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