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Dietary Dried Plum Increases Bone Mass in Adult and Aged Male Mice[1–3]

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Abstract

Bone is progressively lost with advancing age. Therapies are limited and the only effective proanabolic regimen presently available to restore bone is intermittent treatment with teriparatide (parathyroid hormone 1–34). Recent evidence suggests that dietary supplementation with dried plum (DP) can prevent bone loss due to estrogen deficiency. To determine whether dietary DP supplementation can prevent the loss of bone with aging and whether bone that has already been lost can be restored, adult (6 mo) and old (18 mo) male mice were fed a normal diet or isoenergetic, isonitrogenous diets supplemented with DP (0, 15, and 25% DP by weight) for 6 mo. MicroCT analysis and bone histomorphometry were used to assess bone volume, structure, and metabolic activity before, during, and after dietary supplementation. Mice fed the 0% DP diet (control diet) lost bone, whereas both adult and old mice fed the 25% DP-supplemented diet gained bone. Adult but not old mice fed the 15% diet also gained bone. Cancellous bone volume in mice receiving 25% DP exceeded baseline levels by 40–50%. Trabecular structure varied with diet and age and responses in old mice were generally blunted. Trabecular, but not cortical, mineral density varied with age and measures of bone anabolic activity were lower in aged mice. Our findings suggest that DP contains proanabolic factors that can dramatically increase bone volume and restore bone that has already been lost due to aging. In turn, DP may provide effective prophylactic and therapeutic agents for the treatment of osteoporosis. J. Nutr. 140: 1781–1787, 2010.

Introduction

Numerous therapies have been developed for the treatment of osteoporosis (1–4). Most of these, including the bisphosphonates, estrogen, selective estrogen-receptor modulators, calcitonin, and denosumab, a monoclonal antibody directed toward receptor activator of nuclear factor κB-ligand, fall into the category of antiresorptive agents (5,6). The most effective proanabolic agent available for treatment of osteoporosis is teriparatide (parathyroid hormone 1–34) (1,7–13). Teriparatide can increase osteoblast number and activity and dramatically increase cancellous bone mass in patients with osteoporosis. Modest anabolic responses have also been attributed to calcium, vitamin D, insulin-like growth factor-I, and strontium (14).

Epidemiological, clinical, and animal studies suggest that dietary consumption of fruits and vegetables and in particular dried plum (DP)[7] (*Prunus domestica L.*) may be an effective therapy for osteoporosis and has distinct advantages over other treatments (15–24). DP provides an excellent source of polyphenolic compounds, fiber, potassium, and vitamin K, and dietary supplementation has been reported to have both antiresorptive and proanabolic effects (17,18,20,25,26). Furthermore, DP supplementation improves bone strength (19) and is nearly as effective as parathyroid hormone in restoring bone volume in orchidectomized rats (17).

Although DP has been shown to be effective in preventing bone loss and restoring bone already lost due to gonadal hormone deficiency, the beneficial effects of DP on age-related bone loss have not been studied. In the mouse model of age-related bone loss, cancellous bone volume decreases by ~60% between the ages of 6 wk and 24 mo (27–29). Trabecular

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[3] Supplemental Tables 1 and 2 are available with the online posting of this paper at http://jn.nutrition.org.
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[7] Abbreviations used: BFR, bone formation rate; Bone Ar., bone area; BV/TV, bone volume/total volume; Conn.D., connectivity density; Cortical Th., cortical thickness; DA, degree of anisotropy; DP, dried plum; 0% DP, AIN-93 diet alone; 15% DP, AIN-93 diet with 15% dried plum; 25% DP, AIN-93 diet with 25% dried plum; Medullary Ar., medullary area; Mineral Dn., mineral density; Ob.S, osteoblast surface; Oc.S, osteoclast surface; P1NP, N-terminal pro-peptide of type 1 collagen; PYD, pyridinoline; ROI, region of interest; SMI, structure model index; Tb.N., trabecular number; Tb.Sp., trabecular spacing; Tb.Th., trabecular thickness; TFJ, tibia-fibular junction.

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architecture is altered, cortical thickness (Cortical Th.) decreases, and bone strength is compromised (27,30). To determine whether the loss of bone with aging can be prevented and whether bone that has already been lost in aged mice can be replaced, we fed adult and old male mice either a normal diet or diets supplemented with DP for 6 mo. Bone mass and structure were examined before, during, and after dietary supplementation.

Materials and Methods

Mice and diet. Sixty male C57B/6 mice, 6 and 18 mo of age, were obtained from the National Institute of Aging colony of aging rodents (Harlan Sprague Dawley) and divided into 3 dietary groups for each age with 10 animals in each group. The ages were chosen to be representative of adult (6 mo) and old (18 mo) mice. Mice were allowed to acclimate in our animal care facility for at least 3 d before experimentation and were housed in air-filtered, humidity- and temperature-controlled rooms with equal 12-h-light/12-h-dark cycles. The animal protocol for these studies was approved by the Animal Care and Use Committee at the Veterans Affairs Medical Center, San Francisco. Animals were maintained and processed in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Mice were fed either the AIN-93 diet alone (0% DP) or AIN-93 with 15% (15% DP) or 25% (25% DP) DP (*P. domestica L.*, Mayan Sun) by weight for 6 mo (18,31). A detailed description of the DP-supplemented diet can be found in **Supplemental Table 1**. Twenty-five percent DP by weight translates into ~30 prunes/d for a human. A detailed description of the composition of DP can be found in the USDA National Nutrient Database (32). The diets were designed to be isocaloric and isonitrogenous and to have the same concentrations of calcium and phosphorous. Food and water were consumed ad libitum and mice were weighed bimonthly. At baseline and after 3 and 6 mo, bone volume and structure were assessed using in vivo microCT analysis. Each mouse served as its own control. Mice in the 6- and 18-mo-old groups were killed using an overdose of isoflurane followed by bilateral thoracotomy at 12 and 24 mo of age, respectively. At the time the mice were killed, blood was collected from the heart and serum was harvested for assay of pyridinoline (PYD), a degradation product of type 1 collagen reflecting osteoclast activity or bone resorption (Quidel), and N-terminal propeptide of type 1 collagen (P1NP), a product of newly forming collagen reflecting osteoblast activity or bone formation (Immunodiagnostic Systems). The left femur was fixed in 10% phosphate buffered formalin in preparation for histomorphometric analysis. Histomorphometry and measurement of the serum levels of P1NP and PYR were performed at the end of the experiment only (after the mice had been on their respective diets for 6 mo).

MicroCT. The distal femur and tibia-fibular junction (TFJ) were scanned in vivo and analyzed using a Scanco *Viva*CT 40 (Scanco Medical) microCT. The region of interest (ROI) for the distal femur was defined as the cancellous bone compartment beginning 0.6 mm proximal to the most proximal point of the growth plate and extending proximally 1.0 mm. A global threshold, set at 376 mg hydroxyapatite/cm^3, was applied to distinguish mineralized from soft tissue (lean and fatty marrow). The ROI for the TFJ was defined as the region beginning 0.02 mm proximal to the bifurcation of the TFJ and extending proximally 0.2 mm. The threshold was set at 590 mg of hydroxyapatite/cm^3. Trabecular bone volume expressed as a percent of total volume (BV/TV), trabecular number (Tb.N; 1/mm), thickness (Tb.Th; μm), and spacing (Tb.Sp; μm); connectivity density (Conn.D.; 1/mm^3), structure model index (SMI; ranges from 0 to 3 with 0 = plate-like and 3 = rod-like), degree of anisotropy (DA; 1 = isotropic, >1 increasingly anisotropic), and mineral density (Mineral Dn.) (mg hydroxyapatite/cm^3) were calculated using software provided by Scanco (Scanco Medical). Mineral Dn., the average density of the segmented fraction of the ROI not including the marrow fraction, is derived from the linear attenuation coefficient of the X-rays after calibration with a phantom of known hydroxyapatite densities. Total bone area (Bone Ar.) (mm^2), cortical Bone Ar. (mm^2), Cortical Th. (mm), medullary area (Medullary Ar.) (mm^2), and Mineral Dn. (mg of hydroxyapatite/cm^3) were calculated for the TFJ.

Bone histomorphometry. Mice were injected subcutaneously with calcein (10 mg/kg) and demeclocycline (10 mg/kg) on d 7 and 2, respectively, before being killed. The distal end of the right femur was processed undecalcified for quantitative bone histomorphometry. Osteoblast and osteoclast surfaces (Oc.S) were measured in 4-μm-thick sections stained according to the Von Kossa technique with a tetrachrome counterstain. Fluorochrome-based indices of bone formation [mineralizing surface, mineral apposition rate, and surfaced-based bone formation rate (BFR)] were measured in 8-μm-thick, unstained sections. All measurements were performed in the secondary spongiosa of the distal femoral metaphysis with the Osteomeasure System (Osteometrics).

Data analysis. Values are reported as mean ± SD. Differences were considered significant when $P < 0.05$. To assess the effects of diet and age on body weight (**Fig. 1**), we used a 2-way ANOVA combined with the Holm-Sidak post hoc test. To assess the effects of diet, we first used a 1-way repeated measures ANOVA for each diet within an age group (Figs. 2–4). Each diet was examined separately. To assess the between-diet differences at 3 and 6 mo, we used a 2-way ANOVA with time as within-subject factor and diet as a between-subject factor. Analyses to determine interactions of age with diet and time were performed using a 3-way factorial ANOVA with repeated measures with time as within-subject and age and diet as between-subject factors. We compared various residual covariance structures and selected an unstructured covariance matrix, because it appeared to offer the best model fit for the variety of outcomes in this study. Post hoc comparisons using unpaired *t* tests with a Holm-Sidak adjustment were used to identify the specific effects of age, diet, and time. To assess the effects of age and diet on the bone variables at the end of the experiment (Tables 1–4), we used a 2-way ANOVA combined with the Holm-Sidak post hoc test. An unpaired *t* test was used to compare the percent change from 0 to 25% DP between adult and old mice. All statistical analyses were carried out using SigmaStat except for the 3-way factorial ANOVA with repeated measures, which was performed using SAS. We also performed power analyses (ANOVA) to evaluate minimum detectable differences.

Results

The body weights of adult mice fed 25% DP were lower (−23%; $P < 0.01$) than mice fed 0% DP at the end of the experiment (Fig. 1). The diets did not affect body weights of the old mice (data not shown). Body weights in both adult and old mice in

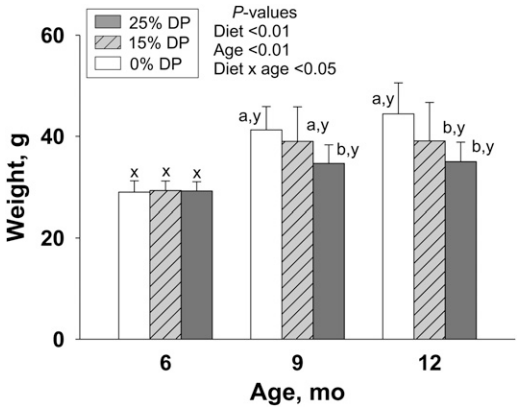

FIGURE 1 Body weights of adult mice after 3 and 6 mo of being fed diets containing 0, 15, or 25% DP. Values are mean ± SD, n = 9–10. Means without a common letter (x, y, z) within a diet group differ, $P <$ 0.05. Means without a common letter (a, b, c) within an age group differ, $P < 0.05$.

each diet group increased with increasing age. During the course of the study, 1 adult mouse died and 5 old mice (2 in the 0% DP, 1 in the 15% DP, and 2 in the 25% DP groups) died. The deaths in the old mice were expected based on the mean life span of male C57B/6 mice (28 mo) (33,34).

Trabecular bone. Dietary supplementation with 25% DP increased cancellous bone volume at the distal femoral metaphysis by 48% in adult mice and by 34% in old mice (**Fig. 2***A*,*B*). These percent changes were not significantly different. Adult and old mice fed 0% DP lost 24 and 28% of their bone, respectively. Bone volume increased in the adult but not old mice fed 15% DP (adult vs. old; $P < 0.03$). The increase in bone volume in the adult mice fed 15 and 25% DP and in the old mice fed 25% DP occurred during the first 3 mo. No further increase occurred between 3 and 6 mo.

The changes in bone volume in the distal femoral metaphysis were associated with changes in Tb.N (Fig. 2*C*,*D*), Tb.Th (**Fig. 3***A*,*B*), and Tb.Sp (Fig. 3*C*,*D*). Tb.N increased in adult (+10%; $P < 0.002$) but not old mice fed 25% DP and decreased in mice of both ages fed 0% DP (Fig. 2*C*,*D*). Tb.N in the 25% DP (but not 15% DP) supplemented adults was significantly greater than in the 0% DP diet group at both 3 and 6 mo but only after 3 mo in the old mice. Tb.Th increased in the adult (+12%; $P < 0.02$; 25% DP only) but not old mice (Fig. 3*A*,*B*). Tb.Sp decreased in adult mice fed either 15 or 25% DP, remained unchanged in old mice fed 25% DP, and increased in both adult and old mice fed 0% DP (Fig. 3*C*,*D*).

Conn.D. increased in adult (+88%; $P < 0.04$) and old (+286%; $P < 0.02$) mice receiving 25% DP, whereas no change occurred in mice fed 0% DP (**Fig. 4***A*,*B*). Conn.D. was greater in mice fed 25% DP than 0% DP for both ages. The SMI decreased in adult (−22%; $P < 0.001$) and old (−19%; $P < 0.01$) mice receiving 25% DP, whereas little (+8%; $P < 0.05$, adult) or no significant change (old) occurred in mice receiving the 0% DP diet (Fig. 4*C*,*D*). The DA increased in both adult and old mice fed all 3 diets (data not shown). The relative responses of bone to 15 and 25% DP were similar in the adults, whereas the responses to the 15% DP diet in old mice more closely tracked old mice receiving the 0% DP diet. Adult mice fed 0 and 15% DP differed significantly in all variables except Tb.Th and Conn.D. Those fed 0 and 25% DP differed significantly in all variables except Tb.Th, and those fed 15 and 25% DP differed significantly in Tb.N., Tb.Sp., and SMI. Old mice fed 0 and 15% DP did not differ significantly in any of the variables. Those fed 0 and 25% differed significantly in BV/TV, Tb.N., Conn.D., and SMI, and those fed 15 and 25% DP differed significantly in BV/TV, Tb.N., and Conn.D. The results of the 3-way ANOVA with repeated measures are given in **Supplemental Table 2**.

At the end of the 6-mo study, cancellous bone volume was higher in the adult mice than in the old mice in all 3 dietary treatment groups ($P < 0.001$) (**Table 1**). Within each age group, increasing DP supplementation was associated with greater bone volume, with differences significant between mice fed 0 and 25% DP ($P < 0.05$) and between those fed 15 and 25% DP ($P < 0.05$) but not between those fed 15 and 0% DP. The differences in magnitude of the percent changes between the mice fed 0 and 25% DP were greater in the adult (+78%) than in the old (+33%) mice (adult vs. old; $P < 0.001$). A comparison was made for measurements of bone volume using microCT analysis and bone histomorphometry. Bone volumes using these 2 techniques were correlated ($R^2 = 0.88$; $P < 0.001$).

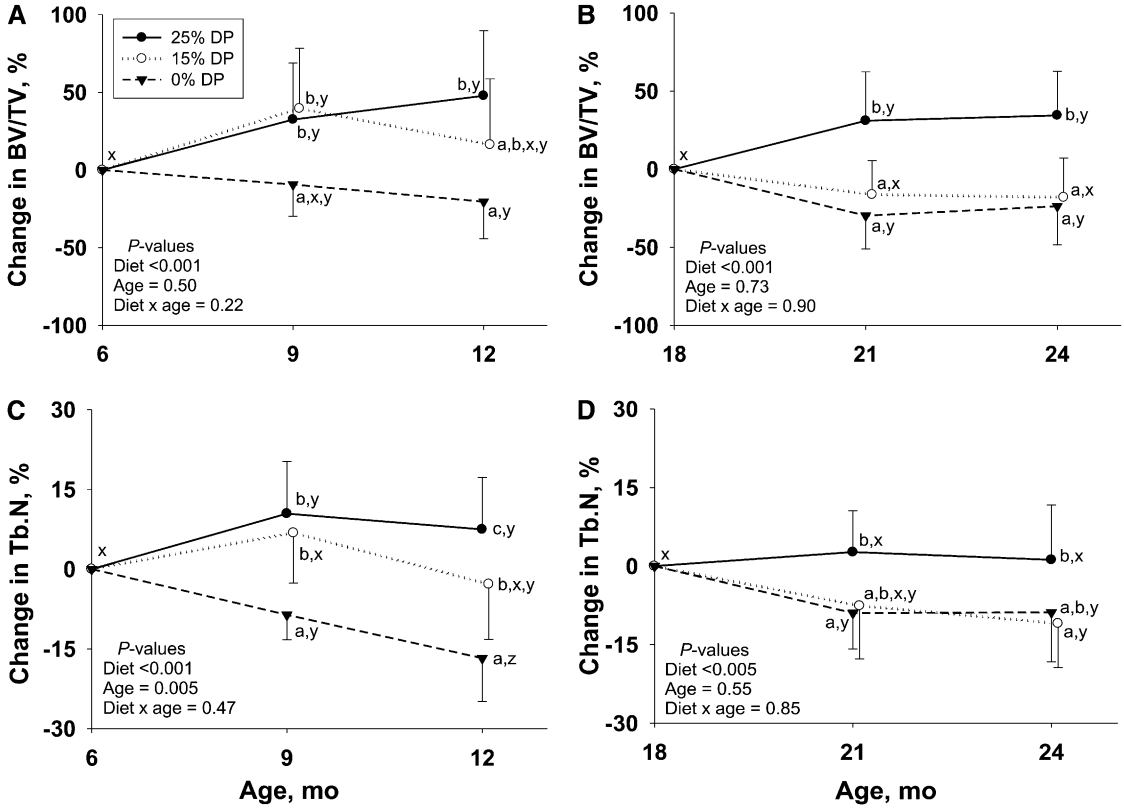

FIGURE 2 BV/TV (*A*,*B*) and Tb.N (*C*,*D*) at the distal femoral metaphysis in adult (*A*,*C*) and old (*B*,*D*) mice after 3 and 6 mo of being fed diets containing 0, 15, or 25% DP. Values are means ± SD, n = 7–10. Means without a common letter (x, y, z) within a diet group differ, $P < 0.05$. Means without a common letter (a, b, c) within an age group differ, $P < 0.05$.

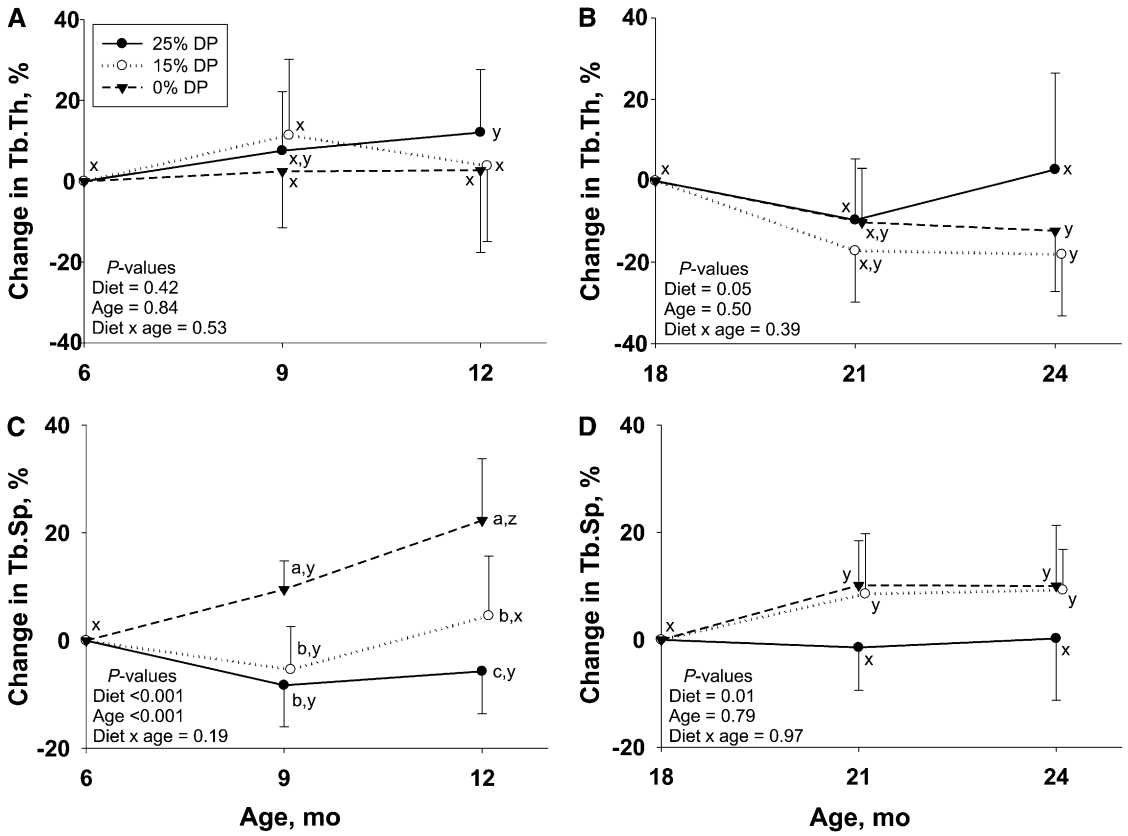

FIGURE 3 Tb.Th (*A,B*) and Tb.Sp (*C,D*) at the distal femoral metaphysis in adult (*A,C*) and old (*B,D*) mice after 3 and 6 mo of being fed diets containing 0, 15, or 25% DP. Values are mean ± SD, n = 7–10. Means without a common letter (x, y, z) within a diet group differ, $P < 0.05$. Means without a common letter (a, b, c) within an age group differ, $P < 0.05$.

Tb.N, Tb.Sp, Conn.D., SMI, and DA were affected by age and diet ($P < 0.01$), but neither age nor diet affected trabecular bone Mineral Dn. (overall mean = 724 ± 9 mg hydroxyapatite/cm^3). Age blunted the responsiveness of BV/TV ($P = 0.02$) and DA ($P < 0.05$) to dietary supplementation with DP.

Osteoblast surface (Ob.S) ($P < 0.01$), BFR ($P < 0.01$), and Oc.S ($P = 0.08$) were all lower in old mice than in adult mice (**Table 2**). Ob.S and Oc.S were unaffected by diet. The mineral apposition rates in mice fed 0, 15, and 25% DP tended to be greater in adult (0.82 ± 0.28, 0.92 ± 0.30, 1.11 ± 0.34, respectively) than in old mice (0.63 ± 0.13, 0.88 ± 0.49, 0.87 ± 0.29, respectively; $P = 0.06$–0.10). The BFR was greater in adult than in old mice ($P < 0.01$) and tended to increase ($P < 0.08$) in response to dietary plum but only in the adult mice ($P < 0.05$; for diet × age interaction).

Cortical bone. During the 6-mo period of dietary plum supplementation, cortical bone thickness (expressed as a percent change from the basal level) decreased by 9% ($P < 0.01$) and 7% ($P < 0.01$) in old mice fed 0 and 15% DP, respectively, but remained unchanged in mice fed 25% DP ($P = 0.19$) (data not shown). Cortical Th. did not change in adult mice fed all 3 diets. Cortical Bone Ar. decreased (−8%; $P < 0.01$) in old mice fed 0% DP but did not change in mice fed either 15 or 25% DP. There were no changes in adult mice. Medullary Ar. did not change in either adult or old mice fed the 25% DP diet but increased in both adult (+13%; $P < 0.01$) and old (+10%; $P < 0.01$) mice fed 0% DP. Mineral Dn. was higher ($P < 0.01$) in the old (1293 ± 8 mg hydroxyapatite/cm^3) than the adult (1265 ± 4 mg hydroxyapatite/cm^3) mice at baseline and increased in the adults fed all 3 diets (2–3%; $P < 0.05$). Mineral Dn. did not change in old mice fed either 15 or 25% DP.

At the end of the 6-mo study, Cortical Th. was less (−10%; $P < 0.03$) and Medullary Ar. greater (+28%; $P < 0.01$) in old nice than in adult mice fed 0% DP (**Table 3**). Compared with the 0% DP group, the 25% DP diet had increased Cortical Th. (+20; $P < 0.01$) and decreased Medullary Ar. (−20%; $P < 0.01$) in the old mice but had little or no effect (+10%, $P = 0.10$, +6%, $P = 0.45$, respectively) in the adults. Mineral densities were unaffected by diet. Bone formation could not be accurately determined on the periosteal surface due to very low levels of bone-forming activity at this site. However, the percentage of the endocortical surface with fluorochrome labeling (mineralizing surface/bone surface) was greater in the adult mice fed 25% DP (25 ± 13%; $P < 0.01$) than in adult mice fed either 15% DP (13 ± 7%) or 0% DP (10 ± 5%). The diets did not affect endocortical bone formation in old mice.

Serum markers of bone metabolism. The serum concentrations of P1NP did not differ among the diet groups for either adult or old mice but were lower in the old than in the adult mice ($P < 0.01$) (**Table 4**). The serum concentrations of PYD were unaffected by diet in the adult mice but decreased (−20%; $P < 0.05$) with increasing dietary plum in the old mice and differed between mice fed the 0 and 25% DP diets.

Discussion

We set out to determine whether the loss of bone with aging can be prevented and whether bone that has already been lost in aged

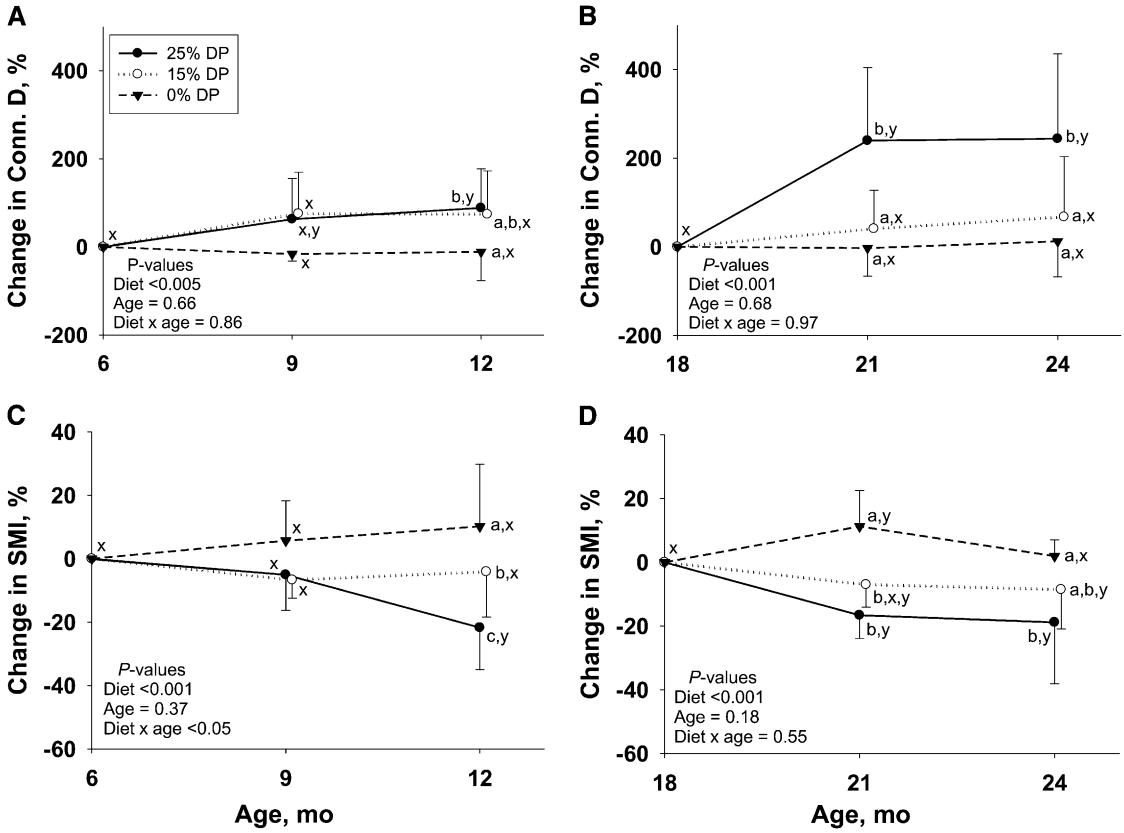

FIGURE 4 Trabecular Conn.D. (*A,B*) and SMI (*C,D*) at the distal femoral metaphysis in adult (*A,C*) and old (*B,D*) mice after 3 and 6 mo of being fed diets containing 0, 15, or 25% DP. Values are mean ± SD, n = 7–10. Means without a common letter (x, y, z) within a diet group differ, $P < 0.05$. Means without a common letter (a, b, c) within an age group differ, $P < 0.05$.

mice can be replaced by dietary supplementation with DP. Normally, beginning around 6–8 wk of age, male mice progressively lose cancellous bone as they age. Loss between 6 and 12 mo and 18 and 24 mo of age is approximately −30 and −20%, respectively (27). The losses in our mice fed 0% DP were comparable (−24 and −28%, respectively). Six months of dietary supplementation with 25% DP increased bone volume above basal levels by nearly 50% in the adults and 40% in the old mice. Dietary DP not only prevented loss but replaced bone that had already been lost due to aging. The magnitude of the changes in bone volume are similar to those in gonadal hormone-deficient male (+36%) and female (+50%) rats fed diets supplemented with 25% DP (17).

The effects of diet were greatest in the adult mice, suggesting that although both adult and old mice can respond to DP, the effects are blunted in the aged mice. In almost all cases, the response of adult mice to 15% DP was similar to the response to 25% DP, whereas old mice showed little or no response to 15% DP. Both the 2-way and 3-way factorial ANOVA showed a clear effect of age on bone responsiveness to dietary DP. The diet-related gain in bone volume during the course of the experiment

TABLE 1 BV/TV, Tb.Th, Tb.N, Tb.Sp, Conn.D., SMI, and DA in the distal femoral metaphysis in adult and old mice fed diets containing 0, 15, or 25% DP for 6 mo[1]

		Diet, % DP			*P*-value
	Age	0	15	25	(age × diet)
BV/TV, *%*	Adult	10.4 ± 2.9[a]	12.0 ± 2.6[a]	18.5 ± 5.7[b]	0.02
	Old	5.2 ± 1.5*	5.8 ± 2.8*	6.9 ± 1.1*	
Tb.N	Adult	4.2 ± 0.7[a]	4.5 ± 0.5[a,b]	4.9 ± 0.6[b]	0.14
	Old	2.9 ± 0.4*	2.9 ± 0.4*	2.9 ± 0.5*	
Tb.Th, *μm*	Adult	54 ± 9	54 ± 7	62 ± 8	0.94
	Old	50 ± 6	52 ± 9	59 ± 10	
Tb.Sp, *mm*	Adult	0.25 ± 0.04	0.23 ± 0.03	0.21 ± 0.02	0.15
	Old	0.35 ± 0.05*	0.34 ± 0.02*	0.36 ± 0.06*	
Conn.D., *1/mm*^{3}	Adult	82 ± 43[a]	104 ± 33[a]	144 ± 59[b]	0.54
	Old	28 ± 13*	37 ± 25*	61 ± 29*	
SMI	Adult	2.9 ± 0.3[a]	2.7 ± 0.3[a]	2.1 ± 0.4[b]	0.26
	Old	3.0 ± 0.2	3.0 ± 0.4	2.6 ± 0.6*	
DA	Adult	1.38 ± 0.09	1.39 ± 0.08	1.41 ± 0.06	0.05
	Old	1.34 ± 0.15[a]	1.38 ± 0.10[a]	1.55 ± 0.16*[,b]	

[1] Values are mean ± SD, n = 7–10. Means in a row with superscripts without a common letter differ, $P < 0.05$. *Different from adult, $P < 0.05$.

TABLE 2 Ob.S, Oc.S, and BFR in the distal femoral metaphysis in adult and old mice fed diets containing 0, 15, or 25% DP for 6 mo[1]

		Diet, % DP			*P*-value
	Age	0	15	25	(age × diet)
Ob.S, *%*	Adult	2.8 ± 2.0	3.7 ± 1.7	3.8 ± 2.5	0.47
	Old	1.8 ± 1.1	1.3 ± 1.0*	1.2 ± 1.0*	
Oc.S, *%*	Adult	0.5 ± 0.3	0.4 ± 0.2	0.4 ± 0.2	0.81
	Old	0.3 ± 0.2	0.4 ± 0.4	0.3 ± 0.2	
BFR, *μm*^{3}*/(μm*^{2}*·d)*	Adult	0.10 ± 0.06	0.11 ± 0.06	0.15 ± 0.06	0.04
	Old	0.03 ± 0.01*	0.09 ± 0.06	0.02 ± 0.01*	

[1] Values are mean ± SD, n = 7–10. *Different from adult, $P < 0.05$.

TABLE 3 Cortical Th., Bone Ar., Cortical Ar., Medullary Ar. and Mineral Dn. at the TFJ in adult and old mice fed diets containing 0, 15, or 25% DP for 6 mo[1]

		Diet, % DP			*P*-value
	Age	0	15	25	(age × diet)
Cortical Th., *mm*	Adult	0.22 ± 0.02	0.23 ± 0.01	0.24 ± 0.02	0.47
	Old	0.20 ± 0.02*,a	0.22 ± 0.01*,a,b	0.24 ± 0.03b	
Bone Ar., mm^2	Adult	1.26 ± 0.09	1.32 ± 0.11	1.28 ± 0.06	0.25
	Old	1.39 ± 0.05*,a,b	1.44 ± 0.16*,a	1.31 ± 0.10b	
Cortical Ar., mm^2	Adult	0.72 ± 0.07	0.76 ± 0.05	0.77 ± 0.05	0.65
	Old	0.70 ± 0.06a	0.78 ± 0.08b	0.76 ± 0.05a,b	
Medullary Ar., mm^2	Adult	0.54 ± 0.05	0.56 ± 0.09	0.51 ± 0.06	0.20
	Old	0.69 ± 0.04*,a	0.66 ± 0.10*,a	0.56 ± 0.09b	
Mineral Dn., mg/cm^3	Adult	1288 ± 23	1302 ± 18	1300 ± 16	0.86
	Old	1295 ± 20	1303 ± 18	1299 ± 27	

[1] Values are mean ± SD, n = 7–10. Means in a row with superscripts without a common letter differ, $P < 0.05$. *Different from adult, $P < 0.05$.

was accompanied by a slowing in the normal gain in body weight (adults only). This suggests that the changes in bone were not a consequence of increased skeletal loading or an overall increase in anabolic activity.

The changes in bone volume, Tb.N, Tb.Th, and Conn.D. occurred primarily during the first 3 mo, suggesting that the effects of DP reach a steady state by 3 mo and that continued consumption can sustain the gains in bone mass but not increase bone mass further. The decrease in the SMI (adult) and increase in DA (old) in mice receiving a DP diet suggest that the changes in bone volume are associated with a shift in trabecular structure from rod-like to more plate-like (adult) and mineral distribution from relatively random to more organized (old). That there was no effect on Mineral Dn. suggests that mineral structure is probably not adversely affected by supplementation of the diet with DP.

We measured indices of bone formation and resorption to determine whether the changes in bone volume induced by dietary supplementation with DP were linked to an increase in bone formation, a decrease in bone resorption, or both. We also measured serum concentrations of P1NP and PYD as global assessments of bone formation and resorption. Consistent with previous findings, trabecular bone formation was lower in the old mice than in adult mice. The serum concentration of P1NP was also lower in old mice and correlated (R^2 = 0.8; $P < 0.02$) with Ob.S. These data confirm that bone anabolic activity decreases with age in the male mouse.

Although dietary supplementation with DP was associated with a trend for increased BFR in adults (P = 0.076), there were no significant effects of diet on Ob.S and serum P1NP. Oc.S was also unaffected by diet, although there was a modest decrease (−18%; $P < 0.05$) in serum PYD (old mice only), suggesting that resorption may decrease with plum supplementation. Indices of bone formation and resorption were similar in mice receiving 0 and 15% or 25% DP for 6 mo. This appears incongruent with the relatively large changes in bone volume. This apparent discrepancy may be related to the time course in the response of bone to DP. Our bone volume data suggest that bone accumulation primarily occurred during the first 3 mo of diets supplemented with DP. Between 3 and 6 mo, there was no further gain in bone, suggesting that bone forming and resorbing activity during this time may not have been different from mice fed the 0% DP diet. It is also possible that there were small dietary effects on Ob.S and Oc.S but that these were obscured by the relatively large variances in these data (minimum detectable difference for Ob.S in the adults was 2.3%).

The larger Medullary Ar. in the old mice compared with the adult mice is consistent with our previous findings and with the normal age-related expansion of the periosteum and erosion of the endocortical surface. In the old mice receiving DP, Cortical Th. increased (+20%) and Medullary Ar. decreased (−20%), but the endocortical MS/BS was not changed. These results are similar to those observed in cancellous bone where, despite increased bone volume, indices of bone formation were not affected. Like cancellous bone, the apparent discrepancy in the changes in Medullary Ar. and mineralizing surface may be related to the time course in the response of bone to DP. That endocortical mineralizing surface increased but there was no significant decrease in Medullary Ar. in the adults is not clear. Taken together, our data suggest that dietary supplementation with DP increases bone formation on the endocortical surface but probably has little or no effect on the periosteal surface. Like trabecular bone, cortical bone Mineral Dn. was unaffected by diet, suggesting that supplementation with DP has little or no effect on mineral structure in cortical bone.

The constituents in DP responsible for the accumulation of bone in supplemented mice are not evident, but polyphenolic compounds may play an important role. DP is rich in polyphenols (180 mg/100 g dried fruit), including neochlorogenic and chlorogenic acid, caffeic acid, and rutin (21). Some of these have been shown to regulate osteoblast and osteoclast activity. Caffeic acid, e.g., can act as an antioxidant to protect against the oxidative stress-induced decrease in alkaline phosphatase and type 1 collagen expression in osteoblasts (22) and rutin has been reported to increase serum levels of osteocalcin and

TABLE 4 Serum concentrations of P1NP and PYD in adult and old mice fed diets containing 0, 15, or 25% DP for 6 mo[1]

		Diet, % DP			*P*-value
	Age	0	15	25	(age × diet)
P1NP, *μg/L*	Adult	40 ± 10	33 ± 11	36 ± 4	0.28
	Old	26 ± 15*	29 ± 9	18 ± 7*	
PYD, *nmol/L*	Adult	1.3 ± 0.2	1.5 ± 0.2	1.4 ± 0.4	0.03
	Old	1.6 ± 0.2*,a	1.5 ± 0.1a,b	1.3 ± 0.2b	

[1] Values are mean ± SD, n = 7–10. Means in a row with superscripts without a common letter differ, $P < 0.05$. *Different from adult, $P < 0.05$.

increase bone mass (23). The high alkaline and potassium contents of DP may also contribute to its beneficial effects on bone (24). Identification of the bioactive components in DP is underway.

Collectively, our data suggest that dietary supplementation with DP can not only prevent bone loss but can also replace bone that has already been lost due to aging. Moreover, both adult and old mice are responsive. This remarkable observation, which has also been shown for gonadal hormone deficiency-induced osteopenia in rats, suggests that dietary DP may provide an effective prophylactic and therapeutic intervention for osteoporosis.

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