Mango Modulates Blood Glucose Similar to Rosiglitazone without Compromising Bone Parameters in Mice Fed High Fat Diet

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Abstract: Both consumption of high-fat diet and one of the commonly used pharmacological therapies for modulating blood glucose, rosiglitazone, are associated with negative effects on bone. Previously, we reported that a diet supplemented with freeze-dried mango modulated blood glucose similar to rosiglitazone in mice fed a high-fat (HF) diet. This study examined the effects of the addition of freeze-dried mango pulp or rosiglitazone to a HF diet on bone parameters in mice. Six week old male C57BL/6J mice were randomly assigned into one of five dietary treatment groups (n=8-9 mice/group): control (9.5% calories from fat), HF (58.9% calories from fat), HF+1% or 10% mango (w/w), and HF+rosiglitazone (50 mg/kg diet) for eight weeks. Bone parameters were assessed *via* dual energy x-ray absorptiometry and micro-computed tomography. Both the HF and HF+rosiglitazone groups had lower whole body, tibial, and vertebral bone mineral density compared to the HF+1% mango group. Trabecular bone volume, number, and separation as well as bone strength were also compromised by HF+rosiglitazone while the mango diets maintained these bone microarchitecture parameters to that observed in the control group. These results suggests that addition of mango to the diet may provide an alternative approach to modulating blood glucose without negatively affecting skeletal health, though human studies are needed to confirm these findings. Additionally, the bioactive component(s) in mango and the mechanisms by which it modulates blood glucose and exerts potentially osteoprotective benefits warrants further investigation.

Keywords: Mango, high fat diet, rosiglitazone, bone.

INTRODUCTION

Certain lifestyle and nutritional factors are associated with an increased risk of developing osteoporosis. Lifestyle factors such as smoking and nutritional factors including excessive alcohol consumption and a diet high in saturated fat, impose long-term consequences on skeletal health and the development of many chronic diseases [1-4]. Indeed, diets high in saturated fat impair bone mineralization in both animal models and humans [1-4] and contribute to impaired glucose tolerance, insulin resistance, and type 2 diabetes mellitus [1]. The use of pharmacological agents for the treatment of chronic conditions such as diabetes also negatively affects bone [5-9]. Although diabetic medications are efficacious at treating abnormalities in glucose homeostasis, they may have other less desirable side-effects. For example, the widely prescribed oral glucose-lowering medication, rosiglitazone (Avandia), used in the treatment of type 2 diabetes mellitus, is associated with increased fracture risk and rapid bone loss in both human and animal studies [5-9]. Therefore, lifestyle and dietary practices that compromise bone health should be avoided whereas practices that maintain or improve bone health should be encouraged.

The general recommendations for a healthy diet include consuming a variety of fresh fruits and vegetables, choosing whole over refined grains, limiting fat intake, and balancing total caloric intake with regular physical activity. Numerous studies have focused on the bioactive components in plants and their mechanisms of action in preventing chronic diseases including the optimal maintenance of bone health [10-12]. Consumption of fruits and vegetables, or bioactive components derived from them, has been associated with reduced risk of osteoporosis in human observational studies and based on animal studies, may actually increase bone mineral density (BMD) [10-11, 13-14]. Fruits and vegetables contain a wide variety of vitamins and minerals as well as phytochemicals which may contribute to maintaining optimal bone health. Bioactive compounds found in fruits and vegetables exhibit antioxidant and anti-inflammatory properties and reduce the renal acid load, actions which may also function as bone-sparing agents [15-17]. Chronic inflammation and conditions linked with increased oxidative stress are also associated with the consumption of a high fat (HF) diet, obesity, diabetes, and in the development of osteoporosis [18-19]. The alkalizing, antioxidant, and anti-inflammatory properties of fruits and vegetables are just a few of the potential mechanisms by which bioactive food components may play a protective role in preventing bone loss.

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Among fruits, mangos (*Mangifera indica L.*) are excellent sources of the antioxidants vitamin C and carotenoids, in addition to being good sources of dietary fiber and vitamin K [20]. Mangos also contain minerals such as potassium, magnesium, zinc, copper, manganese, and selenium. These fruits are good sources of phenolic compounds such as tannins, mangiferin and flavonoids, which may contribute to its antioxidant and anti-inflammatory properties [20]. Despite the number of bioactive compounds found in mango, relatively few studies have been conducted to investigate the potential health benefits of regular mango consumption. Although mango is usually obtained from the diet in the form of the pulp, other parts of the mango fruit (e.g., skin) and the tree (e.g., bark and leaves) have been found to exhibit potent antioxidant, antibacterial, anti-viral, anti-HIV, anti-cancer, anti-diabetic, anti-atherogenic properties, anti-inflammatory, and immune-modulatory properties [21-25]. Additionally, mangiferin, a major phenolic compound found in mango, is capable of reducing alveolar bone loss in a rat model of experimental periodontitis [26] and suppressing activation of a key signaling pathway in osteoclastogenesis [27]. The majority of the research on mango has focused on the stem bark extract of mango that is particularly rich in mangiferin. To our knowledge, there are no studies investigating the effect of mango fruit on bone health in the context of HF diet. Therefore, this study compared the effects of freeze-dried mango pulp to the hypoglycemic drug rosiglitazone on skeletal health in mice fed HF diet. We hypothesized that freeze-dried mango pulp, in addition to lowering blood glucose to a similar level as rosiglitazone as we have previously reported [28], would not exert the same negative consequences of rosiglitazone on bone mass and structural parameters in mice fed HF diet.

MATERIALS AND METHODS

Diet and Animal Care

Three month-old male C57BL/6J mice were obtained from Harlan Teklad (Indianapolis, IN). All animal handling and procedures were approved by the Institutional Animal Care and Use Committee of the Oklahoma State University. Mice were acclimated to a standardized powdered rodent diet (AIN 93M; control) [29] for three days. After acclimation, mice were weighed and randomly assigned to one of five dietary treatment groups (n=8-9 mice/ group): control, HF, HF+1% or 10% (w/w) freeze-dried mango pulp, and HF+rosiglitazone (50 mg/kg diet; Cayman Chemicals, Ann Arbor, MI). Mice were group-housed in a humidity and temperature-controlled room with a 12 hr light:dark cycle. The HF diets were based on formulation of Molnar and colleagues [30] and adjusted to have the same macronutrient composition, as well as calcium and phosphorus content (Table **1**). The energy contribution of the control versus HF diets were: fat (9.5% vs. 58.9% of total kcal), carbohydrates (75.8% vs. 27.7% of total kcal), and protein (14.7% vs. 13.4% of total kcal).

Mangos (Tommy Atkins variety) were obtained from U.S. importers, ripened to a soluble solids content of >10%, and peeled. A portion of the mango pulp was used for characterization of ascorbic acid, carotenoids, and phenolic compounds [31] and the remainder was freeze-dried, ground, and analyzed for its nutrient composition prior to incorporation into the powdered diet at 1% or 10% concentration by weight. The doses of freeze-dried mango were based on our earlier animal study with dried plum [32] and those of others that investigated the effects of dried fruits on clinical parameters [13, 33-34]. Based on the limited information available with freeze-dried mango pulp, we chose to explore the effect of the 1% and 10% doses. The dose and mode of administration of rosiglitazone (50 mg/kg diet) was based on the studies by Chao *et al.* [35].

Food and deionized water were provided *ad libitum*. Food intake was monitored daily for eight weeks during the mid-portion of the light cycle. Mice were weighed weekly.

Glucose Tolerance Test and Plasma Glucose

After seven weeks of dietary treatment, an intraperitoneal (IP) glucose tolerance test (IPGTT) was performed. Six mice were randomly selected from each group and fasted for 12 hours. Mice were injected IP with a 20% glucose solution at a dose of 2 g glucose/kg body weight. Blood glucose concentration was determined in tail vein blood samples collected at 0, 5, 15, 30, 60 and 120 minutes after glucose injection using a glucose testing kit (Onetouch Ultra, LifeScan, Inc. Milpitas, CA). To examine glucose tolerance, differences in blood glucose concentration between groups at each time point were evaluated and the area under the glucose curve (AUC) was calculated by the trapezoidal rule [36]. An Alfa Wassermann (West Caldwell, NJ) clinical chemistry analyzer was used to determine plasma glucose concentration according to the manufacturer's instructions. Insulin resistance was estimated by the homeostasis model assessment of

Table 1: Composition of the Experimental Diets

Ingredient	Normal diet[a] (AIN-93M)	High fat (HF) diet[b,c]	HF diet + 1% mango	HF diet + 10% mango
Amount g/kg diet				
Mango[d,e]	0	0	10	100
Total carbohydrate	720	370	370	370
Cornstarch	620	100	94	25.2
Sucrose	100	270	270	270
Total fat	40	350	350	350
Soybean Oil	40	40	39.4	33.5
Lard	0	310	310	310
Total Protein	140	180	180	180
Casein	140	180	179.6	176.1
Total fiber	50	50	50	50
Cellulose	50	50	49.6	31
Vitamin Mix (AIN 93VX)	10	10	10	10
Mineral Mix (AIN 93 MX)	35	35	35	35
Choline Bitartrate	2.5	2.5	2.5	2.5
L-cysteine	1.8	1.8	1.8	1.8
Tert-butylhydroquinone	0.008	0.008	0.008	0.008
Total calories[e] *kcal/ 100g diet*	379	549	553	546

[a]Based on AIN-93M formulations with 75.8%, 14.7%, and 9.5% of total calories coming from carbohydrate, protein, and fat, respectively [29].
[b]HF diet was based on formulation of Molnar *et al.* [20] with 27.7%, 13.4% and 58.9%, of total calories coming from carbohydrate, protein, and fat, respectively.
[c]Rosiglitazone (Cayman Chemicals, Ann Arbor, MI) was added to HF diet at a dose of 50 mg/kg diet.
[d]Freeze-dried Tommy Atkins variety mango with the following nutrient composition (g/100 g) carbohydrates, 74.8; protein, 3.9; fat, 6.5; fiber, 3.8; calcium, 0.0424; phosphorus, 0.0941.
[e]Analyzed by NP Analytical Laboratories (St. Louis, MO).

insulin resistance (HOMA-IR) as previously described [37] using the following equation:

HOMA-IR = [fasting insulin (μU/mL) x fasting glucose (mmol/L)] / 22.5.

Although originally developed and validated in humans, HOMA-IR is an accepted measure that can be used to estimate insulin sensitivity and resistance in mice [38].

Necropsy, Tissue Processing and Assessment of Bone Mineral Area, Content, and Density

After 8 weeks of treatment, mice were anesthetized with a ketamine/xylazine cocktail (70 and 3 mg/kg body weight, respectively). Whole body bone mineral area (BMA), content (BMC) and density (BMD) were measured using a GE Lunar PIXImus series densitometer (Madison, WI) prior to collection of tissues. Blood was drawn from the carotid artery into EDTA-coated tubes. Bones were harvested, cleaned of adhering tissues and stored at -20°C freezer until analyzed. BMA, BMC, and BMD of the left and right tibiae as well as the lumbar vertebrae (L4-L6) were also assessed by the PIXImus densitometer. Daily quality control procedures were followed before scanning according to the manufacturer's guideline.

Assessment of Bone Microarchitecture Using Micro-Computed Tomography (μCT)

Tibiae and L4 vertebrae were scanned using μCT (MicroCT40, SCANCO Medical, Switzerland) to examine trabecular and cortical bone microarchitecture. All scans were performed using a 1024 x 1024 matrix resulting in an isotropic voxel resolution of 22 μm^3. An integration time of 70 milliseconds per projection was used with a rotational step of 0.36 degrees resulting in total acquisition time of approximately 150 minutes/sample.

The proximal tibial metaphysis and mid-diaphysis regions were scanned to assess microarchitectural properties of trabecular and cortical bone, respectively.

The proximal tibia was scanned from the growth plate in the proximal direction to acquire 350 slices (~16 μm/slice). Contours were semi-automatically placed to incorporate the secondary spongiosa beginning 25 slices (400μm) from the growth plate and 150 images (2400 μm) identified in the volume of interest (VOI). The midshaft of the tibia was evaluated by acquiring 34 slices at the midpoint and evaluating 30 (480 μm) of these slices.

The L4 vertebrae were scanned in a cranial-caudal direction so that 530 transverse slices were acquired to evaluate trabecular bone microarchitectural changes of the spine associated with a HF diet and mango supplementation compared to rosiglitazone. The VOI included the secondary spongiosa in the vertebral body or approximately 300 slices (4800 μm).

The bone morphometric parameters assessed with μCT included trabecular bone volume expressed as a percent of total volume (BV/TV), trabecular number (TbN), trabecular thickness (TbTh) and trabecular separation (TbSp). Non-metric parameters of the trabecular bone included structure model index (SMI) and connectivity density (Conn). Four cortical parameters were assessed at the midshaft of the tibia, including cortical area, porosity, thickness and medullary area. Coefficients of variations were 2.0% (BV/TV), 1.1% (TbN), 0.66% (TbTh) and 1.30% (TbSp) for morphometric and 4.6% (Conn Den) and 2.7% (SMI) for non-metric parameters.

Simulated Biomechanical Testing of Tibia and L4 with Finite Element Analysis

μCT analyses allowed for the development of finite element (FE) models by converting bone voxels (from the VOI) into 8-node brick elements [39]. The apparent mechanical properties chosen for each bone included: linear, elastic and isotropic with a Poisson's ratio of 0.3 and a Young's modulus of 10 GPa [39]. Compression testing was simulated on the region of interest from the scan of each tibia and vertebral body. The apparent stiffness, strains and stresses for a given force were calculated from the results of FE analyses.

Biomarkers of Bone Formation and Resorption

The bone formation marker, N-terminal propeptide of procollagen type 1 (PINP), and bone resorption marker, pyridinoline (PYD), were assessed in plasma samples. The concentrations of both biomarkers were determined using enzyme-linked immuno-assays (ELISA) following the manufacturer's specifications. The ELISA kits to assess PINP and PYD were purchased from Immunodiagnostic Systems Ltd. (Fountain Hills, AZ) and Quidel Corporation (San Diego, CA), respectively.

Statistical Analyses

Statistical analyses involved computation of means and standard error (SE) for each of the treatment groups using SAS version 9.1 (SAS Institute, Cary, NC). The significance of treatment effects was analyzed by the one-way ANOVA model followed by *post-hoc* analysis using Fisher's least square means separation test when F values were significant. Differences were considered significant at $P < 0.05$.

RESULTS

Glucose Parameters and Whole Body, Tibial, and Lumbar BMC, BMA, and BMD

The fasting plasma glucose of mice in the HF+1% mango group, but not those in the HF+10% mango group, was statistically similar to the HF+rosiglitazone group (Table **2**). The effect of dietary treatments on HOMA-IR corresponded with the changes observed in plasma glucose (Table **2**). In agreement with a lower plasma glucose levels, insulin resistance was lowest in the HF+rosiglitazone group as indicated by low HOMA-IR values. Similar to the plasma glucose values, HOMA-IR value of the control group was not statistically different from the HF group. Mice receiving both doses of freeze-dried mango diets exhibited HOMA-IR values that were similar to the HF+ rosiglitazone group.

To examine glucose tolerance and clearance from the plasma, an IGTT was performed following a 12 hr fast. The 1% dose of freeze-dried mango normalized blood glucose concentrations in response to HF diet as indicated by approximately 35% reduction in the glucose AUC compared to mice in the HF group (Table **2**). The effect of 1% mango on AUC was similar to that observed with the rosiglitazone; however, the response to rosiglitazone can be considered intermediate since mice in 10% freeze-dried mango, the control, and rosiglitazone groups were all statistically similar to the HF diet in terms of their effect on glucose tolerance.

Whole body, tibial, and vertebral BMA were similar among all the treatment groups (Table **2**). BMC of the whole body (P=0.0559), tibia (P=0.0597), and lumbar vertebra (P=0.0911) tended to be higher in the mice receiving either dose of the freeze-dried mango compared to the HF+rosiglitazone group. BMD of the

Table 2: Glucose Parameters and Whole Body, Tibial, and Lumbar Bone Mineral Content (BMC), Area, and Density (BMD)

Parameters	Control	High fat diet (HF)	HF diet+ 1% Mango	HF diet+ 10% Mango	HF diet+ Rosiglita-zone	*P* value
Glucose parameters[1]						
Plasma glucose (mg/dl)	235.7± 25.6^{a}	231.9± 10.2^{ab}	153.8± 20.7^{cd}	179.8± 21.2^{bc}	122.1± 9.3^{d}	0.0002
HOMA-IR	2.98±0.39^{ab}	3.36±0.32^{a}	2.08±0.30^{bc}	2.11±0.27^{bc}	1.38±0.20^{c}	0.0006
AUC (mg/dl•min)	36031± 1351^{ab}	43289± 1926^{a}	27710± 3035^{b}	38740± 5111^{a}	36246± 3098^{ab}	0.0170
Whole body						
BMC (mg)	458.6 ± 11.5	450.7 ± 19.8	486.8 ± 23.1	505.3 ± 24.4	425.5 ± 15.5	0.0559
Area (cm^2)	9.118 ± 0.178	9.182 ± 0.289	9.346 ±0.324	9.944 ±0.379	8.916 ±0.230	0.1388
BMD (mg/cm^2)	50.28 ± 0.49^{ab}	48.96 ± 0.69^{bc}	51.96 ±0.9^{a}	50.91 ±1.07^{ab}	47.65 ±0.89^{c}	0.0094
Tibia						
BMC (mg)	23.6 ± 0.64	22.5 ± 0.69	25.6 ± 1.1	25.0 ± 1.0	22.7 ± 0.88	0.0597
Area (cm^2)	0.492 ± 0.008	0.478 ± 0.009	0.498±0.012	0.498 ± 0.013	0.494 ± 0.012	0.7353
BMD (mg/cm^2)	48.11 ± 0.72^{bc}	46.83 ± 0.75^{c}	50.91 ±1.22^{a}	49.93 ±1.03^{ab}	46.09 ± 0.84^{c}	0.0026
Lumbar						
BMC (mg)	20.9± 0.64	19.9± 0.52	21.7± 0.97	21.6± 1.2	18.8± 0.75	0.0911
Area (cm^2)	0.425± 0.006	0.419± 0.004	0.423± 0.007	0.430± 0.009	0.428± 0.007	0.7739
BMD (mg/cm^2)	48.81 ± 0.96^{ab}	47.36 ± 1.09^{b}	51.24 ±1.52^{a}	49.86 ±1.68^{ab}	43.80 ± 1.51^{c}	0.0072

Values are mean ± SE, n=8-9 mice/group; within a row, values that do not share the same superscript letters are significantly (P<0.05) different from each other. HOMA-IR, homeostasis model assessment of insulin resistance; AUC, area under the curve.
[1]Glucose parameters have been previously reported [28].

whole body, tibia, and lumbar vertebra was significantly higher in mice fed the HF+1% mango compared to mice on the HF and HF+rosiglitazone treatments. Compared to the HF group, mice in the HF+1% mango exhibited 6.1%, 8.7%, and 8.2% higher whole body, tibial, and vertebral BMD, respectively. In comparison to the HF+rosiglitazone group, mice in the HF+1% mango had 9.0%, 10.4%, and 17.0% higher whole body, tibial, and vertebral BMD, respectively. The whole body, tibial, and vertebral BMD were significantly higher in the HF+10% mango group than the HF+rosiglitazone group but not statistically different from the HF group except for the tibial BMD. These findings provide further confirmation that consumption of HF diet has a negative effect on BMD which was exacerbated if combined with rosiglitazone. Furthermore, supplementation of the HF diet with 1% mango appeared to exert positive effects on BMD.

Microarchitectural Properties of the Tibia and Vertebra

Representative 3D images generated from the µCT scans of the proximal tibia metaphysis indicate that HF diet negatively affects trabecular microarchitecture (Figure **1a-e**). Moreover, the negative effect of the HF diet on bone microarchitecture is further aggravated by the addition of rosiglitazone (Figure **1e**). Both doses of mango were able to improve trabecular microarchitecture (Figure **1c & d**) as shown by the distribution of the trabecular network, and what appears to be increased connections between struts when compared to the microarchitecture of mice in the HF and HF+rosiglitazone groups.

Mice in the HF+1% mango group had higher BV/TV (30.4%) and TbN (7.7%), and lower (7.9%) TbSp in the proximal tibia metaphysis in comparison to the HF group (Figure **2a-c**); however, these changes were not statistically significant. Similar to BMD, the negative effects of HF diet on microarchitectural properties were exacerbated when combined with rosiglitazone. When compared to the HF +rosiglitazone group, the mice in the HF+1% mango group exhibited significantly higher tibial BV/TV (85.6%) and TbN (42.0%), and decreased (37.3%) TbSp (Figure **2a-c**). Tibial SMI of the HF+1% mango group tended (P=0.0795) to be lower compared to the HF+rosiglitazone group, indicating a more plate-like arrangement of the trabeculae and a stronger bone

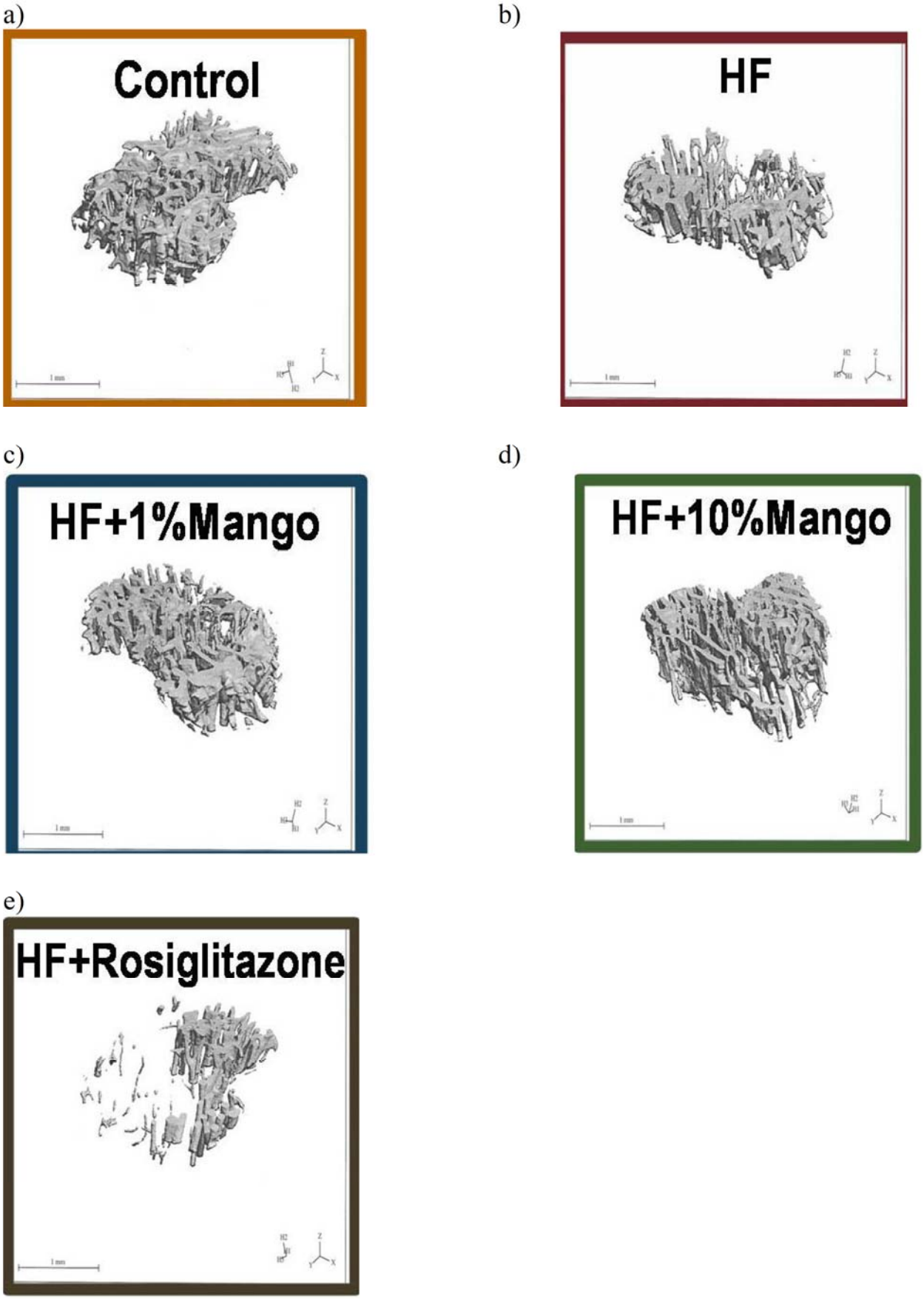

Figure 1: Representative images of the 3D structure of the trabecular bone in the proximal tibia metaphysis of mice given **a**) control, **b**) high fat (HF), **c**) HF+1% mango, **d**) HF+10% mango, or **e**) HF+rosiglitazone diet for two months.

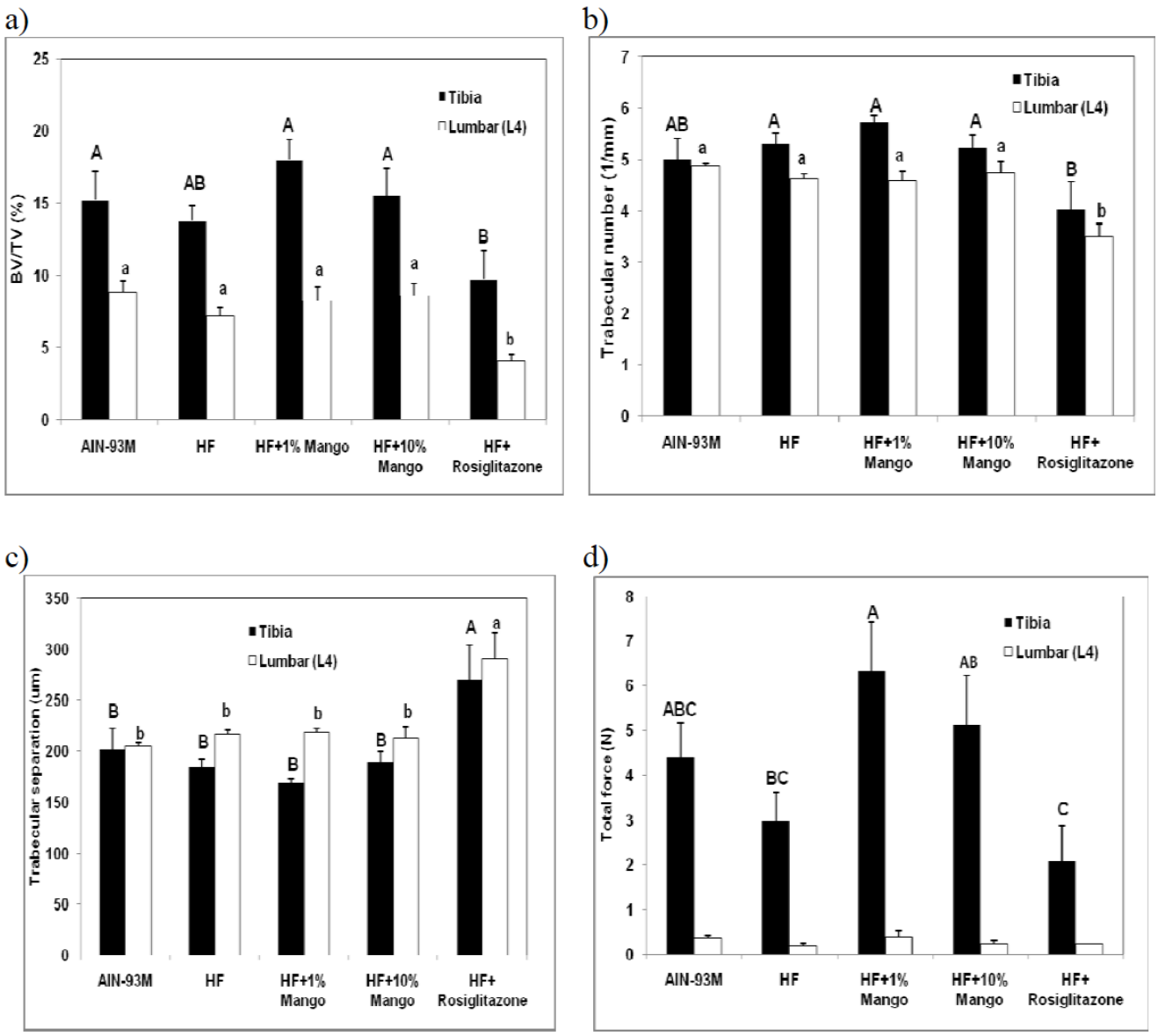

Figure 2: Alterations in **a**) trabecular bone volume (BV/TV), **b**) trabecular number (TbN), **c**) trabecular separation (TbSp), and **d**) total force at the proximal tibial metaphysis and vertebral body following 2 month consumption of control (AIN-93M), high fat (HF), and HF diets in combination with freeze-dried mango pulp or rosiglitazone in mice.

Bars are mean ± SE, n=8-9 mice/group; upper case and lower case letters are comparison between groups of the tibial and vertebral bone, respectively; within a set of bars, values that do not share the same superscript letters are significantly (P<0.05) different from each other.

in the proximal tibia (Table **3**). No significant differences in bone microarchitecture of the cortical bone at the tibial mid-diaphysis were detected (Table **3**).

Similar to the tibia, mice in the HF+rosiglitazone group had significantly higher vertebral TbSp and lower BV/TV, TbN, and connectivity compared to the other groups (Figure **2a-c** and Table **3**). HF+rosiglitazone group tended (P=0.0616) to have higher vertebral SMI, indicating a more rod-like arrangement of the trabeculae and a weaker bone, compared to the other groups. Aside from the effects of the combination of HF diet and rosiglitazone, no significant differences with the other dietary treatment groups were observed in the aforementioned lumbar microarchitectural parameters.

Biomechanical Assessment by Finite Element Analysis

The influence of dietary treatments on bone biomechanical properties of the tibia and vertebra was evaluated by the simulated compression testing with the μCT generated data (Table **4** and Figure **2d**). Mice in the HF+1% mango group had approximately 2- and

Table 3: Trabecular and Cortical Bone Microarchitecture

Parameters	Control	High fat diet (HF)	HF diet+ 1% Mango	HF diet+ 10% Mango	HF diet+ Rosiglita-zone	*P* value
Proximal tibia metaphysis						
TbTh (um)	54.0± 2.4	49.4± 1.5	51.1± 2.1	50.7± 2.0	55.0± 3.5	0.4415
SMI	2.24± 0.22	2.41± 0.13	1.90± 0.16	2.07± 0.21	2.61± 0.18	0.0795
Connectivity ($1/mm^3$)	99.2± 22.4	93.3± 15.5	143.4± 13.1	110.9± 16.6	75.7± 25.2	0.1745
Tibial middiaphysis						
Cortical thickness (mm)	0.219± 0.004	0.224± 0.005	0.229± 0.008	0.2270± 0.009	0.218± 0.008	0.7389
Cortical porosity (%)	0.82± 0.03	0.87± 0.04	0.80± 0.05	0.81± 0.06	0.85± 0.04	0.7647
Cortical area (mm^2)	0.741± 0.013	0.738± 0.0354	0.767± 0.053	0.799± 0.046	0.749± 0.037	0.7792
Medullary area (mm^2)	0.0064± 0.0003	0.0066± 0.0003	0.0060± 0.0002	0.0063± 0.0002	0.0065± 0.0002	0.5774
Lumbar						
TbTh (um)	33.3± 0.58	31.7± 0.70	34.2± 1.8	34.6± 1.2	31.5± 1.2	0.2913
SMI	2.52± 0.14	2.62± 0.07	2.54± 0.12	2.57± 0.07	2.95± 0.07	0.0616
Connectivity ($1/mm^3$)	114.7± 16.6[a]	94.4± 13.6[a]	102.3± 16.8[a]	111.52± 13.8[a]	21.1± 18.3[b]	0.0024

Values are mean ± SE, n=8-9 mice/group; within a row, values that do not share the same superscript letters are significantly (P<0.05) different from each other. TbTh, trabecular thickness; SMI, structure model index.

Table 4: Biomechanical Properties of the Tibia and Vertebral Body Using Finite Element Analysis

Parameters	Control	High fat diet (HF)	HF diet+ 1% Mango	HF diet+ 10% Mango	HF diet+ Rosiglita-zone	*P* value
Tibia						
Stiffness ($N/m \times 10^3$)	2467.9± 434.0	3376.2± 1425.4	6715.2± 2926.2	2870.6± 631.2	1161.0± 455.4	0.1385
Size independent stiffness (N/mm^2)	447.6± 80.5[abc]	320.3± 76.1[bc]	649.8± 100.8[a]	524.0± 98.3[ab]	206.4± 78.8[c]	0.0163
Average Von Mises stresses (MPa)	4.21± 0.21	3.28± 0.23	4.12± 0.35	4.15± 0.18	3.95± 0.33	0.2444
Lumbar						
Stiffness ($N/m \times 10^3$)	54.7± 12.5	31.6± 6.6	60.4± 24.3	36.3± 12.8	3.9± 1.8	0.0990
Size independent stiffness (N/mm^2)	93.6± 23.7	52.3± 10.7	95.6± 37.2	56.9± 19.9	5.9± 2.8	0.0863
Average Von Mises stresses (MPa)	1.85± 0.20[a]	1.53± 0.12[a]	1.83± 0.40[a]	1.31± 0.25[a]	0.69± 0.20[b]	0.0342

Values are mean ± SE, n=8-9 mice/group; within a row, values that do not share the same superscript letters are significantly (P<0.05) different from each other.

3-fold increase in tibial total force in comparison to the HF and HF+rosiglitazone groups, respectively (Figure **2d**). Size independent stiffness of the proximal tibia metaphysis was significantly higher in the HF+1% mango group in comparison to the HF and HF+rosiglitazone groups (Table **4**). There were no significant differences in the stiffness and average Von Mises stresses in the proximal tibia metaphysis.

There were no significant differences in vertebral total force (Figure **2d**) among the groups; however, stiffness (*P*=0.0990) and size independent stiffness

(*P*=0.0863) tended to be lower in the HF+rosiglitazone group (Table **4**). Vertebral average Von Misses stresses was significantly lower in the HF+rosiglitazone group compared to all the other treatment groups.

Bone Biomarkers

To determine if alterations in bone microarchitecture were associated with systemic measure of bone metabolism, plasma concentrations of PINP, an indicator of bone formation and PYD, a by-product of bone resorption, were assessed. Mice in the HF+1% mango group had the highest plasma concentrations of PINP while those in HF group had the lowest (Figure **3**). Plasma PINP of the HF+rosiglitazone group was statistically similar to the HF+1% mango and the control groups. Despite the negative effects of HF diet with or without rosiglitazone on bone microarchitecture in the tibia, none of the dietary treatments had a significant effect on the plasma PYD concentrations.

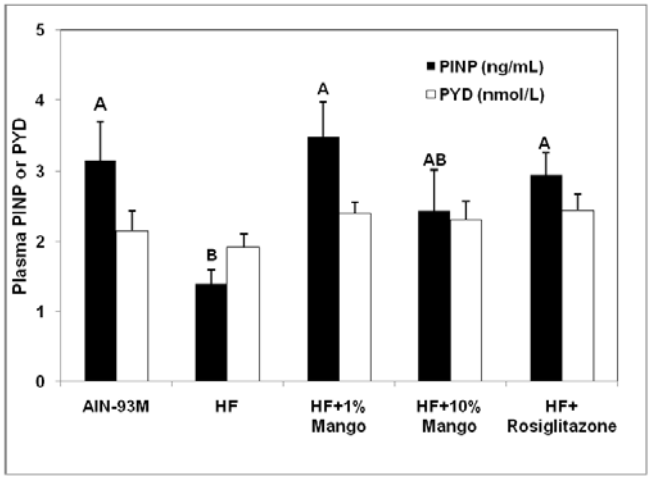

Figure 3: Effects of freeze-dried mango pulp and rosiglitazone on plasma N-terminal propeptide of type I procollagen (PINP) and pyridinoline cross-link (PYD) of mice fed high fat (HF) diet for two months.

Bars are mean ± SE, n=8-9 mice/group; within a set of bars, values that do not share the same superscript letters are significantly (*P*<0.05) different from each other.

DISCUSSION

This study investigated the effects of freeze-dried mango on bone parameters in mice fed HF diet. As we previously reported [28], our data demonstrates that freeze-dried mango (i.e., l% dose) was able to modulate glucose parameters similar to, or even to a greater degree, than that observed with rosiglitazone. Results of the IGTT and HOMA-IR calculations show that the mice receiving mango-supplemented diets may be as efficacious as rosiglitazone in improving glucose tolerance and decreasing insulin resistance.

Our data also provide some evidence that consumption of HF diet is associated with alterations in bone quality and strength although not as significant as those observed by other investigators [3, 19, 40]. Several studies have demonstrated that HF diet exhibits deleterious effects on BMC, microarchitectural parameters, and mechanical properties [3, 19, 40]. Parhami and colleagues [3] reported that a HF atherogenic diet (containing 15.8% fat, 1.25% cholesterol, and 0.5% cholate) inhibits osteoblastic differentiation which could be attributable to increased bioactivity of oxidized lipids. Furthermore, HF diet may also promote osteoclastic differentiation which is mediated by T lymphocytes [19]. Our data on plasma PINP concentrations supports reduced bone formation due to HF diet; however, we did not observe changes in the bone resorption marker, PYD.

Rosiglitazone added to the HF diet has the most negative effects on BMD, micro-architectural parameters, and strength. Mice in the HF+rosiglitazone diet had the lowest BV/TV and TbN and highest TbSp. Our findings on the negative effects of rosiglitazone are consistent to earlier findings. Rzonca and colleagues [8] reported that mice given rosiglitazone for 7 weeks had whole body BMD and proximal tibia BV/TV and TbN decreased by 10%, 24.1% and 11.2%, respectively. In our study, the addition of rosiglitazone to HF diet reduced whole body BMD and proximal tibia BV/TV and TbN by 3%, 29.6% and 24.3%, respectively. The slight differences in these parameters may be due to the age of the animals and the dose of rosiglitazone. We used 3-m old mice with an approximate intake of 5 µg rosiglitazone/g body weight/day compared to 6-m old male mice with an intake of 20 µg rosiglitazone/g body weight/day used by Rzonca and colleagues [8]. Similarly, Ali and colleagues [41] demonstrated that bone loss occurred in mice given rosiglitazone for 28 d which was attributed to lower ratio of osteoblasts to osteoclasts, bone formation rate, expression of key osteoblastic transcription factors and an increased diversion of progenitors from the osteoblast to the adipocyte lineage. Our findings, combined with an accumulating body of scientific evidence, indicate that while rosiglitazone may improve glucose tolerance, its detrimental effects on trabecular bone are likely to increase fracture rates long-term.

The addition of mango to the HF diet, particularly at the 1% dose, was able to modulate plasma glucose similar to rosiglitazone without the negative effects on bone. Mice in the HF+1% mango group had whole body, tibial, and vertebral BMD that were higher than

the mice in the HF and HF+ rosiglitazone groups. This protective effect of mango on bone and its ability to improve insulin resistance may be due to its antioxidant and anti-inflammatory properties [20, 24, 26]. Mango provides a number of bioactive compounds including carotenoids, ascorbic acid, and phenolic compounds. Many of the compounds identified in mango have been shown to exhibit both antioxidant and anti-inflammatory properties [20, 24, 26]. In particular, mango stem bark extract exhibits anti-inflammatory properties capable of inhibiting ovalbumin-induced paw edema in rats [24]. Furthermore, mango juice prepared from mango pulp inhibits free radical production and neoplastic transformation in both BALB/3T3 and HL-60 cell lines [25]. Another mechanism by which mango may have modulated bone loss and blood glucose is through the decreased activation of peroxisome proliferator-activated receptor (PPAR) γ which has also been shown to alter the commitment of marrow mesenchymal stem cells by favoring conversion to the adipocyte lineage instead of the osteoblastic lineage. This is supported by our observation that the relative gene expression of the adipogenic gene, aP2, tended to be higher in the HF+rosiglitazone group compared to the HF+1% mango group (data not shown). Additionally, studies focused on the polyphenolic components of the mango fruit, quercetin and the aglycone derivative of mangiferin (i.e., norathyriol), have revealed that these components inhibit the activation of all three isoforms of PPAR [42]. However, these bone protective mechanisms of mango are all speculative and need to be further investigated.

There are few studies that demonstrate that one of the major phenolic compounds in mango, mangiferin, has positive effects on bone. A study by Li and colleagues [43] showed that mangiferin may be partly responsible for the inhibitory effects on bone resorption of a traditional Chinese medicine (Kampoe formulae) used for the treatment of osteoporosis. Oral administration of mangiferin reduced alveolar bone loss in a rat model of experimental periodontitis which was attributed to its anti-inflammatory property and its ability to accelerate repair and healing of injured areas [26]. In an *in vitro* study, mangiferin suppressed receptor activator of nuclear factor kappa B (NF-κB) ligand (RANKL)- induced NF-κB activation, a key signaling pathway involved in osteoclastogenesis [27]. From these studies, it is clear that mangiferin has an important role in preventing bone loss. To what extent it contributes to the effect of the freeze-dried mango we have observed is not known. Based on the findings on mangiferin, it seems logical to infer that freeze-dried mango may act by inhibiting bone resorption. However, our data on the bone resorption marker, PYD, does not support that mango inhibit bone resorption but rather might have an effect on bone formation. As mentioned earlier, more studies are needed to identify the bioactive component(s) in mango and their mechanism(s) of action.

There are many limitations that need to be taken into account when dealing with dietary interventions such as mango supplementation for the prevention of chronic disease such as osteoporosis. Some of these challenges include determining the exact dose and identifying the bioactive compound and the possible mechanisms of action. The observed positive effects of mango may be attributed to synergistic actions of different bioactive compounds and not just due to one individual component. For example, Berardini and colleagues [44] established that the antioxidative capacity of mango peel total phenolic extract was higher than isolated mangiferin and quercetin 3-O-glucoside. Another challenge is to identify the exact dose that will exert the most beneficial effect. As shown by our data, the 1% mango was more efficacious than the 10% dose. This finding demonstrates that there is a maximum dose that is effective and any additional amount will not provide any additional benefits. The phenomena that lower doses can be more effective than the higher doses have been observed with other phenolic compounds. For example, blueberry purified anthocyanin at a dose of 0.2 mg/ml added to drinking water was more effective than 1.0 mg/ml in modulating glucose and other clinical parameters of mice fed high fat diet [45]. A study by Marugundanan and colleagues [23] demonstrated that mangiferin at 10 mg/kg is as effective as the 20 mg/kg dose for its antidiabetic, antihyperlipidemic and antiatherogenic activities in streptozotocin-diabetic rats. These findings demonstrate the need to identify the optimal dose of foods and their bioactive compounds that exert health benefit and not to work under the assumption that more is better. Another limitation of our study is whether we would see the same effects in humans and what will be the most effective dose and if this dose is reasonable for human consumption. In humans, the 1% dose used in this animal study is equivalent to eating approximately 10 g/day of freeze dried mango (about 50-100 g of fresh fruit or half a fruit) when calculated on a diet w/w basis. Regardless of the aforementioned limitations, our findings are very exciting in that mango may be used as a cheap and safe dietary intervention for modulating blood glucose and preventing bone loss.

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STATEMENT OF AUTHORS' CONTRIBUTIONS TO THE MANUSCRIPT

E.A.L, P.P, S.L.C, and B.J.S designed the research and wrote the paper; A.B., W.L., S.K.P, Y.W conducted the research; E.A.L had primary responsibility for final content. All authors read and approved the final manuscript.

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