Title of Study: Effects of Mango on Bone Parameters in Mice Fed High Fat Diet

Principal Investigator: Dr. Edralin A. Lucas
Nutritional Sciences Department
Oklahoma State University
422 HES
Stillwater, OK 74078
Phone: 405-744-3132
Fax: 405-744-1357
Email: edralin.a.lucas@okstate.edu

Co-Investigators: Dr. Penelope Perkins-Veazie,[1] Dr. Brenda J. Smith,[2] Dr. Stephen Clarke,[2] Dr. Solo Kuvibidila, [2] Dr. Stanley A. Lightfoot, [3]
[1]North Carolina State University Research Campus, Kannapolis, NC; [2]Nutritional Sciences Department, Oklahoma State University, Stillwater, OK, 74078; [3]Veterans Affairs Medical Center, Oklahoma City, OK

Introduction

Osteoporosis, characterized as a reduction in bone mass and increased susceptibility to fracture, afflicts 10 million individuals in the U.S. today.[1] Alarmingly, another 34 million individuals have low bone mass or osteopenia.[1] The direct healthcare costs associated with osteoporosis were estimated to be between $12-18 billion every year.[1] In 2005, it was estimated that the incident of fractures was more than 2 million and a total cost of $19 billion.[1] By 2025, it is predicted that fractures will increase to more than 3 million and the annual projected fracture costs will be $25.3 billion.[1] Though there are several treatment options available approved by the Food and Drug Administration (FDA), long term adherence to current treatments is not reasonable.[2] Hence, there is a need for alternative therapies that has fewer side-effects. With our increasing knowledge of the role of diet in the treatment or prevention of chronic disease, we believe that an attractive treatment option is one that can be included as part of the daily diet. Investigating the role of dietary interventions that are effective, inexpensive, and easily incorporated into an osteoporosis treatment regimen is a very appealing alternative.

Bone is a dynamic tissue which is constantly remodeled throughout life. A delicate balance is maintained between bone formation and bone breakdown (resorption). Bone cells known as osteoblasts are responsible for bone formation while the activity of osteoclasts leads to bone resorption. Osteoporosis develops when the bone remodeling process favors the activity of osteoclasts. Osteoporosis predisposes one to an increased risk for bone fracture.

Lifestyle (e.g. not smoking and increased physical activity) and nutritional factors (e.g. increased calcium and vitamin D intake) are known to reduce the risk of osteoporosis. Diets high in saturated fat have also been shown to increase the risk of osteoporosis.[3] Saturated fat has been shown to inhibit the absorption of dietary calcium thereby decreasing bone mineralization.[3] In mice, a high fat diet was also shown to convert cells that would normally become bone forming osteoblast to fat cells resulting to a decrease in bone formation.[4,5] Type 2 diabetes has also been shown to increase an individual's risk for fracture beginning 5-10 years after diagnosis, although little is known as to why this occurs.[6]

In addition to lifestyle factors and diet, certain medications are also associated with increased risk of bone loss and fracture. For example, rosiglitazone, also known as Avandia™, is an oral medication used to lower blood glucose for the treatment of type 2 diabetes mellitus. One way by which rosiglitazone reduces blood glucose is by increasing the uptake of glucose by fat

and muscle. Rosiglitazone also converts osteoblast precursor cells to fat cells instead of mature osteoblast resulting to increased total body fat and a decrease in bone formation leading to an increase risk for osteoporosis.[7] Another drug that is widely prescribed is fenofibrate, a drug used to lower elevated cholesterol and/or triglyceride levels. The use of fenofibrate is also often associated with undesirable side effects including skin and digestive problems, muscle pain, sweating, and dizziness.[8] A study using mice, has shown that fenofibrate also decreased bone mineral density.[9] Options that can lower blood glucose and lipids without the associated side effects, particularly those that affect bone, need to be explored.

Active compounds found in foods may offer a more appealing therapeutic option for many chronic diseases.[10-13] In particular, fruits and vegetables have been investigated for their potential health benefits.[10-13] Among these fruits, mangos are rich source of vitamins A and C and contain over 20 different vitamins and minerals.[14] Vitamin A plays an important role in vision and bone growth while vitamin C promotes healthy immune function and is important in collagen formation, a protein that supports and connects tissues such as skin and bone. One way by which mango may protect bone is through their antioxidant activity. Vitamins A and C and other anti-oxidant compounds found in mango can protect the cells in our body from the damaging effects of free radicals. For example, mango juice has been shown to inhibit the formation of free radicals and delay the development of cancer cells.[15] However, to our knowledge, there are no studies that have investigated the effects of mango on bone parameters.

The ***objective*** of this project was to investigate the effect of freeze-dried mango pulp on bone parameters in mice fed a high fat diet and to compare these effects to fenofibrate and rosiglitazone. Our ***hypothesis*** is that compounds in mango can counter the negative effects of high fat diet on bone.

Fresh ripe Tommy Atkins mangos where peeled, cut, freeze-dried, and ground to a fine powder. Mango samples were analyzed for carbohydrate, fat, protein, calcium, and phosphorus content and added to a standard mouse diet. Dietary treatment consisted of control (regular mouse diet, AIN-93M), high fat (HF), high fat plus 1% mango powder (HF+1% mango), high fat plus 10% mango powder (HF+10% mango), high fat plus fenofibrate (HF+fenofibrate), and high fat plus rosiglitazone (HF+rosiglitazone). All high fat diets were adjusted to have similar carbohydrate, fiber, protein, fat, calcium, and phosphorous content. Mice were assigned to one of the six diets (8-9 mice per group) for 2 months and were allowed to drink and eat without

restriction. After two months of treatment, mice were anesthetized and whole body composition and bone density were measured. Blood and tissues including bones were collected. Bones were cleaned of adhering tissues and used for the analyses of bone parameters.

Results:

We have previously reported that incorporation of mango in the mouse diet improved blood glucose and reduced body fat associated with consuming a high fat diet. Here we present our findings on the effect of mango on bone parameters of mice fed a high fat diet.

Whole Body, Tibia, and Spine Bone Mineral Content (BMC), Area (BMA), and Density (BMD)

The effects of mango, fenofibrate, and rosiglitazone on the bone mineral content and area of the whole body, tibia and spine is shown in Table 1. The bone mineral content of the whole body, spine, and tibia was consistently lower in the mice fed the HF+rosiglitazone diet and higher in the HF+mango groups. There were no significant differences in the bone mineral area of the whole body, spine, and tibia.

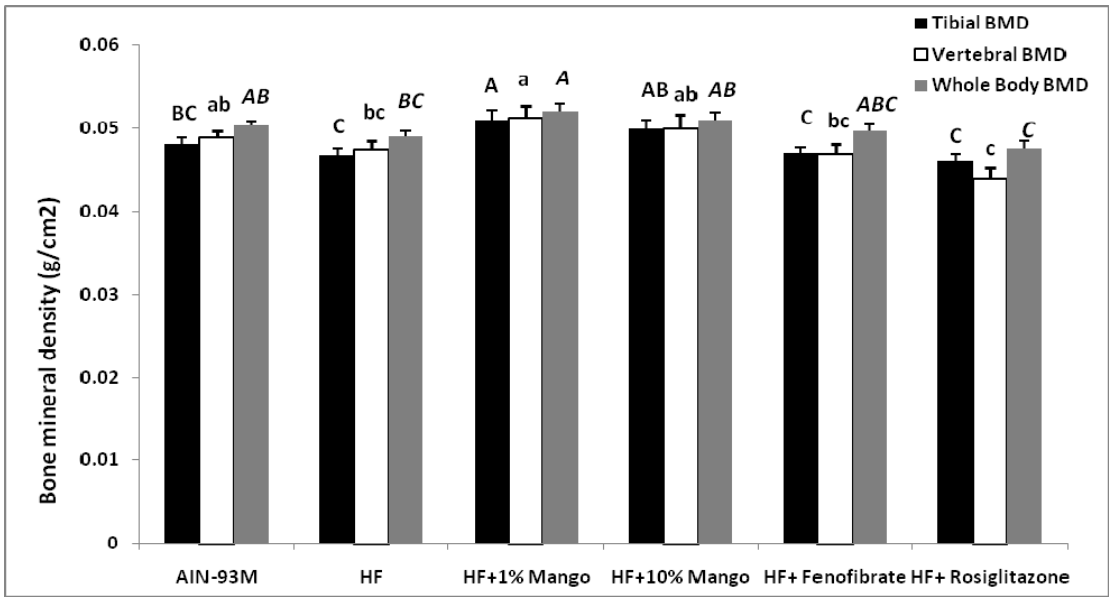
Table 1: Effects of mango, rosiglitazone and fenofibrate on whole body, tibia, and spine bone mineral content (BMC) and area of mice fed high fat diet for two months

Para-meters	**Normal Diet AIN-93M**	**HF**	**HF + 1% Mango**	**HF + 10% Mango**	**HF + Feno-fibrate**	**HF + Rosi-glitazone**
Whole body						
BMC† (mg)	458.6± 11.5	450.7± 19.8	486.8± 23.1	505.3± 24.4	447.3± 14.0	425.5± 15.5
Area (cm^2)	9.12± 0.18	9.18±0.29	9.35±0.33	9.94±0.38	8.99±0.25	8.92±0.23
Tibia						
BMC (mg)	$23.6\pm0.6^{AB C}$	$22.5\pm0.7^{B C}$	25.6 ± 1.1^{A}	$25.0\pm1.0^{A B}$	22.4 ± 0.8^{C}	$22.7\pm0.9^{B C}$
Area (cm^2)	0.492± 0.008	0.478± 0.009	0.498± 0.012	0.498± 0.013	0.477± 0.011	0.444± 0.012
Spine						
BMC‡ (mg)	20.9±0.6	19.9±0.5	21.7±1.0	21.6±1.2	19.6±0.5	18.8±0.7
Area (cm^2)	0.425± 0.006	0.419± 0.004	0.423± 0.007	0.430± 0.009	0.418± 0.005	0.428± 0.007

Values are mean ± SE, n=8 or 9; within a row, values that do not share the same letters are significantly ($P<0.05$) different from each other. †$P=0.0537$; ‡ P= 0.0636

Similar patterns were observed for the bone mineral density (BMD) with the mice receiving the HF+1% mango diet having consistently higher BMD of the whole body, spine, and tibia and consistently lower for the mice given the HF+rosiglitazone diet (Figure 1).

Figure 1. Effects of mango supplementation compared to rosiglitazone and fenofibrate on bone mineral density of the whole body, spine, and tibia of mice fed high fat (HF) diet for two months[1]

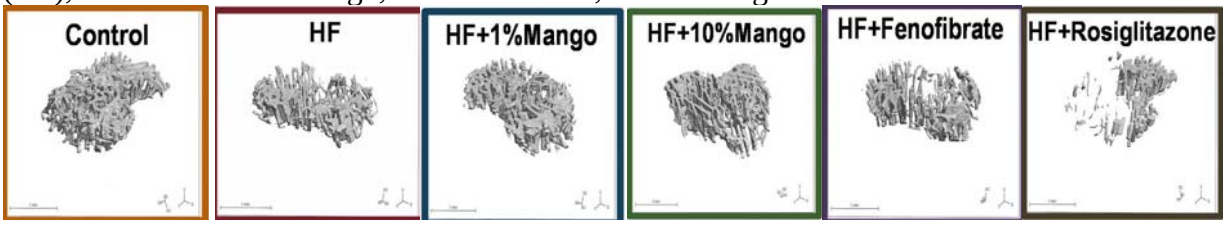


[1]Bars are mean ± SE, n=8-9/group; bars that do not share the same letters are significantly (P<0.05) different from each other.

Three dimensional structure (microarchitecture) of the bone

In addition to bone mass (BMC and BMD), X-ray microcomputed tomography analyses gives as 3-D structure of the bone as well as other indicators of bone quality. As shown in Figure 2, a deterioration of quality of the tibia was observed in mice receiving the HF, HF+fenofibrate, and HF+rosiglitazone. There was no apparent deterioration of the bone quality of the mice in the mango groups and is similar to the control group.

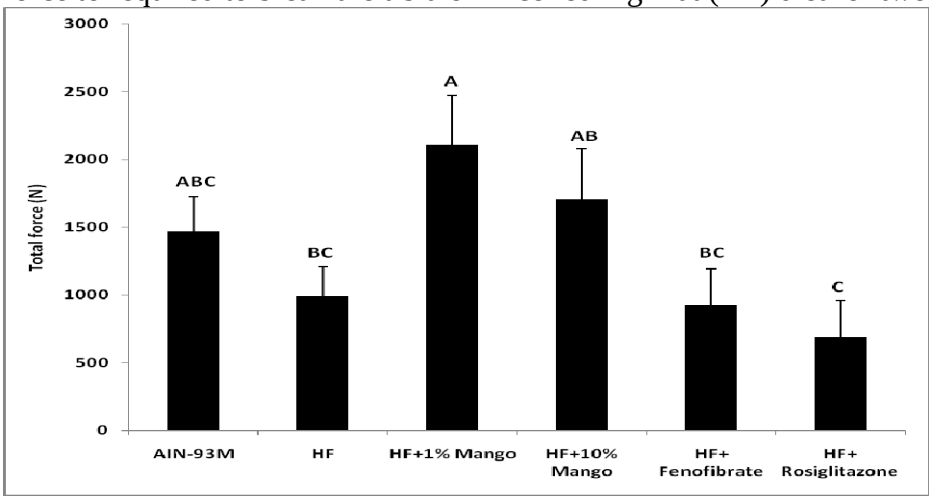
Figure 2. Representative images of the 3-D structure of the tibia of mice given control, high fat (HF), HF+1% or 10% mango, HF+fenofibrate, or HF+rosiglitazone diet for two months



Bone strength using simulated compression testing

The strength of the bone was also assessed using simulated compression testing. Poor quality bone takes a lower amount of force required to break the bone which also means increase risk for fracture. Figure 3 shows that 1% mango improved bone strength compared to the animals consuming the high fat (HF) diet. It also required a smaller amount of force to break the tibia of the mice fed HF+rosiglitazone diet in comparison to those mice that received the HF+1% mango diet. The tibia for the mice fed the HF+1% mango diet is the strongest bone as shown by the higher total force. The 10% mango has an intermediate effect on bone strength.

Figure 3. Effects of mango supplementation compared to rosiglitazone and fenofibrate on total force to required to break the tibia of mice fed high fat (HF) diet for two months[1]



[1]Bars are mean ± SE, n=8-9/group; bars that do not share the same letters are significantly (P<0.05) different from each other.

Summary:

The results of our study demonstrate that mango, particularly the 1% dose, counteracted the negative effects of HF diet on bone. Mice receiving the HF+1% mango diet had the highest BMD of the whole body, spine, and tibia. Importantly, mango not only improved BMD but also the quality of the bone as shown by improvement in the microarchitecture and strength. HF diets compromised bone quality and strength, but high fat diet in combination with rosiglitazone is the most detrimental to bone quality and strength.

This study together with our earlier findings, demonstrate that mango was as effective as rosiglitazone (a glucose lowering drug) in lowering blood glucose and reducing body fat due to

consumption of a high-fat diet. However, mango provides the benefits of glucose control and the prevention of an increase in body fat without compromising bone quality as seen with rosiglitazone. These findings could be very important for prevention and treatment strategies for individuals who are at risk for or have type 2 diabetes. Studies using a suitable animal model of osteoporosis as well as human studies are needed to confirm our findings and to begin to understand how mango works. Moreover, the component(s) of mango responsible for its positive effects on bone needs to be further investigated.

References:

1. National Osteoporosis Foundation (NOF). Osteoporosis: A debilitating disease that can be prevented and treated. 2008.
2. Biskobing DM, Novy AM, Downs R. Novel therapeutic options for osteoporosis. *Curr.Opin.Rheumatol.* 2002; 14(4):447-52.
3. Wohl GR, Loehrke L, Watkins BA, Zernicke RF. Effects of high-fat diet on mature bone mineral content, structure, and mechanical properties. *Calcif Tissue Int*. 1998; 63:74-79.
4. Parhami F, Tintut Y, Beamer WG, Gharavi N, Goodman W, Demer LL. Atherogenic high-fat diet reduces bone mineralization in mice. *J Bone Miner Res*. 2001;16:182-188.
5. Parhami F, Jackson SM, Tintut Y, Le V, Balucan JP, Territo M, Demer LL. Atherogenic diet and minimally oxidized low density lipoprotein inhibit osteogenic and promote adipogenic differentiation of marrow stromal cells. *J Bone Miner Res.* 1999;14(12):2067-78.
6. Melton LJ 3rd, Leibson CL, Achenbach SJ, Therneau TM, Khosla S. Fracture risk in type 2 diabetes: update of a population-based study. *J Bone Miner Res.* 2008;23(8):1334-42
7. Kalaitzidis RG, Sarafidis PA, Bakris GL. Effects of thiazolidinediones beyond glycaemic control. *Curr Pharm Des* 2009;15:529-536.
8. Roberts WC. Safety of fenofibrate--US and worldwide experience. *Cardiology* 1989; 76; 169-179.
9. Toda K, Okada T, Miyaura C, Saibara T. Fenofibrate, a ligand for PPARalpha, inhibits aromatase cytochrome P450 expression in the ovary of mouse. *J Lipid Res.* 2003;44(2):265-70.
10. Ullah MF, Khan MW. Food as medicine: potential therapeutic tendencies of plant derived polyphenolic compounds. *Asian Pac J Cancer Prev* 2008; 9:187-195.

11. Jew S, AbuMweis SS, Jones PJ. Evolution of the human diet: linking our ancestral diet to modern functional foods as a means of chronic disease prevention. *J Med Food* 2009; **12**: 925-934.
12. Wu H, Dai Q, Shrubsole MJ, *et al.* (2009) Fruit and vegetable intakes are associated with lower risk of colorectal adenomas. *J Nutr* **139**, 340-344.
13. Iriti M & Faoro F (2006) Grape phytochemicals: a bouquet of old and new nutraceuticals for human health. *Med Hypotheses* **67**, 833-838.
14. U.S. Department of Agriculture, Agricultural Research Service (2005) USDA National Nutrient Database for Standard Reference, Release 18. http://www.ars.usda.gov/ba/bhnrc/ndl
15. Percival SS, Talcott ST, Chin ST, Mallak AC, Lounds-Singletone A, Pettit-Moore J. Neoplastic transformation of BALB/3T3 cells and cell cycle of HL-60 cells are inhibited by mango (*Mangifera indica* L.) juice and mango juice extracts. *J Nutr.* 2006; 136 (5): 1300-4.