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Role of vitamins other than vitamin D on bone health

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Abstract
Nutrition is one of the several factors that determine the health of the animal. Good bone health increases the quality of life as bone forms a major connective tissue in the body. The role of vitamin D and calcium is a well-established fact from earlier researches. Other vitamins such as vitamin A, B, C, E and K also play an important part in contributing to the bone health. Hypovitaminosis of retinal causes cortical thinning of the periosteal bones. The B complex vitamins are important cofactors for various enzymatic reactions for example; folate plays a crucial role in homocysteine metabolism thereby contributing to the bone formation. The antioxidant nature of vitamin E helps to reduce the oxidative damage as well as protects the chondrocyte membrane from lipid peroxidation. Vitamin K primarily plays a key role in coagulation of blood but also has a role in bone health along with other vitamins. The water-soluble vitamin C is involved in the synthesis of collagen by influencing the function of prolyl hydroxylase domain (PHD) protein, which hydroxylates prolines that are important for collagen assembly which contributes to long term bone health. The supplementations of both fat soluble and water-soluble vitamins are necessary for maintaining the optimal bone health along with calcium and vitamin D.

Keywords: Bone health, vitamin A, B complex vitamins, vitamin E, collagen

1. Introduction
Bone serves a variety of roles in the body, including mobility, soft tissue support and protection, calcium and phosphate storage. Bone is a completely mineralized connective tissue. Inorganic salts and organic matrix make up the majority of bone. Collagenous proteins (90%), primarily type I collagen, and non-collagenous proteins such as osteocalcin, osteonectin, osteopontin, fibronectin, bone sialoprotein II, bone morphogenetic proteins (BMPs), and growth factors make up the organic matrix. Phosphate and calcium ions make up the majority of bone's inorganic composition.
Osteoblasts, bone lining cells, osteocytes, and osteoclasts are the four types of cells found in bones. Bone undergoes both resorption and absorption at the same time.
One of the most important factors that determine bone mass and fragility is nutrition. Inorganic minerals (calcium, magnesium, phosphorus, sodium, potassium, and different trace elements) and vitamins (vitamins A, D, E, K, C, and some B vitamins), as well as macronutrients like protein and fatty acids, all influence bone health. Bone health is greatly enhanced by good dietary supplements.

2. Role of Fat-Soluble Vitamins on Bone Health
2.1 Vitamin A
Vitamin A refers to substances with biological activity similar to retinol. Sporn J in 1976 coined the name "retinoid". They're made up of four isoprenoid units linked together in a head-to-tail fashion. Preformed vitamin A and provitamin A carotenoids are both sources of vitamin A in the diet. Preformed vitamin A is found in foods including eggs, liver, butter, milk, and fortified cereals as long chained fatty acids of retinol (retinyl esters). Carotenoids (e.g., α-carotene, β -carotene, and β -cryptoxanthin) are provitamin A carotenoids found in plants like carrots, spinach, collards, and pumpkins.
Vitamin A is consumed by the animals in the form of preformed vitamin or provitamin A carotenoids. Long-chained retinol fatty acids make up preformed vitamin A. The pancreatic and intestinal enzymes hydrolyze the ingested retinyl esters, and the free retinol is absorbed by the intestinal mucosal cells Retinol being water insoluble binds to cellular binding protein II (CBPII) in enterocytes. In mucosa cells, half of provitamin A carotenoids is absorbed intact, while the other half is oxidized to retinal and then converted to retinol. Long chain fatty acids are used to esterify retinol produced from retinyl esters and provitamin A carotenoids.
The retinyl esters, along with intact carotenoids, are integrated into chylomicrons, which are

transported by the lymphatics, along with lipids such as cholesterol, cholesterol esters, and triglycerides. Some unesterified retinol is directly absorbed into portal system. Vitamin A absorption is considerably aided by the presence of fat in the diet. Fat activates enzymes that hydrolyze dietary retinyl esters, enhances micelle formation for retinol and carotenoids solubilization in the intestinal lumen, and boosts chylomicron production.

Following hydrolysis of chylomicron triglyceride by lipoprotein lipase and addition of apolipoprotein E, chylomicron remnants containing retinyl esters develop in the bloodstream. The retinyl esters are hydrolyzed and the remains are taken up by hepatocytes via receptor-mediated endocytosis. Retinol is re-esterified and stored in liver stellate cells when it is no longer needed by the organism. Smaller amounts of retinyl esters and carotenoids are also transported to extrahepatic tissues by chylomicrons and remnants for use and storage.

Retinoids are principally mediated by two nuclear hormone receptor families, retinoic acid receptors (RARs) and retinoid X receptors (RXRs). Each receptor family has three isotypes (a, ß, and ɣ) that are generated by different genes. Retinoids have been found to bind retinoid-related orphan receptors (ROR) and retinoid-related orphan receptors (ROR) in addition to RARs, RXRs, and PPAR (peroxisome proliferator-activated receptors) [1].

2.1.1 Vitamin A and Its Relationship with Bone

In experimental animals, hypervitaminosis A causes thinning of the cortex of long bones at the diaphysis, as well as an increased frequency of fractures. Reduced endosteal/marrow blood flow and pathological endosteal mineralization were found in rats with hypervitaminosis A [2].

In foetuses of pregnant rats with hypervitaminosis A, a significant reduction of bone growth was observed [3]. Excessive vitamin A or synthetic retinoids fed to diverse species of animals were linked to poor bone growth and radiolucency, rapid bone re-modelling with subsequent loss of bone mineral content, and an increased incidence of spontaneous fractures [2].

Collagen synthesis in embryonic chick calvaria was considerably suppressed after 24 hours of retinol culture [4]. The terminal cartilage of mouse bone rudiments cultured in a medium with a high concentration of vitamin A lost its metachromasia, shrunk, and eventually dissolved. Vitamin A deprivation in growing chicks affected both osteoclastic and osteoblastic activity, resulting in abnormal basioccipital bone and spine formation [5].

Vitamin A deficiency caused reversible bone alterations [5]. When these animals were given vitamin A, the osteoclasts and osteoblasts, becomes active resulting in removal of superfluous bone deposited or not absorbed during vitamin deficiency. More studies are needed to discover the safest dose of vitamin A that does not harm skeletal health.

2.2 Vitamin K

Vitamin K was first discovered to be necessary for blood coagulation. However, vitamin K has been discovered to have a variety of additional roles, and there is some evidence that it may protect against bone loss. This is accomplished mostly by the gamma-carboxylation of osteocalcin by vitamin K [6]. The major dietary sources include green leafy vegetables, meat and milk products.

2.2.1 Mechanism of Action of Vitamin K on Bones

Several coagulation factors, including factors II, VII, IX, protein C, and protein S, require vitamin K to function biologically. This is vitamin K's most well-known metabolic function.

The vitamin K–dependent carboxylase, a microsomal enzyme that enables the posttranslational conversion of glutamyl to alpha-carboxyglutamyl residues, requires vitamin K as a cofactor. Alpha-carboxyglutamyl–containing proteins are prevalent in bone tissue, in addition to the hepatic tissue where clotting factors are synthesized. Up to 80% of the total alpha-carboxyglutamyl content of adult bone is made up of osteocalcin. Human osteocalcin is mostly produced by osteoblasts. The precise role of osteocalcin in bone metabolism is still unknown. The current mechanistic evidence suggests that osteocalcin plays a regulatory role in bone mineral maturation.

Newly generated osteocalcin is largely integrated into the extracellular matrix of bone, although a little amount is liberated into the bloodstream and can then be measured. Osteocalcin is generally recognized as a bone turnover marker. In fact, the degree to which osteocalcin is carboxylated is thought to be a more sensitive indicator of vitamin K status than traditional blood coagulation tests. Low vitamin K status is associated with high serum levels of undercarboxylated osteocalcin (ucOC), and vice versa.

Vitamins K and D may function in tandem on bone metabolism [7]. They demonstrated that the bone Gla protein (osteocalcin) is a good indicator of bone metabolism. They discovered that bone Gla protein may be found in serum and plasma and created a radioimmunoassay to detect it. They also discovered that the vitamin K-dependent gamma-carboxylation of this bone-specific protein generated by osteoblasts.

Anti-vitamin K impairs gamma-carboxylation, undercarboxylated osteocalcin increases with vitamin K deficiency and anti-vitamin K therapy. Vitamin K's antiresorptive properties are due to its geranyl-geranyl side chain, which works through mechanisms other than gamma carboxylation [8].

One of the mechanisms of vitamin K's action on bone is the activation of the steroid and xenobiotic receptors, which increases collagen formation [6]. Furthermore, vitamin K may alter bone metabolism by blocking the development of certain bone-resorbing factors such as prostaglandin E_2 and interleukin 6 or by influencing urinary calcium excretion. In rats, vitamin K treatment increased total and carboxylated osteocalcin while decreasing uncarboxylated osteocalcin and urine calcium and hydroxyproline [9].

Vitamin K has a favorable effect on calcium balance in ovariectomized rats [10]. In ovariectomized rats, supplementing with vitamin K increased calcium retention. Alpha-carboxyl glutamic acid may contain proteins in the kidney that contribute to this action. These proteins may be implicated in the kidney's calcium homeostasis.

2.3 Vitamin E

Vitamin E is a fat-soluble antioxidant vitamin with considerable antioxidant effects. Vitamin E is found in eight different isoforms in nature: tocopherols and tocotrienols. An aromatic chromanol ring and a side chain make up each isomer of vitamin E. In contrast to tocopherols, which have a saturated phytyl side chain, tocotrienols have an unsaturated

farnesyl (isoprenoid) side chain [11]. Tocotrienols' unsaturated side chains help them to enter the membrane lipid bilayer more effectively.

Vitamin E's antioxidant effectiveness varies depending on which isomer you use. Vitamin E is a powerful antioxidant that aids the body's natural defense against membrane lipid peroxidation. Vitamin E is most commonly found in the form of -tocopherol, which is used as an antioxidant supplement. -Tocopherol is the most prevalent form of vitamin E and has the highest biological activity. Tocopherols are abundant in polyunsaturated vegetable oils and in the germ of cereal seed and tocotrienols are abundant in palm oil, cereal grains, and rice bran.

2.3.1 Effect of vitamin E on bones

Tocotrienol has piqued scientists' curiosity due to its significant antioxidative property. Free radicals are implicated in osteoblast and osteocyte death, as well as osteoclastogenesis and thus bone resorption. Through the activation of nuclear factor-kappa B, which normally governs osteoclast differentiation and consequently bone resorption and re-modeling, oxidative stress accelerates bone resorption. Vitamin E has been shown to have an inhibitory effect on collagen synthesis in various mouse tissues [12].

Intramuscular injections of vitamin E showed a protective impact on chondrocyte membranes during maturation and differentiation in suckling lambs [13]. Vitamin E is thought to prevent cartilage resorption and protect chondrocyte membranes by lowering free radical production and lipid peroxidation.

Vitamin E was found to play a function in enhancing bone density in nicotine-treated rats, Vitamin E increased trabecular bone and reduced calcium loss in rats without ovaries by neutralising antioxidants and increasing trabecular bone [14]. Vitamin E administration protected bones from oxidative damage, maintained bone matrix trophism, and accelerated trabecular bone growth. Vitamin E supplementation, protects against bone loss and damage due by oxidative stress, which is triggered by a lack of sex hormones [15].

A vitamin E-deficient diet causes bone deterioration, which is caused by a decrease in calcium absorption. Increased levels of bone-resorbing cytokines, particularly interleukin 1 and 6 (IL-1 and IL-6), have been linked to faster bone resorption. In oestrogen deficiency, monocytes release IL-1, which causes osteoblasts to secrete IL-6. IL-6 promotes osteoclast growth, which leads to increased bone resorption. Tocotrienol was found to be effective in preventing the rise of serum IL-1 as well as the negative effects of free radicals on trabecular bone formation [14]. Supplementation with alpha-tocotrienol successfully prevented osteoporosis in adrenalectomized rats substituted with dexamethasone [16].

Role of Water- Soluble Vitamins on Bone Health

2.4 Vitamin B_2 – Riboflavin

Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) are the most physiologically active forms of vitamin B_2 or riboflavin, both participate in oxidation-reduction reactions in metabolic pathways involved in energy production. Senile animals are prone to biological shortage of vitamin B_2 due to ageing and diminished absorption efficiency. It is transformed to lumiflavin when exposed to UV rays of sunshine.

Riboflavin is phosphorylated in the intestinal wall during absorption and transported to the cells by the blood. Cereals, meat, fatty fish, dark-green vegetables, animal and fish by products, distiller's grain, yeast, and oil meals are all good sources of riboflavin. Fair sources include green grass and leaves. The richest natural source of this vitamin is yeast. Riboflavin is abundant in leaves, whereas it is scarce in cereals and its byproducts.

Vitamin B_2 is abundant in oil seed cakes. Riboflavin is phosphorylated in the intestinal wall during absorption and delivered by the blood to the cells of the tissues, where it exists as phosphate or flavoprotein. Lactochrome/lactoflavin is a greenish yellow fluorescent pigment found in milk and whey.

2.4.1 Effect of riboflavin on bones

The offspring of rats fed vitamin B_2-deficient diets caused aberrant foetal development and skeletal deformity [17]. In an *in vitro* experiment on pre-osteoblastic cells, discovered that vitamin B2 and its photoproducts improved cell proliferation and alkaline phosphatase activity, and decreased the RANKL/OPG (osteoprotegerin) ratio by increasing OPG expression [18].

The coenzymatic form flavin adenine dinucleotide (Methylenetetrahydrofolate reductase enzyme) is related to bone [19]. Vitamin B_2 may interact with the MTHFR gene polymorphism, causing hyperhomocysteinemia to damage bone cells.

2.5 Vitamin B_3 – Niacin

Niacin, often known as vitamin B_3, is the generic name for nicotinic acid and nicotinamide, which are both substrates for the active coenzymes nicotinamide adenine dinucleotide (NAD) and NAD phosphate, respectively (NADP). NAD also serves as a substrate for biological processes such as DNA processing, cell differentiation, and calcium mobilization in cells. The biosynthesis of tryptophan-niacin synthesizes vitamin B_3, and its effectiveness is influenced by other dietary and hormonal factors such vitamin B_6, vitamin B_2, and iron. Rich food sources include yeast, meats, cereals, legumes and seed

2.5.1 Effect of Niacin on Bones

The direct effect of vitamin B_3 on bones has been studied in only a few research. In all age groups of chicks, a larger dose of vitamin B_3 decreased bone strength and increased bone breaking, implying that excessive niacin consumption may promote bone fractures, however the mechanism is unclear [20].

2.6 Vitamin B_6 - Pyridoxine

The three pyridines pyridoxine, pyridoxal, and pyridoxamine, as well as their 5'-phosphorylated derivatives, make up vitamin B_6. Pyridoxal phosphate (PLP) is a cofactor for approximately 100 enzyme processes in glycogen, phospholipid, and amino acid metabolism. PLP affects nucleic acid biosynthesis and immune system function through playing essential roles in neurotransmission, amino acid metabolism, homocysteine and cystathionin metabolism, and one-carbon metabolism. The uptake and metabolism of amino acids are tightly linked to PLP (transamination, decarboxylation, deamination). PLP is required for the

synthesis of CoA from pantothenic acid, as well as the synthesis of sphingolipids and δ aminolevulinic acid (haeme precursor). Pyridoxine is required for full tryptophan metabolism; otherwise, the aberrant metabolites xanthurenic acid and kynurenic acid are produced and expelled. Good dietary sources include meats, whole grain products, vegetables, bananas and nuts.

2.6.1 Effect of Pyridoxine on Bones

Vitamin B_6 involve independently in lysyl oxidase activity, collagen cross-linking, and bone mechanical properties [21]. PLP, as a cofactor for lysyl oxidase, is required for the enzymatic action of lysyl oxidase in the synthesis of collagen cross-links. Vitamin B_6 deficiency lowered the activity of lysyl oxidase, an enzyme that catalyzes the oxidative deamination of lysine residues in tropoelastin and tropocollagen to create aldehydes for the crosslinks in collagen, in chick cartilage and chick aorta *in vitro* models [22]. This could be the reason that vitamin B_6 can impair the mechanical properties of the bone.

Apart from its role in lysyl oxidase, vitamin B_6 may also play a role in bone formation as a substrate of alkaline phosphatase and in the coupling of osteoblasts and osteoclasts, where calcification is dependent on periosteal glucose 6-phosphate dehydrogenase (G6PD) activity, which is induced by putrescine, a compound derived from a vitamin B_6-dependent enzyme, orninithine decarboxylase [23]. Vitamin B_6 deficiency may thus result in alterations in the bone, implying an imbalance in the coupling between osteoblasts and osteoclasts, resulting in greater bone cavities and decreased new bone formation.

Pyridoxine shortage in chicks resulted in aberrant excitability, aimless movements, convulsions, tiredness, and decreased development and egg production [24].

2.7 Vitamin B_9 – Folate

Vitamin B_9, often known as folate, is essential for one-carbon metabolism, nucleotide synthesis, homocysteine metabolism, and the methylation of DNA, RNA, proteins, and phospholipids. Re-methylation of methionine in the methylation pathway is dependent on both folate and B_{12}. Vitamin B_9 deficiency causes megaloblastic alterations in the bone marrow and other tissues, and homocysteine can be converted to cysteine via the trans-sulphuration route, which requires vitamin B_6 as a cofactor for the enzymatic process from cystathionine to cysteine. Major food sources of folate include citrus fruits, dark green leafy vegetables, and legumes.

2.7.1 Mechanism of Action of Folate on Bones

The combined effects of B vitamin deficiency (B_6, folate, and B_{12}) on the activity of osteoblasts and osteoclasts, bone turnover biomarkers, bone strength and area, and fracture healing were investigated in several *in vitro* and *in vivo* studies [19]. Although vitamin B_9 deficiency resulted in a considerably higher serum level of homocysteine, there was no influence on bone strength and area, mineral matrix, callus stiffness, size or tissue composition, or bone turnover, according to the findings. Hyperhomocysteinemia may cause tissue-specific accumulation of homocysteine in bone as a result of its binding to collagen in the extracellular matrix, reducing bone growth and strength.

Resorption activity was shown to be considerably higher in folate-deficient osteoclasts as compared to folate-treated cells [19]. Folate was thought to have a similar effect as tetrahydrobiopterin, a cofactor for the nitric oxide synthase enzyme. In turn, the endothelial isoform of nitric oxide synthase promotes bone density maintenance and is a critical mediator of the anabolic effects of mechanical stress and estrogens on bone. Folate may thus aid in the preservation of bone density by assisting in the preservation of optimal nitric oxide synthase activity in bone cells [25].

2.8 Vitamin B_{12} – Cyanocobalamin

Vitamin B_{12}, also known as cobalamin, was first discovered in 1855 as a therapy for pernicious anaemia and demyelinating diseases of the central nervous system. In individuals with pernicious anaemia, vitamin B12 was first linked to osteoporosis and fractures. Vitamin B_{12} is physiologically connected to the function of two enzymes, L-methyl malonyl-coenzyme A (CoA) mutase and methionine synthase, the latter of which is involved in homocysteine metabolism. The mechanisms linking vitamin B12 to several aspects of bone physiology are yet unknown. The dietary sources include animal sources like liver, meat and shell fish.

2.8.1 Effect of Cobalamin on Bones

Vitamin B_{12} boosted osteoblastic proliferation and alkaline phosphatase activity in human bone marrow stromal osteoprogenitor cells and osteoblastic cells showing that B_{12} has a direct effect on osteoblast proliferation and development [12]. They hypothesized that vitamin B_{12} deficiency could boost osteoclast production indirectly by increasing MMA (methylmalonic acid) and homocysteine levels. Several studies have examined the effect of B vitamins on osteoblasts and osteoclasts *in vitro* and on bone quality in animal models using a combination of vitamin B_{12}, folate, and vitamin B_6, and found that deficiency of these vitamins, including vitamin B_{12}, increased osteoclast resorption activity [26].

2.9 Vitamin C- Ascorbic acid/ antiscorbutic factor

Vitamin C, often known as ascorbic acid, is an essential vitamin that animals cannot synthesize. It is necessary for the hydroxylation of lysine and proline, which is required for the creation of stable collagen triple helixes and, as a result, for normal bone growth. Scurvy is a condition caused by a deficiency of ascorbic acid, and it has been linked to lower bone marrow density and mineral content in guinea pigs, especially during the skeletal development stages [27].

ASC (reduced form) and DHA (complete form) are the two most common forms of vitamin C. (oxidized form). Facilitated diffusion across membranes is facilitated by carrier proteins, yet it is dependent on an electrochemical gradient, just like passive diffusion.

Vitamin C is abundant in citrus fruits (lemon, gooseberry, guava, and so on), tomatoes, and potatoes. Humans, guinea pigs, subhuman primates, certain birds, certain fishes, bats, and some invertebrates all require vitamin C. The enzyme L-Gluconolactone oxidase is required for vitamin C production from six carbon sugars in these species. Vitamin C is an excellent reducer. Vitamin C is highly thermolablile. Alkali destroys vitamin C activity, which is missing in dried foods. Spongy gums, loose teeth, anaemia, swollen joints, weak blood vessels, impaired immunocompetence, delayed wound healing, sluggish hormonal function (adrenal cortex and gonads), bleeding, and osteoporosis are all symptoms of vitamin C deficiency.

2.9.1 Effect of Vitamin C on Bone

Ascorbic acid (AA) has a role in collagen synthesis by altering the function of the protein prolyl hydroxylase domain (PHD), which hydroxylates prolines that are necessary for collagen assembly. The guinea pig was one of the first model organisms for exploring the effects of vitamin C on bone because it lacks the GULO gene (L-gulonolactone oxidase) and hence cannot catalyse the final step in vitamin C production.

The GULO gene is dormant in guinea pigs and higher primates due to changes that happened around 20 million years ago. An early guinea pig study discovered that AA deficiency prevented normal collagen synthesis, and that lower hydroxyproline content resulted in defective femoral bone collagen synthesis [27]. This led to more studies and observed that AA-deficient guinea pigs had fewer and thicker bone trabecular layers, but more trabecular spacing in the tibia. In scorbutic animals, femur length and bone volume density were likewise reduced, but bone strength was unaltered. When compared to guinea pigs fed a vitamin C-rich diet, Scorbutic guinea pigs have significantly lower ALP activity in both bone and serum [28].

Reduced circulating osteocalcin levels in GULO mice also indicated defective osteogenesis, signifying a failure in the bone production process [29]. GULO mice had aortic wall injury in addition to diminished trabecular bone volume. Despite the fact that there are few publications addressing the skeletal characteristics of GULO animals, we and others demonstrated that deletion of the whole GULO gene causes poor osteoblast development, reduced bone production, and spontaneous fractures in mice.

2.10 Future Prospective

A well-established study should be carried taking into consideration the different age groups, the nutritional value of feed given as well as, a reliable method to diagnose the levels of vitamins in body. Certain vitamins have a co-relation with genetic constitution of the animal. Research can be conducted to manipulate the gene and thereby aids in treating the bone deformities. The administration of fat soluble and water-soluble vitamins as a supportive therapy in companion animal treatment can be practiced with enough research. The complex nature of vitamin interaction is yet to be understood fully for arriving to a definite conclusion.

4. Conclusion

The benefits of nutrition on bone health are not restricted to vitamin D and calcium alone, as this article demonstrates. Retinol can be harmful to your health if you consume too much or too little of it. B complex vitamins, as well as vitamins C, E, and K, have been shown in studies to have a beneficial relationship with bone marrow density, lowering the incidence of fractures and subsequent abnormalities. Other vitamins' interactions with bone are complicated, and genetic factors, gender, animal age, hormonal therapy, and even calcium consumption all play a role. Bone is no exception to the rule that all tissues require distinct nutrients. Providing a well-balanced diet is the best way to avoid deficiency disorders and thus extend life expectancy.

5. References

1. Bastien J, Rochette EC. Nuclear retinoid receptors and the transcription of retinoid-target genes. Gene. 2004;328:1-16.
2. Kneissel M, Studer A, Cortesi R, Susa M. Retinoid-induced bone thinning is caused by subperiosteal osteoclast activity in adult rodents. Bone. 2005;36(2):202-214.
3. Johansson S, Lind PM, Ha Kansson H, Oxlund H, Melhus H. Subclinical hypervitaminosis A causes fragile bones in rats. Bone. 2002;31(6):685-689.
4. Dickson I, Walls J. Vitamin A and bone formation. Effect of an excess of retinol on bone collagen synthesis *in vitro*. Biochemical Journal. 1985;226(3):789-795.
5. Mellanby E. Vitamin A and bone growth: the reversibility of vitamin A deficiency changes. The Journal of Physiology. 1947;105(4):382-399.
6. Binkley N, Harke J, Krueger D. Vitamin K treatment reduces undercarboxylated osteocalcin but does not alter bone turnover, density, or geometry in healthy postmenopausal North American women. Journal of Bone and Mineral Research. 2009;24(6):983-991.
7. Peterkofsky B. Ascorbate requirement for hydroxylation and secretion of procollagen: relationship to inhibition of collagen synthesis in scurvy. The American journal of Clinical Nutrition. 1991;54(6):1135-S1140.
8. Ducy P, Desbois C, Boyce B. Increased bone formation in osteocalcin deficient mice. Nature. 1996;34(6590):382-448.
9. Schug TT, Berry DC, Shaw NS, Travis SN, Noy N. Opposing effects of retinoic acid on cell growth result from alternate activation of two different nuclear receptors. Cell. 2007;129(4):723-733.
10. Anderson JB. Calcium, phosphorus and human bone development. Journal of nutrition. 1996;126(4):1153-1158.
11. Xu H, Watkins BA, Seifert MF. Vitamin E stimulates trabecular bone formation and alters epiphyseal cartilage morphometry. Calcified Tissue International. 1995;57(4):293-300.
12. Kim GS, Kim CH, Park JY, Lee U, Park CS. Effects of vitamin B12 on cell proliferation and cellular alkaline phosphatase activity in human bone marrow stromal osteoprogenitor cells and UMR106 osteoblastic cells. Metabolism. 1996;45(12):1443-1446.
13. Maiorano G, Manchisi A, Salvatori G, Filetti F, Oriani G. Influence of multiple injections of vitamin E on intramuscular collagen and bone characteristics in suckling lambs. Journal of Animal Science. 1999;77(9):2452-2457.
14. Reddi K, Henderson B, Meghji S. Interleukin 6 production by lipopolysaccharide-stimulated human fibroblasts is potently inhibited by naphthoquinone (vitamin K) compounds. Cytokine. 1995;7(3):287-290.
15. Shearer MJ. The roles of vitamin D and K in bone health and osteoporosis prevention. Proceedings of the Nutrition Society. 1997;56(3):915-937.
16. Nirwana A, Kiftiah AG, Zainal M, Norazlina MT, Gapor, Khalid BAK. Palm vitamin E prevents osteoporosis in orchidectomized growing male rats. Natural. Product. Sciences. 2000;6(4):155-160.
17. Warkany J, Nelson RC. Congenital malformations induced by rats by maternal nutritional deficiency. Journal of Nutrition. 1942;23(4):321-333.
18. Neto A, Yano CL, Paredes-Gamero EJ, Machado D, Justo GZ, Peppelenbosch M, *et al*. Riboflavin and

photoproducts in MC3T3-E1 differentiation. Toxicology *in vitro*. 2010;24(7):11-1919.
19. Herrmann M, Tami A, Wildemann B, Wolny M, Wagner, and Schorr H. Hyperhomocysteinemia induces a tissue specific accumulation of homocysteine in bone by collagen binding and adversely affects bone. Bone. 2009;44(3):467-475.
20. Haliloglu B, Aksungar FB, Ilter E. Relationship between bone mineral density, bone turnover markers and homocysteine, folate and vitamin B12 levels in postmenopausal women. Archives of Gynecology Obstetrics. 2010;281(4):663-668.
21. Murray JC, Levene CI. Evidence for the role of vitamin B-6 as a cofactor of lysyl oxidase. Biochemical Journal. 1997;167(2):463-467.
22. Bird TA, Levene CI. Lysyl oxidase: Evidence that pyridoxal phosphate is a cofactor. Biochemical and biophysical research communications. 1982;108(3):1172-1180.
23. Fedde KN, Lane CC, Whyte MP. Alkaline phosphatase is an ectoenzyme that acts on micromolar concentrations of natural substrates at physiologic pH in human osteosarcoma (SAOS-2) cells. Archives of Biochemistry and biophysics. 1988;264(2):400-409.
24. Dodds RA, Catterall A, Bitensky L, Chayen J. Abnormalities in fracture healing induced by vitamin B6-deficiency in rats. Bone. 1986;7(6):489-495.
25. McCarty MF. Supplemental arginine and high-dose folate may promote bone health by supporting the activity of endothelial-type nitric oxide synthase in bone. Medical Hypotheses. 2005;64(5):1030-1033.
26. Holstein JH, Herrmann M, Schmalenbach J, Obeid R. Deficiencies of folate and vitamin B12 do not affect fracture healing in mice. Bone. 2010;47(1):151-155.
27. Tsuchiya H, Bates CJ. Ascorbic acid deficiency in guinea pigs: contrasting effects of tissue ascorbic depletion and of associated inanition on status indices related to collagen and vitamin D. British Journal of Nutrition. 1994;72(5):745-752.
28. Kipp DE, McElvain M, Kimmel DB, Akhter MP, Robinson R, Lukert B. Scurvy results in decreased collagen synthesis and bone density in the guinea pig animal model. Bone. 1996;18(3):281-288.
29. Sugimoto T, Nakada M, Fukase M, Imai Y, Kinoshita Y, Fujita TL. Effects of ascorbic acid on alkaline phosphatise activity and hormone responsiveness in the osteoblastic osteosarcoma cell line UMR-106. Calcified Tissue International. 1986;39(3):171-174.