Mediterranean Journal of Nutrition and Metabolism 7 (2014) 87–93
DOI:10.3233/MNM-140009
IOS Press

Vitamin E and bone health: Not just alpha-tocopherol

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Abstract. Vitamin E collectively refers to eight chemically distinct isoforms: α, β, γ and δ tocopherols and tocotrienols. Because α-tocopherol is the most abundant form of vitamin E in human tissues, most studies of vitamin E's antioxidant effect on bone have only investigated the effects of α-tocopherol and many supplements do not contain the other isoforms. Despite this, alpha-tocopherol shows mixed results in animal and human studies of bone, with the most positive outcomes obtained under conditions of oxidative stress. Some studies even show a detrimental effect but because so few human studies measure baseline or follow-up blood levels, it is impossible to determine whether the study population were in deficiency or excess. The only human intervention study to measure blood levels showed that supplementation caused an excess, which was dose-dependently associated with suppression of vitamin K-dependent proteins, thereby increasing risk of osteoporosis. It is likely that α-tocopherol will prove to have a U-shaped dose/benefit curve, as with some other fat-soluble vitamins. The remaining isoforms show greater promise than alpha-tocopherol for bone health, particularly because supplementation of α-tocopherol can suppress their bioavailability. In particular, γ-tocopherol and α- and γ-tocotrienol have shown improved results for bone relative to α-tocopherol.

Keywords: Vitamin E, tocopherols, tocotrienols, bone

1. Introduction

Vitamin E collectively refers to the eight fat-soluble, chemically distinct isoforms: α, β, γ and δ tocopherols (saturated) and tocotrienols (unsaturated) [1]. Tocopherols are abundant in the leaves and seeds of most plants and polyunsaturated vegetable oils, while tocotrienols are mainly found in palm oil, cereal grains, and rice bran [1, 2]. The commercial availability of vitamin E is mostly in the form of α-tocopherol, which is taken as an antioxidant supplement; it has the highest biological activity of all isomers and is the most abundant form of vitamin E in human tissues and serum, as it is selectively retained in the body [2]. This paper, although not a systematic review, will consider the evidence for the beneficial properties of all the vitamin E isoforms for bone health and osteoporosis prevention in an attempt to make sense of the somewhat contradictory evidence.

2. *In vitro* studies

In vitro studies of the various forms of vitamin E have shown no effect of α-tocopherol on osteoblasts or their expression of bone sialoprotein mRNA or alkaline phosphatase activity, although high dose α-tocopherol decreased expression of osteocalcin mRNA, suggesting inhibition of differentiation [3–6]. Similarly, δ-tocopherol had no effect on expression of bone sialoprotein mRNA or alkaline phosphatase activity [4]. α-tocotrienol, however, inhibited RANKL expression in osteoblasts and osteoclastogenesis and RANKL-induced osteoclast differentiation by

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ISSN 1973-798X/14/$27.50

suppression of Fos expression, possibly by inhibiting ERK and NF-kB activation. It could thereby reduce bone resorption in mature osteoclasts without affecting their survival [5, 7]. γ-tocopherol, however, proved to be toxic to osteoblasts in high concentrations but at low concentrations it protected osteoblasts from hydrogen peroxide toxicity, displaying improved antioxidant activity compared to α-tocopherol [6]. When cultured with osteoclasts, γ-tocopherol inhibited osteoclast formation and activity to a greater extent than α-tocotrienol [7].

3. Animal studies

A 2012 systematic review of vitamin E supplementation found that among the eight animal studies, four showed positive changes in bone structure [8–11], with three more showing improvement of biochemical results, although there was no improvement in bone structure parameters [10, 12, 13], and only one [14] reporting no positive findings. Better effects were obtained from tocotrienols than α-tocopherol [2].

Among the α-tocopherol studies, many are carried out in ovariectomised rodents, where it prevented the induced reduction in trabecular bone volume and number and reduced osteoclast surface relative to osteoblast surface [15]. α-tocopherol also maintained bone mineral density and calcium content, raised alkaline phosphatase and reduced TRAP activity [12]; it improved early stage fracture healing, when there is excessive production of free radicals, so that blood results in animals with fractures was comparable to the sham-operated group, with increased activity of SOD and glutathione peroxidase [16]. In male rats fed nicotine (to simulate smoking and oxidative stress, a known risk factor for osteoporosis), α-tocopherol had no significant effect on the increased serum IL-1, IL-6 (known to be associated with accelerated bone resorption after menopause [2]) and pyridinoline, the reduced osteocalcin or trabecular thickness [9, 17] but it increased osteoprotegerin and restored the nicotine- or vitamin E deficiency-induced bone calcium loss [18, 19]; it also reversed the lowered trabecular bone volume, mineral appositional rate and bone formation rate [9] and reduced urinary deoxypyridinoline [18]. Another study found that α-tocopherol could reduce oxidative stress-induced elevated IL-6 and osteoclast number and restore age-related low osteocalcin but had no effect on IL-1, osteoblast number, bone formation rate or the ratio of eroded surface/bone surface [8]. α-tocopherol also had no effect on lowering thiobarbituric acid-reactive substance (TBARS), a measure of lipid peroxidation, or glutathione peroxidase or superoxide dismutase (*SOD) activity* in male rats [20] but in chicks high dose α-tocopherol did succeed in lower TBARS and raising mineral apposition rate but there was no effect on bone length [21]. Higher doses of α-tocopherol in orchidectomised rats did not improve bone mineral density (BMD) or content (BMC) or reverse the deterioration in trabecular microarchitecture compared to adequate vitamin E and nor were serum osteocalcin and urinary deoxypyridinoline improved [14]. In mice deficient in α tocopherol transfer protein, α-tocopherol stimulated osteoclast fusion independent of its antioxidant capacity by inducing the expression of dendritic cell-specific transmembrane protein [22].

Palm oil, rich in α- and β-tocotrienol, could reverse nicotine-induced down-regulation of gene expression of BMP-2, osterix and runx-2 [23]. It could also prevent iron-induced elevated IL-1, IL-6, urine deoxypyridinoline cross-links and osteoclast number, lower serum osteocalcin and the ratio of eroded surface/bone surface and increase osteoblast number and bone formation rate and prevent the reduction in trabecular bone volume thickness [8]. In ovariectomised rats palm oil prevented the reduction in trabecular bone volume and number reduced osteoclast surface relative to osteoblast surface [15], and restored normal BMD and alkaline phosphatase activity but only high doses restored BMC [12]. It improved bone calcification in vitamin E-deficient mice [19]. Tocotrienols could also restore nicotine-induced bone calcium loss in male rats [18], while γ-tocotrienol could reduce nicotine-induced elevated IL-1 and IL-6 and pyridinoline and increase lowered osteocalcin [17], as well as reversing the lowered trabecular bone volume, mineral appositional rate and bone formation rate [1]. In comparison studies, α- and γ-tocotrienol were more effective than α-tocopherol in improving static and dynamic bone histomorphometric parameters [1, 10, 11, 13, 20, 24].

4. Epidemiological studies

A 2012 systematic review of vitamin E supplementation found that among the three epidemiological studies of women, none showed any association between vitamin E intake and BMD changes [2]. A more detailed examination,

however, shows that in two studies vitamin E consumption was assessed by questionnaire, which may have failed to distinguish between the different isomers of vitamin E. Furthermore, since most vitamin E supplements contain only α-tocopherol, it is reasonable to assume that these studies generally investigated only α-tocopherol supplementation. Wolf et al. conducted a very large cross-sectional study of intake of all antioxidants in women aged >50 and found that increasing intakes were not independently associated with BMD [25], however serum levels were not assessed to determine whether the study population was deficient or replete in vitamin E; if already replete, additional vitamin E would not be expected to improve bone parameters. Similarly, Macdonald et al. investigated intake of various foods in women during the menopausal transition, followed them up after 5–7 years and found that increased dietary intake of vitamin E was inversely associated with BMD although the addition of intake from supplements removed this association; the authors point out that since vitamin E was highly correlated with polyunsaturated fatty acids (PUFAs), it may simply be a surrogate marker for them. Again, intake was assessed by questionnaire and no baseline blood levels were tested [26]. In a smaller study, Maggio et al. investigated elderly women with and without osteoporosis and found that plasma antioxidants, including vitamin E, were consistently lower in osteoporosis patients but this study did not measure dietary intake. Far from showing no association with bone health, this study clearly indicates that women with osteoporosis have lower plasma vitamin E, although there was no difference in plasma malondialdehyde, a byproduct of lipid peroxidation [27].

Other epidemiological studies have found that in postmenopausal Korean women, intake of vitamin E was inversely associated with risk of osteoporosis and positively associated with BMD [28], although in Japanese women vitamin E intake was inversely associated with BMD [29]. A small study of postmenopausal women found that use of vitamin E supplements was negatively associated with serum C-telopeptides (marker of bone turnover) but there was no difference in serum bone specific alkaline phosphatase or BMD [30]. A US study of fracture patients showed that among ever smokers, those in the highest quintile of vitamin E intake had a lower risk of hip fracture compared to the lowest quintile [31]. Similarly, Melhus et al. showed that among female current smokers, the odds ratio for first hip fracture with low vitamin E intake was 3.0 but decreased to 1.1 with high intakes of vitamin E [32].

Among studies investigating blood levels of vitamin E, Mata-Granados et al. found that in early postmenopausal women, a lower α-tocopherol/lipid ratio was associated with greater risk of osteoporosis and lower BMD [33], while Ostman et al. showed that elderly men with serum α-tocopherol levels below the median combined with high oxidative stress had significantly lower BMD after five years [34]. Another study found that in metabolic syndrome patients, serum vitamin E levels were associated with BMD of the lumbar spine in men only [35]. In the only study to consider other vitamin E isomers, Hamidi et al. showed that high serum γ-tocopherol and a low ratio of serum α-tocopherol to γ-tocopherol were associated with increased bone specific alkaline phosphatase, a marker for bone formation, although there was no association with urinary N-telopeptides, a marker for bone resorption [36].

5. Intervention studies

Intervention studies mostly involve vitamin E given with vitamin C. A small study of postmenopausal women given placebo, 600 mg/d α-tocopherol plus 1000 mg/d vitamin C, exercise plus placebo or exercise plus supplements for 6 months showed a significant decrease in lumbar spine BMD in the placebo group but it remained stable in all treatment groups [37]. Another study gave 500 mg/d vitamin C plus 100 mg/d vitamin E (probably α-tocopherol) and an aerobic training programme for 8 weeks to elderly women and found significantly decreased serum bone-specific alkaline phosphatase but no change in osteocalcin or urinary type I collagen C-telopeptide, although the dosage was small [38]. Baseline and follow-up blood levels were not measured in either study. Booth et al. carried out a small study giving 335.5 mg/d α-tocopherol to rheumatoid arthritis (RA) patients and 671 mg/d to healthy subjects for 12 weeks. Mean baseline α-tocopherol levels were 30 mcmol/l in the RA patients and 24 mcmol/l in the healthy subjects and after supplementation they rose to 69.8 mcmol/l and 55.8 mmol/l respectively, with the RA treatment group moving from within to outside the normal range (25 to 60 mcmol/l). Supplementation caused a significant increase in proteins which are induced by the absence of vitamin K (PIVKA II), such as undercarboxylated osteocalcin, from within normal range to above normal range, with a larger increase in the group taking higher supplement doses, but levels were unchanged in the placebo group. This suggests that high dose α-tocopherol may antagonise vitamin K, another fat soluble vitamin, which is necessary for healthy bone formation [39]. Although the authors did not

comment on supplementation taking RA patients above the normal range and healthy subjects close to the upper limit for plasma α-tocopherol, this may indicate that in already replete subjects, supplementation could have adverse consequences.

6. Discussion

In vitro studies show very little effect of α-tocopherol or δ-tocopherol on expression of bone biomarkers although low dose γ-tocopherol protected osteoblasts from oxidative damage, while high doses of α-tocopherol and γ-tocopherol proved detrimental to bone. γ-tocopherol inhibited osteoclast formation and activity to a greater extent than α-tocotrienol, which also proved able to inhibit osteoclast differentiation and RANKL expression in osteoblasts.

Animal studies are somewhat divided over whether α-tocopherol is beneficial or has no effect. In healthy unstressed rats it appears to have little effect but high doses may nevertheless lower markers of oxidative stress and improve mineral apposition rate. Consequently, although outcomes vary, it appears that the best results for α-tocopherol are obtained under conditions of oxidative stress or oestrogen deficiency. Most of the tocotrienol studies were also carried out under conditions of oxidative stress or oestrogen deficiency, when they proved effective in restoring markers of bone formation and resorption and inflammation, reducing the ratio of osteoclasts to osteoblasts and improving bone calcium loss and bone formation rate. In comparison studies, α- and γ-tocotrienol were more effective than α-tocopherol in improving static and dynamic bone histomorphometric parameters.

Large epidemiological studies have shown no association between vitamin E dietary and supplementary intake assessed by questionnaire and BMD, although blood levels were not assessed. Curiously, in one study dietary intake alone had a negative association with BMD, a finding which was echoed in a smaller study of Japanese women. For Korean women, however, intake was inversely associated with osteoporosis risk and positively associated with BMD. Use of vitamin E supplements (generally only α-tocopherol) was inversely associated with C-telopeptides but there was no association with BMD or bone formation markers. Higher vitamin E intake seems particularly helpful for smokers, where it confers a lower risk of fracture. Investigation of blood levels appears to provide greater clarity of results, with postmenopausal women and elderly men with lower plasma α-tocopherol having lower BMD and greater risk for osteoporosis, particularly in the presence of high oxidative stress. Among the other vitamin E isomers, only γ-tocopherol was studied and showed that higher serum levels and a higher ratio of γ-tocopheral to α-tocopherol were associated with increased markers of bone formation.

Intervention studies of α-tocopherol given with ascorbic acid show that at 600 mg/d they can prevent the menopause-induced BMD reduction but at 100 mg/d and 50% of the dose of ascorbic acid there was significantly reduced bone-specific alkaline phosphatase, although blood α-tocopherol concentrations were not measured. γ-tocopherol supplementation took plasma α-tocopherol levels close to or outside the normal range and dose-dependently increased PIVKA II, suggesting that in excess α-tocopherol antagonises vitamin K.

A growing number of studies are now showing the detrimental effect of supplemented α-tocopherol on serum levels of other vitamin E isoforms. Hamidi et al. found that among postmenopausal women, supplement users had significantly lower serum γ-tocopherol than those not supplementing [36], a finding supported by an earlier study [40], while among healthy volunteers α-tocopherol reduced the rate of tocotrienol absorption [41]. Studies by Uchida et al. in rats showed that administration of α-tocopherol enhanced γ-tocopherol catabolism [42] and decreased the α-tocotrienol and γ-tocopherol concentrations in tissues and inhibited uptake of γ-tocotrienol, possibly due to α-tocopherol having the highest affinity for α-tocopherol transfer protein (αTTP) [43]. Others have shown that α-tocopherol dose-dependently decreased δ-tocotrienol [44] and α-tocotrienol [45] uptake into cells due to competitive transport [46], although there was no effect on γ-tocotrienol [45]. Gee points out that this has caused an apparent paradox in that in human randomized controlled trials α-tocopherol supplementation significantly increases all-cause mortality, which is due to α-tocopherol significantly depressing the bioavailability of other vitamin E isoforms which have greater health benefits. Furthermore, he believes there is no necessity to supplement with α-tocopherol since from a pharmacologic viewpoint it does more harm than good [47]. These findings may explain why some of the studies of α-tocopherol have a negative result.

These studies have shown that tocopherols and tocotrienols both exhibit antioxidant activities with respect to bone and are most effective under conditions of oxidative stress. Some have found that the antioxidant properties of

tocotrienols are mediated through induction of antioxidant enzymes such as superoxide dismutase, NADPH:quinone oxidoreductase and glutathione peroxidase, as well as by suppression of the inflammatory transcription factor NF-kB, and are considered to have superior antioxidant properties to tocopherols [1]. Furthermore, among those studies specifically relating to bone, γ-tocopherol has been found to act by inhibiting RANKL expression.

7. Conclusion

Overall, it appears that α-tocopherol has little effect *in vitro* and studies are divided over whether it has proved beneficial in animal experiments, although under conditions of oxidative stress or oestrogen deficiency α-tocopherol and tocotrienols improved bone biomarkers and histomorphometric measures, with tocotrienols generally proving the more effective. Epidemiological studies also show mixed results for vitamin E intake but higher quantities appeared helpful in reducing osteoporosis risk among smokers. Studies of blood levels show that lower α-tocopherol levels are independently associated with lower BMD and osteoporosis, particularly in oxidative stress. Only γ-tocopherol was studied among the isomers and showed that higher serum levels and a higher ratio of γ-tocopherol to α-tocopherol were associated with increased markers of bone formation. Of the three intervention studies, those combining α-tocopherol and ascorbic acid supplementation failed to test blood levels but found a benefit to postmenopausal BMD loss, while a single study of α-tocopherol supplementation, which took blood levels outside normal range, was found to depress levels of vitamin K-dependent proteins. Given the studies showing that α-tocopherol suppresses bioavailability of other vitamin E isoforms that may have a greater benefit on bone, it appears that α-tocopherol should not be supplemented alone unless blood levels are below normal range, which may occur under conditions of oxidative stress or oestrogen deficiency. It is likely that α-tocopherol will prove to have a U-shaped dose/benefit curve, as with some other fat-soluble vitamins. The remaining tocopherols and tocotrienols show greater potential for promoting bone health and more studies are undoubtedly needed.

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