

## Review Article

# Association of the B-complex vitamins with osteoporosis and osteoporotic fractures

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Osteoporosis is a bone metabolic disease associated with low bone mass and deterioration of bone microarchitecture, thus increasing the risk for potential fractures. There is emerging evidence supporting the role of vitamin B in bone health and the prevention of fractures. In this review we evaluated both observational studies and studies assessing the genetic association of the group of B vitamins with the risk for osteoporosis and osteoporotic fractures. We retrieved 25 relevant studies and according to the results, future research is necessary to determine if dietary intake and supplementation of vitamin b may have a potential influence on osteoporosis and osteoporotic fractures.

**Keywords:** Fractures, MTHFR, Osteoporosis, RANK, Vitamin B

**Introduction**

As life expectancy increases, so does the number of elderly that are prone to osteoporosis and osteoporotic fractures. Osteoporosis is a metabolic skeletal disease causing deterioration of bone microarchitecture, reduction of bone mass and increased risk of fractures<sup>1</sup>. Our skeleton is in a constant phase of remodeling during our lifetime, balancing between bone formation and bone resorption. During the first three decades of our life, bone formation outweighs bone absorption from birth to skeletal maturation until reaching peak skeletal growth and bone mass. Peak bone mass is the maximum bone mass that a person can attain by the end of skeletal growth. After that period, and especially after menopause in women, bone absorption supersedes bone formation due to decreased levels of estrogen, causing bone loss and possibly bone fragility<sup>1,2</sup>. Factors such as age, gender, ethnicity, race, weight, physical inactivity, smoking, use of glucocorticoids also increases the risk of subsequent fractures. It is presumed that 1 in 3 women and 1 in 5 men over the age of 50 will experience an osteoporotic fracture in their lifetime, usually of the spine, hip and wrist<sup>2</sup>. Unfortunately hip fractures have a higher rate of morbidity and institutionalization, influencing the quality of life of older people. The cost for surgery, hospitalization and medication constitutes a significant economic burden regarding national health systems<sup>3-5</sup>.

Vitamins are important for several biochemical functions and vitamin deficiency can cause health issues<sup>4</sup>. Depending

on how they are absorbed and stored in the body, vitamins are classified into two groups: water soluble and fat soluble<sup>6</sup>. Since they cannot be synthesized in the body at all or only in adequate quantities, they have to be obtained through diet<sup>7</sup>. Nutrition supplementation especially with calcium and vitamin D has an important role in reducing bone fragility and fractures<sup>2,4</sup>. There are eight water soluble B Vitamins (B1, B2, B3, B5, B6, B7, B9 and B12)<sup>7</sup>. Lately there is an emerging interest in the effect of B vitamins on bone health and the prevention of osteoporotic fractures. High levels of plasma homocysteine concentration in elderly has been linked to an increase of bone fragility and risk of fractures<sup>2</sup>. Homocysteine has a metabolic relationship with vitamin B12, B6 and folate and supplementation with folic acid can reduce the levels of homocysteine<sup>2</sup>. Further on we will discuss the effect of the vitamin B group on osteoporosis and osteoporotic fractures.

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## Materials and methods

In this review we researched the PubMed database (Medline), for articles related to the effect of B complex vitamins on osteoporosis and osteoporotic fractures by using key words: vitamin B1, thiamine, vitamin B2, riboflavin, niacin, vitamin B5, pantothenic acid, vitamin b6, pyridoxine, biotin, folate, vitamin B9, vitamin B12, cobalamin, osteoclastogenesis, RANKL, ROS, MTHFR, osteoporosis, fracture. Our inclusion criteria were: a) studies published in the English language b) publication dates between 2001 and September 2021 c) original articles, including reviews d) prospective or retrospective studies e) studies in humans f) in vitro studies and g) animal studies. We excluded case-reports.

## Results

Our original search provided 201,621 articles, and after implementing our inclusion and exclusion criteria, we selected 25 articles exploring the effect of B-complex vitamins on bone fragility and low impact fractures. In the following paragraphs we briefly summarise basic knowledge on the various vitamins of the B-complex as discussed on the reviews that we selected, as well as present data concerning their effects on bone health and disease.

### Vitamin B1 (Thiamine)

Thiamine was one of the first vitamins that was discovered during the 17<sup>th</sup> century and was able to be synthesized<sup>7</sup>. The structure of thiamine is made of a thiazolium ring (4-methyl-5-hydroxy-ethyl thiazole) and a pyrimidine ring (2.5-dimethyl 1-6-aminopyrimidine) linked by a methylene bridge<sup>7</sup>. There are five thiamine phosphate derivatives: thiamine monophosphate (ThMP), thiamine diphosphate (ThDP) (thiamine pyrophosphate), adenosine thiamine triphosphate and adenosine thiamine diphosphate. Thiamine pyrophosphate is a cofactor for enzymes such as pyruvate dehydrogenase, 2-oxoglutarate dehydrogenase (OGDC) and 2-hydroxyphytanol-coA lyase which are important in carbohydrate, amino acid and lipid metabolism<sup>6,7</sup>. Thiamine is also important in the synthesis of neurotransmitters acetylcholine and  $\gamma$ -aminobutyric acid (GABA)<sup>7</sup>. Natural food sources of vitamin B1 are unrefined whole grain cereal, pork, nuts, fruit and yeast products<sup>4,7</sup>. Malnutrition, malabsorption and alcoholism are the main reasons for low levels of vitamin B1<sup>7</sup>. Thiamine deficiency causes nutritional polyneuropathy (dry beriberi) resulting in symmetric neuronal loss, numbness of the feet and hands and confusion<sup>6,7</sup>. Wernicke encephalopathy causes ataxia, ophthalmoplegia and confusion seen mostly in alcoholics with thiamine deficiency<sup>6,7</sup>. Wet beriberi affects the heart causing fluid retention, irregular heart beat and dyspnea<sup>7</sup>.

The impact of thiamine on osteoporosis and osteoporotic fractures is not yet clearly understood. In the Singapore Chinese Health study, a cohort study of 63257 Chinese men

and women living in Singapore, between the ages of 45 and 74, that were evaluated after a period of 13.8 years for the relationship of vitamin b levels and hip fractures, showed that vitamin B1 did not have a protective role neither a harmful impact on hip fractures<sup>8</sup>.

In another study 60 orthopedic patients were equally divided in two groups. Each group had their thiamine levels evaluated for 14 days. The 30 patients in one group with femoral neck fracture had thiamine deficiency throughout the period of 14 days after surgery. On the other hand, even though the 30 orthopedic patients in the other group needing a total hip replacement had low thiamine levels after surgery, thiamine had returned to normal levels after two weeks<sup>4</sup>. Thiamine deficiency was thought to be a common finding present in patients with femoral neck fracture before surgery, suggesting a relation between osteoporotic fractures and thiamine levels. Another possible mechanism correlating confusion in the elderly due to thiamine deficiency, was regarded as a causative role in the occurrence of falls leading to hip fractures<sup>4,8</sup>.

Tae-Keun Ahn et al findings, in 301 Korean postmenopausal women over the age of 50, using a multifactor dimensional reduction method (MDR) comparing gene allele combinations suggest a genetic association of specific allele combinations of four single nucleotide polymorphism within the 3'-untranslated region, including SLC19A2 (encodes thiamine carrier), TCN2 (encodes transcobalamin II), CD320 (encodes transcobalamin II receptor) and SLC19A1 (encodes reduced folate carrier protein 1). The allele CD320C-TCN2T-SLC19A1T-SLC19A2C was associated with the prevalence of osteoporosis and osteoporotic vertebral compression fractures<sup>9</sup>.

Thiamine has a dose dependent effect on osteoclast differentiation<sup>10</sup>. Osteoclasts are large multinucleated cells that are responsible for the absorption and resorption of bone matrix. Derived from the hematopoietic stem cells the osteoclasts need the presence of RANKL (receptor activator of nuclear kB ligand), a membrane protein type II and M-CSF (macrophage colony stimulating factor), a secreted cytokine, in order to differentiate into a mature osteoclast. RANKL binds with RANK activating a downstream of signaling pathways. A recent study showed that thiamine and the non-coenzyme role of ThDP inhibit RANKL induced osteoclast differentiation<sup>10</sup>.

Reactive oxygen species (ROS) and the production of oxidative stress is important for different cellular signaling events, including osteoclastogenesis.  $H_2O_2$  is the main ROS capable of inducing osteoclast formation and activation<sup>11</sup>. RANKL induced osteoclastogenesis produces ROS via the Rac1-Nox 1/2/4 signaling. Thiamine suppresses the production of reactive oxygen species inhibiting the Rac1-Nox 1/2/4 signaling pathway<sup>10</sup>.

The unfolded protein response (UPR) is activated in response to the unfolded proteins that compile in the endoplasmic reticulum. The inositol-requiring protein-

1a /X-box-binding protein (IREa/XBP1) branch of the UPR is activated during osteoclast differentiation<sup>12</sup>. Thiamine inhibits this signaling pathway constraining osteoclast formation and bone resorption.

Estrogen deficiency leads to bone absorption and osteoporosis. In a study with ovariectomized mice (OVX) thiamine had a protective role against bone loss caused by low estrogen levels after ovariectomy. In this study the ovariectomized mice that were given a diet rich in vitamin b1 had higher bone mineral density (BMD), compared to the mice given a diet lacking thiamine<sup>10</sup>.

In conclusion, studies in people aged over 50 showed that thiamine rich diet may have a protective role in osteoporotic fractures, in vitro studies have shown that thiamine inhibits osteoclast differentiation and ROS accumulation and in animal studies thiamine inhibits bone loss due to estrogen deficiency. Therefore, there is a significant likelihood that thiamine is important in bone health, osteoporosis and fractures due to bone loss.

## Vitamin B2 (Riboflavin)

Vitamin B2 (7.8-dimethyl-10-ribitylisoalloxazine), also called riboflavin, is a water soluble vitamin that acts as a cofactor for the metabolism of macronutrients<sup>7</sup>. Through its active forms, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) that act as electron carriers, riboflavin participates in redox reactions for the production of energy, biosynthesis and detoxification<sup>4,6</sup>. Riboflavin is important for the production of glutathione, an antioxidant, neutralizing reactive oxygen species<sup>7</sup>. Milk, eggs, dairy products, enriched cereal and fatty fish are excellent sources of riboflavin. Green leafy vegetables, nuts and fruit have also a considerable amount of vitamin B2<sup>4,7,13</sup>. Deficiency due to malnutrition, and decreased absorption, especially in the elderly as well as eating disorders result in cheilosis, seborrheic dermatitis, conjunctivitis<sup>7,13</sup>.

According to a study of 5035 men and women, over the age of 55, who were genotyped for the MTHFR C677T variant, riboflavin has an impact on osteoporosis and fractures<sup>14</sup>. Women with the MTHFR TT genotype and low levels of riboflavin, had elevated homocysteine levels and a higher risk of osteoporotic fractures<sup>14</sup>. In order for homocysteine to metabolize to methionine, methyltetrahydrofolate is required, which is catalysed by MTHFR (methylene tetrahydrofolate reductase)<sup>14</sup>. The polymorphism of MTHFR C677T, with the replacement of alanine (Ala) with valine (Val) at the 222<sup>th</sup> position of the MTHFR gene, results in the decreased activity of the enzyme and leads to elevated homocysteine levels. To enhance the activity of the enzyme, a derivative of vitamin B2, riboflavin adenosine dinucleotide (FAD) is needed as a binding co factor. The T allele contrast to the C allele inhibits the proper positioning of co factor FAD, resulting in a thermolabile enzyme, lowering its activity, especially in people with the TT genotype<sup>14</sup>.

Low levels of riboflavin had no effect on osteoporotic

fractures in men with the MTHFR C677T variant, seemingly due to an altered threshold in the levels of riboflavin, below which the polymorphism causes a risk for fractures<sup>14</sup>.

In a longitudinal study of 1241 Scottish women living in Aberdeen, 45-54 years old, that performed a bone mineral density scan in 1990-1993, a decreased femoral bone mineral density after a period of 6.6 years, was associated with women with low levels of riboflavin and TT homozygotes<sup>1,15</sup>.

In an in vitro study riboflavin and its photoderivatives (formylmethylflavin, lumiflavin, 2-ketoriboflavin and 4-ketoriboflavin) have a positive impact on ascorbate and  $\beta$ -glycerophosphate induced osteoblast differentiation of MC3T3-E1 cells<sup>16</sup>. Furthermore, riboflavin and irradiated riboflavin increased the expression of osteoprotegerin (OPG), lowering the RANKL/OPG ratio, stimulated transcription factor Runx-2 and  $\beta$ -catenin, therefore affecting osteoclastogenesis<sup>16</sup>. Osteoprotegerin binds with RANKL receptor inhibiting RANK-RANKL interaction suppressing osteoclastogenesis. In the same study an increase of alkaline phosphatase activity was observed and attenuated osteoblast proliferation. It also activated different signaling pathways such as the AKT kinase inhibiting GSK3, subsequently activating and stabilizing the wnt/ $\beta$ -catenin signaling pathway<sup>16</sup>.

Therefore it is essential for other studies to take place in order to understand the importance of riboflavin in osteoporosis and upcoming fragile fractures.

## Vitamin B3 (Niacin)

Vitamin B3 (pyridine-3-carboxylic acid) is a water soluble vitamin that can be produced in the liver by the aminoside tryptophan<sup>7</sup>. Its amide derivative nicotinamide is a component for the co enzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), which are electron carriers for redox reactions<sup>6</sup>. NAD and NADP are important cofactors for the synthesis of fatty acids, nucleic acids and DNA repair<sup>7</sup>. Niacin rich sources are liver, meat, poultry, peanuts and eggs<sup>7</sup>. Since the human body cannot produce the necessary amounts of niacin, daily consumption of rich dietary sources is essential. Niacin deficiency causes the disease pellagra, that is characterized by four symptoms: diarrhea, dementia, dermatitis and death<sup>7</sup>.

In a cross-sectional study of a total of 380 women, between the ages of 30 and 60 years old, there was a positive correlation of niacin intake and calcaneus BMD (bone mineral density) in premenopausal women<sup>17</sup>. BMD was measured at a heel scan, at the right calcaneus, using dual energy X-ray densitometry. Osteoporosis is defined as a BMD of equal or less than 2.5 SD below the reference mean of young adults with peak bone mass. However, a large percentage of fractures has been observed also in women with osteopenia<sup>1</sup>.

In the Singapore Chinese health study, 1630 Chinese

women, aged 45-74, identified with hip fracture after 13.8 years, there was no positive or negative association between hip fractures and niacin intake<sup>8</sup>.

In a longitudinal study with 5187 American participants, including both men and women, over the age of 65, the intake of niacin through diet had an inverse effect with femoral BMD measured after a period of 13 years, but not with total body BMD<sup>18</sup>. Another possible explanation for an increase of hip fractures in subjects receiving higher levels of niacin was thought by the authors to be niacin's ability to stimulate serotonin and prostaglandin release<sup>18</sup>.

Due to the adverse effect of niacin on osteoporosis and fractures further studies should take place in the near future.

### Vitamin B5 (pantothenic acid)

Pantothenic acid is a component of coenzyme A and has a vital role in fatty acid, carbohydrates, proteins metabolism and the synthesis of hormones<sup>7</sup>. Co enzyme A is essential for the citric acid cycle and the production of energy<sup>7</sup>. It is also very important for the regulation of ROS accumulation<sup>7</sup>. Milk, meat, dairy products, eggs are rich sources of vitamin B5. Deficiency of pantothenic acid is rare, since small quantities are present in every food source<sup>7</sup>.

Qinyu Ma et al found that pantothenic acid can enhance RANKL induced osteoclastogenesis in low quantities (<200  $\mu\text{M}$ ), using tartrate resistant acid phosphatase (TRAP) stain, whereas in excessive dosages (>500  $\mu\text{M}$ ) it suppresses osteoclastogenesis<sup>19</sup>. The low concentration of pantothenic acid stimulates phosphatidylinositol 3 kinase-protein kinase B (PI3K-Akt) pathway contributing to osteoclast proliferation and differentiation<sup>11,19</sup>. Elevated concentrations of pantothenic acid prohibits osteoclastogenesis by accumulating reactive oxygen species (ROS). ROS is fundamental for the stimulation of osteoclasts and bone resorption by activating MAP kinases, JNK, ERK and P38. Moreover higher concentrations of vitamin B5 activate the transcription factors of the forkhead box, class O, family, FoxO1, FoxO3 and nuclear factor erythroid 2 (Nrf2) promoting the production of antioxidant enzymes<sup>19</sup>.

High dietary consumption of pantothenic acid using ovariectomised (OVX) mice, an osteoporotic model of mice, contributed to elevated bone mineral density (BMD)<sup>19</sup>. This suggests that pantothenic acid has a positive impact on BMD despite estrogen deficiency<sup>19</sup>.

Further investigation is needed in order to uncover the role of vitamin B5 in osteoporosis and osteoporotic fractures.

### Vitamin B6 (Pyridoxine)

Vitamin B6 consists of a group of 6 derivatives: pyridoxine, pyridoxal, pyridoxamine, pyridoxine-5-phosphatase, pyridoxal-5-phosphatase and pyridoxamine-5-phosphatase<sup>4,7</sup>. Pyridoxal phosphate (PLP) is the active compound of pyridoxine that acts as a cofactor for numerous enzymes especially in decarboxylation of amino acids, amino acid biosynthesis, transamination, deamination, as well as

glycolysis and gluconeogenesis<sup>7</sup>. Vitamin B6 is fundamental for the conversion of homocysteine to cystathionine and finally to cysteine<sup>1,4</sup>. Pyridoxine is found in ample amounts in meat, chickpeas, avocado, poultry and fish<sup>4,7</sup>. Vitamin B6 deficiency can cause seborrheic dermatitis-like eruption, cardiovascular, hematologic or central nervous symptoms. Usually pyridoxine insufficiency occurs in patients with renal dysfunction, malabsorption, malnourishment and alcoholism<sup>7</sup>.

A study of 1002 people, aged 75, that had no hip fractures in 1987-1989 and also had minimum of one vitamin b level measured or homocysteine, showed that after a period of 4 years elevated concentrations of homocysteine have been related to an increase of osteoporotic fractures, especially hip fractures<sup>20</sup>.

Vitamin B6, vitamin B12 and folate play a significant role in the metabolism of homocysteine<sup>1</sup>. Low concentrations of these vitamins lead to homocysteine abundance, resulting in hyperhomocysteinemia (HHCY), reduced BMD and decreased bone quality<sup>1,21</sup>.

In the longitudinal review of the Framingham Osteoporosis Study, where 5209 participants took place in 1948, 1401 that were alive in 1987-1989 showed that low levels of pyridoxine were associated with a reduction of femoral neck bone mineral density and an increase in hip fractures after a period of 4 years<sup>20</sup>.

The Singapore Chinese Health study showed that there was an inverse association between hip fractures of Chinese women and pyridoxine dietary rich consumption<sup>8</sup>. These findings were restricted only in elderly women without a history of diabetes, furthermore implying a connection between pyridoxine, estrogen deficiency and diabetes mellitus<sup>8</sup>. Since previous epidemiologic studies have showed a positive influence of vitamin B6 on breast cancer, it is assumed that pyridoxine could interact with hormone synthesis<sup>8</sup>. Diabetes mellitus has a negative impact on osteoporosis and results in an increase of osteoporotic fractures, suggesting a protective effect of pyridoxine on elevated glucose plasma levels<sup>8</sup>.

Vitamin B6 is a cofactor for lysyl oxidase activity and collagen cross linking formation. Lysyl oxidase is an enzyme that catalyzes the formation of aldehydes from oxidative lysine residues. Aldehydes then form enzymatic cross links with other lysyl oxidase derived aldehydes creating collagen fibril<sup>4,21</sup>. Inhibiting the formation of collagen can result in poor bone microarchitecture and subsequently to osteoporosis and risk for fragility fractures<sup>21</sup>. In a study using chicks, as in vitro models, the low concentration of pyridoxine reduced the formation of enzymatic cross links<sup>4</sup>.

Future clinical studies may provide deeper insight into the relationship of vitamin B6, osteoporosis and fragility fractures for the prevention of bone deterioration.

### Vitamin B7 (Biotin)

Biotin is important for the metabolism of carbohydrates, protein and fats<sup>6</sup>. Vitamin B7 serves as a coenzyme for

the gluconeogenesis, carbohydrate catabolism and the biosynthesis of fatty acids<sup>7</sup>. Rich sources of biotin are poultry, liver, eggs, peanuts and sunflower seeds<sup>7</sup>. Deficiency can result to hair thinning, brittle nails and skin rashes. Biotinidase deficiency is an autosomal metabolic disorder due to the inability of biotin digestion in the diet<sup>6,7</sup>.

The effect of biotin on osteoporosis and osteoporotic fractures has not yet been evaluated in human studies. Biotin deficiency has been reported to lead to osteopenia in animal models<sup>22</sup>. In an animal study, where hamsters, chickens and mice were used, low levels of biotin led to teratogenesis. Mice fetuses showed bone malformations, such as limb deformities, cleft lip and a narrow jawline<sup>23</sup>. Since the model of mice used in this study is appropriate for human gestation, further studies should determine the impact of biotin on bone formation and resorption.

### Vitamin B9 (Folate)

Folate is a water soluble vitamin that is naturally found in dark leafy vegetables and legumes<sup>24</sup>. Folic acid is the synthetic active form of folate, that is found in fortified food and supplements<sup>25</sup>. Folic acid is firstly reduced in the liver to dihydrofolate by the enzyme dihydrofolate reductase, to tetrahydrofolate (THF) and then is converted to 5-10-methylenetetrahydrofolate (5-10-MRTHF)<sup>25</sup>. Folate is essential for the synthesis and the methylation of DNA and the metabolism of homocysteine to methionine.<sup>(26)</sup> Folate deficiency results in macrocytic anemia, elevated homocysteine levels, fatigue, depression, neurological defects and fetus development malformations<sup>24</sup>.

Vitamin B9 has been linked to osteoporosis and osteoporotic fractures due to the metabolic relation folate has with homocysteine<sup>1,27</sup>. Homocysteine metabolizes to methionine by the methylation of 5-methyltetrahydrofolate. Folic acid is converted to 5-10-methyltetrahydrofolate and 5-methyltetrafolate by the enzyme 5-10-methyltetrahydrofolate reductase (MTHFR). The C677T polymorphism of MTHFR reduces the activity of the enzyme, resulting in the excessive intracellular accumulation of homocysteine<sup>27</sup>.

In a study of 307 postmenopausal Japanese women, the TT genotype was associated with a decreased BMD compared to the CC or CT genotype<sup>1</sup>.

In a study regarding 328 postmenopausal British women there was no significant association between the TT, CC, CT genotype and folate concentration or BMD<sup>27</sup>.

In the Hordaland Homocysteine Study women with elevated plasma homocysteine levels and low folate consumption have a higher risk of hip fracture compared to men<sup>28</sup>.

The Singapore Chinese study showed no apparent association between folate and the risk for hip fractures<sup>8</sup>.

In the Danish Osteoporosis Prevention study, 1869 women, just before menopause, were measured at baseline and after a period of 5 and 10 years for vitamin b intake and

bone mineral density (BMD). There was a positive effect of folate dietary intake and femoral BMD, at the cross-sectional analysis after 5 years. However the longitudinal study after a 10 year period showed no positive or risk association between folate intake and femoral BMD<sup>29</sup>.

The B-PROOF randomized controlled trial showed no potential relationship between folate and vitamin B12 supplementation and reduction of osteoporotic fractures in hyperhomocysteinemic elderly aged >65 years<sup>30</sup>.

In the Framingham Osteoporosis study folate plasma concentration had no effect on hip fracture<sup>20</sup>.

Also in a randomized trial of women with cardiovascular disease, supplementation with folic acid was not associated with a higher risk of non-spine fractures after a 7.3 year period<sup>31</sup>.

Furthermore in another cohort study of 188 postmenopausal women situated in Morocco in 2008, the association of asymptomatic vertebral fractures with folate concentration showed that folate deficiency was not a determinant for asymptomatic vertebral fractures<sup>32</sup>.

Due to the inconsistencies of the clinical results, more clinical studies are needed for the evaluation of vitamin B9 with osteoporosis and osteoporotic fractures.

### Vitamin B12 (Cobalamin)

There are four different compounds of cobalamin: cyanocobalamin, hydroxocobalamin, adenosylcobalamin and methylcobalamin that are necessary cofactors for the biosynthesis of DNA, erythropoiesis, the activation of the enzyme L-methylmalonyl-coenzyme A (CoA) mutase and the enzyme methionine synthase<sup>33</sup>. Rich dietary sources of cobalamin are meat, meat products, milk, fortified cereal and eggs<sup>34</sup>. Deficiency of vitamin B12 leads to pernicious anemia and neurological symptoms, usually caused by malabsorption, chronic acid-reducing agents and malnutrition<sup>34</sup>.

Hyperhomocysteinemia has been related to an increased risk for osteoporosis and osteoporotic fractures<sup>35</sup>. The elevated levels of plasma homocysteine can be controlled by folate and cobalamin supplementation. Methylcobalamin is used as cofactor for the metabolism of homocysteine to methionine by the enzyme methionine synthase<sup>36</sup>. Therefore, this reaction converts 5-methyltetrahydrofolate to tetrahydrofolate with the assistance of vitamin B12. Methylmalonyl-CoA is converted to succinyl-CoA, catalyzed by the enzyme methylmalonyl-CoA synthase, with adenosylcobalamin used as cofactor<sup>36</sup>. Increased levels of methylmalonic acid (MMA) have been related to osteoporosis<sup>37</sup>. Vitamin B12 deficiency results in elevated levels of MMA and homocysteine.

In another study of 328 postmenopausal British women, cobalamin can prevent the risk for fractures due to its metabolic relationship with homocysteine<sup>27</sup>.

There has been no positive association of Vitamin B12 intake and risk of hip fractures in the Singapore Chinese study nor in the Hordaland study<sup>8,28</sup>.

The B-PROOF study only showed a positive association in elderly >80 years old with high levels of homocysteine<sup>30</sup>. Vitamin B12 deficiency especially in the elderly leads to neurological symptoms, such as motor deficiencies, numbness of the feet that can result to a tendency to falls<sup>20</sup>.

A genetic analysis study in 301 Korean women over the age of 69, as well as the individuals with elevated homocysteine levels, found a correlation between osteoporosis and osteoporotic vertebral fractures and the genotype CD320rs9426CT+TT, compared to the CC genotype. The CD320 gene encodes transcobalamin II receptor, which binds with transcobalamin II for the cellular absorption of vitamin B12<sup>9</sup>. Further research is obligatory in order to determine the positive or negative effect of cobalamin on fractures and bone deterioration.

In a cell culture study MMA and homocysteine stimulates osteoclastogenesis, resulting in bone absorption and supplementation with cobalamin can alter the outcome<sup>3</sup>.

In relation to osteoporosis and osteoporotic fractures thiamine in humans may have a protective role. Riboflavin deficiency, especially in people with the MTHFR TT genotype, can result in an increase in fractures. Higher levels of niacin in the elderly are probably linked to hip fractures. Folic acid and cobalamin are possibly linked to bone deterioration through the homocysteine metabolism. Given the fact that osteoporosis has been on the increase during the last few decades, it is essential that further human studies are conducted, in order to determine the exact impact of the vitamin B complex on osteoporosis and osteoporotic fractures for its prevention.

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