Association of hyperhomocysteinemia with osteoporosis: a systematic review

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Osteoporosis, especially in postmenopausal women, is a major health and economic concern in the world. Environmental, metabolic and genetic factors play roles in determination of bone mass and health. Over the past several years, evidence has been growing regarding the negative effects of homocysteine on bone health. The objective of this paper was to provide a review of the state of the science of homocysteine and osteoporosis. Medline, Index Medicus, Scopus, Google Scholar and Web of Sciences were searched for any paper regarding the effects of homocysteine on osteoporosis using key words of osteoporosis, bone health, homocysteine, hyperhomocysteinemia, and methylentetrahydrofolate reductase polymorphism. Most of the relevant citations from human literature were studied and summarized. Regarding bone mineral density, risk of fracture and bone markers that have been studied, the negative effects of homocysteine on bone health seems considerable. Vitamin B deficiency may play an important role in bone metabolism, which has to be further investigated. According to the differences in genetic predisposition, environmental, sex differences and nutritional factors, further studies are needed to explore the effective mechanisms of association between homocysteine and osteoporosis.

Osteoporosis is one of the most common illnesses in the elderly, and has recently been connected to hyperhomocysteinemia (HHCY) [1–3]. Frequent fracturing of bones is one of the main problems in osteoporotic patients, which often leads to substantial damage, disability and need for care (there are approximately 2.5 million osteoporotic fractures per year in the USA) [4].

Homocysteine is a sulfur-containing amino acid intermediate formed during the metabolism of methionine. In normal kidney function, its high serum level is mostly related to enzyme deficiency or B vitamin deficiency (folate, B12 and B6) [5]. Age and gender are two major determinants of homocysteine (HCY) concentrations in humans. Plasma concentration of HCY increases with age, and young men (aged 30–40 years) normally have higher HCY levels than women (approximately 2 µmol/l higher). Estrogen is involved in the differentiation between the sexes; therefore, during the menopause the plasma HCY level increases [6], whereas the age-dependent elevation of HCY concentration is mostly related to physiological decrease in kidney function [7,8].

Initially, in the early 1960s, HCY was reported as a pathogenic agent. It has been shown that a high circulating HCY level is an independent risk factor for several chronic diseases, including cardiovascular and Alzheimer diseases [9], and moderate HCY levels carry approximately 10% of the total risk of cardiovascular diseases [6,7,10]. In addition, in patients with homocystinuria, the prevalence of skeletal deformities such as osteoporosis has been increased [11–13]. HHCY is also considered as a risk factor for neurodegenerative diseases, osteoporotic fractures and pregnancy complications. In fact, elevated plasma HCY concentration increases the risk of hip fracture, causing disability [14], high medical costs [15] and death [16].

In 1957, HCY was first recognized as a responsible factor for osseous mutations in patients with homocystinuria [17]. These patients showed accelerated bone growth, skeletal deformities, flattened vertebral bodies and a low bone density [18].

The association of plasma HCY and folate on bone mineral density (BMD) has been investigated in previous studies [1,2,19,27,28]. *In vitro* studies indicated the probable interference between HCY and the formation of collagen cross-links, prevention of fibrils' insolubilization, inhibition of lysyl oxidase and delay in the synthesis of more complex cross-links in collagen [20–22]. However, whether HCY is the causative agent or whether osteoporosis is caused by various elements of HCY metabolism has not been fully evaluated yet. Folate and vitamin B12 are major components of HCY metabolism. Concentrations of folate and its metabolites differ in the several

Keywords: bone health, homocysteine, osteoporosis

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body compartments, and not only plasma but also red blood cells (RBCs) carry folate. RBCs act as a folate reservoir and maintain folate homostasis. Unlike plasma folate, RBC folate is not affected by exogenous factors such as diet and drugs [23]. Therefore, there is the possibility that RBC folate as a long-term marker of the body folate better predicts BMD than plasma folate.

Taken together, it seems necessary to determine the exact effects of HCY on senile conditions such as osteoporosis. There is convincing evidence regarding the association of HHCY with osteoporosis, which starts with the next part of the paper containing the most reliable human, cell culture and animal studies.

Study observations

The relation between HHCY and bone diseases had not been recognized for decades, until two prospective population-based studies determined a potential relationship between moderately elevated plasma HCY and the frequency of osteoporotic fractures in the elderly population [1,24].

van Meurs *et al.* evaluated 2404 elderly patients (aged 55 years and older) over a period of 11,253 man-years in Amsterdam and Rotterdam (The Netherlands). They observed an association between HCY level and risk of fracture that was independent of BMD and other potential risk factors for fracture [1].

McLean *et al.* observed similar results in a subgroup of the Framingham study [2]. The men in that study (average age: 70 years; range: 59–91 years) with an HCY level in the third quartile (mean value: 13.4 µmol/l) had a fracture risk 2.07-times higher than men with an HCY level in the first quartile. The fracture risk for men in the fourth quartile (mean HCY value: 20.8 µmol/l) was 3.84-times higher than for men in the first quartile. This relationship in women was less clear. However, Périer *et al.* obtained different results in 671 postmenopausal women who were followed prospectively during a mean follow-up of 10 years, and concluded that HCY is not an independent risk factor of osteoporotic fractures in healthy postmenopausal women with a broad age range [25].

The mechanisms of the link between HHCY and fracture risk are unknown [26]. Some studies analyzed HCY in relation to BMD, but they showed no or only a weak correlation between these two variables [27–29].

In fact, the weak correlation between HCY and BMD is not unexpected, since BMD is only an integral measurement of bone metabolism over a longer period of time. There are studies in which biochemical bone markers have been measured that demonstrate a real-time monitoring of bone metabolism [27,30].

Dhonukshe-Rutten *et al.* showed an increased level of bone formation and resorption markers in patients with HHCY [30]. Herrmann *et al.* investigated post- and pre-menopausal women, and reported that HCY concentration correlated positively with bone-resorption marker urinary deoxypyridinoline crosslinks, but not with the bone-formation marker osteocalcin in serum. They suggested that bone metabolism has been shifted towards bone resorption by HCY [27]. In addition, they stimulated osteoclast activity in cultured human osteoclasts with increasing doses of HCY, which is in agreement with the hypothesis of increased bone resorption in the presence of HHCY. Existing results propose that HHCY can affect osteoclast activity, but these data are not adequate to conclude that osteoclasts are the principal target of HCY in human bone.

On the other hand, Gerdhem *et al.*, who conducted the study in 996 women from the Osteoporosis Prospective Risk Assessment study, suggested HCY as a risk factor. They observed the link between high bone-marker levels and low BMD at baseline. During a mean 7-year follow-up, high HCY concentration was associated with mortality, but no obvious link to fracture risk was observed [31].

Kim *et al.* investigated the effects of HCY on human bone marrow stromal cells. They observed that HCY induces apoptosis in primary human bone marrow stromal cells via the reactive oxygen species-mediated mitochondrial pathway and NF-κB activation in human bone marrow stromal cells. HCY was found to contribute to the development of osteoporosis by reducing bone formation. It was concluded that antioxidants may have a role in preventing bone resorption in patients with HHCY [32]. Koh *et al.* performed a similar study on osteoclasts and suggested that HCY directly activates formation of osteoclasts by generation of intracellular reactive oxygen species. Thus, an antioxidant seems to attenuate bone loss in HHCY [33]. However, Herrmann *et al.* demonstrated that accumulation of HCY by decreasing concentration of folate, vitamin B12 and B6 does not affect the activity of human osteoblasts [34]. Furthermore, the association between HHCY and reduced bone quality and disturbed bone metabolism was confirmed in animal studies by Herrmann *et al.* and Ozdem *et al.* suggesting HHCY as a causal osteoporotic factor in rats [35,36].

While in the elderly, HHCY is principally caused by vitamin B deficiency, it is not well known whether these vitamins play a significant role in bone health. Considering the mechanisms, previous studies proposed a reduction in osteoblast activity in association with low vitamin B12 concentrations [37,38].

Goerss *et al.* observed that in patients with pernicious anemia (caused by vitamin B12 deficiency), the risks of proximal femur, vertebral and forearm fractures were 1.9-, 1.8- and 2.8-times more than controls, respectively [39].

In one prospective trial on 600 patients with osteopenia and osteoporosis, the important role of B vitamins in bone health was studied. Sato *et al.* treated the patients with 5 mg of folic acid and 1500 µg of vitamin B12 or placebo for 2 years. They observed an approximately 75% decrease in the incidence of fractures in the treatment group, which was comparable with that of alendronate [40].

Considering the various folate concentrations in different compartments of the body, Golbahar *et al.* suggested RBC folate as a better predictor of BMD than plasma folate, for which deficiency may be associated with the pathogenesis of osteoporosis in postmenopausal women [23]. Approximately 1 year later, Gjesdal *et al.* performed another study on 5338 elderly patients to examine the association between hip BMD and plasma levels of HCY, folate, vitamin B12 and the methylenetetrahydrofolate reductase (*MTHFR*) polymorphism. They concluded that elevated HCY and low folate levels were associated with reduced BMD in women but not in men [41].

In another recent study, Green *et al.* investigated 276 healthy older subjects who were randomly assigned to receive either daily supplement of folate, vitamin B12 and vitamin B6 or placebo for 2 years. By measuring bone-specific alkaline phosphatase and bone-derived collagen fragments at baseline and the end of study, they concluded that supplementation with folate and vitamin B6 and B12 can lower plasma HCY but has no effect on bone turnover [42].

Concerning the significance of folic acid, many groups studied the effect of the C677T *MTHFR* polymorphism on bone. An increase of fracture incidence was detected with every T allele, especially in patients with low folate levels [43–45]. There are some contradictory results in this issue. Li *et al.* reported no association between *MTHFR* (C677T) and the BMD of Chinese men or women. They performed the study on postmenopausal women, elderly women and elderly men. High folate and vitamin B intake in the study population, added to the low number of patients and low prevalence of the TT genotype, should be taken into account [46].

Abrahamsen *et al.* confirmed that in the lowest quartile of riboflavin, B12, B6 and folate intake, BMD in *MTHFR* TT genotype is only significantly reduced, at least at the time of menopause, and vitamin B supplementation would only be anticipated to affect BMD in approximately 2% of the population, such as those with the TT genotype and low vitamin B intake [47]. They also observed significant skeletal effects of this common polymorphism at lumbar spine in men at the age of 25 years [48].

Hong *et al.* obtained similar results. They included 1899 Chinese postmenopausal women to verify the association of the *MTHFR* polymorphism with BMD and fractures. They demonstrated that the *MTHFR* C677T polymorphism is an independent predictor of fracture risk, although it only had a weak effect on BMD [49].

Other than higher fracture risk, low circulating levels of vitamin B12 and folic acid are also associated with low BMD, which is in agreement with the link between HCY and BMD [50–53].

Baines *et al.* studied the link between plasma HCY, its determinant folate, vitamin B12, vitamin B6, *MTHFR* genotype and BMD in 328 postmenopausal women. According to the baseline BMD, the subjects were assigned to three groups of control, osteopenic and osteoporotic. The osteoporotic patients showed a significantly lower serum folate and a higher incidence of recent fracture. In conclusion, they found that low serum folate is an important risk factor for osteoporosis, with HCY level having a lesser importance. Both vitamins B12 and B6, by affecting HCY, may also have an effect on the skeleton, although a weaker one than folate [54].

More details of the above-mentioned studies are illustrated in **Tables 1 & 2**.

Discussion

Although it generally seems that HHCY contributes to osteoporosis development, there is little evidence regarding the direct effects of HCY on bone. While some studies concern the strong link between HCY and osteoporosis, others consider other factors such as folate. As we can see, different studies evaluated the effects of HCY on bone loss from different viewpoints such as

Table 1. Effects of homocysteine on bone: human studies.

Study	Study population	Results	Ref.
van Meurs *et al.* (2004)	2404 elderly patients (≥55 years)	↑ 1.4-fold in total fracture risk per SD increase in HCY	[1]
Li *et al.* (2004)	657 Chinese men and women	No association between *MTHFR* polymorphism and BMD	[46]
McLean *et al.* (2004)	825 men and 1174 women aged 59–91 years	Higher HCY level → higher fracture risk especially in men	[2]
Dhonukshe-Rutten *et al.* (2005)	615 men and 652 women (age: 76 ± 6.6 SD years)	High HCY and low vitamin B12 levels → ↓ BUA, ↑ OC and DPD, ↑ fracture risk	[30]
Abrahamsen *et al.* (2005)	1700 postmenopausal women	↓ riboflavin, B12, B6 and folate + *MTHFR* TT genotype → ↓ BMD	[47]
Dhonukshe-Rutten *et al.* (2005)	73 adolescents (9–15 years)	Sign of an impaired cobalamin status is associated with low BMD	[50]
Hermann *et al.* (2005)	143 peri- and post-menopausal women	Weak but significant relationship between HCY and bone resorption	[27]
Golbahar *et al.* (2005)	366 post-menopausal women	RBC folate deficiency is associated with low BMD	[23]
Abrahamsen *et al.* (2006)	780 healthy Danish men (20–29 years)	Significant skeletal effects of *MTHFR* polymorphism at lumbar spine in men at 25 years	[48]
Gjesdal *et al.* (2006)	5338 men (47–50 years) and women (71–75 years) (Hordaland study)	HHCY and low folate levels were associated with low BMD in women	[41]
Gerdhem *et al.* (2007)	996 women aged 75 years	↑ HCY → ↑ bone marker and ↓ BMD	[31]
Hong *et al.* (2007)	1899 Chinese postmenopausal women	MTHFR polymorphism is an independent risk factor of fracture risk, had a weak effect on BMD	[49]
Baines *et al.* (2007)	328 postmenopausal British women	Low serum folate level is more important than HCY for osteoporosis	[54]
Green *et al.* (2007)	276 healthy subjects	Supplementation with folate and vitamins B6 and B12 lowered plasma HCY, but had no effect on bone turnover after 2 years	[42]
Périer *et al.* (2007)	671 postmenopausal women (OFELY study)	HCY is not an independent risk factor of osteoporotic fracture	[25]

BMD: Bone mineral density; BUA: Broadband ultrasound attenuation; DPD: Deoxypyridinoline; hBMSC: Human bone marrow stromal cell; HCY: Homocysteine; HHCY: Hyperhomocysteinemia; MTHFR: Methylenetetrahydrofolate reductase; OC: Osteocalcin; RBC: Red blood cell; ROS: Reactive oxygen species; SD: Standard deviation.

BMD, fracture risk, bone markers, serum levels of vitamin B groups and the *MTHFR* C677T polymorphism. The probable mechanisms of action of HCY on bone are illustrated in **Figure 1**.

Concerning various parameters measured in different studies, some limitations can be numbered that should be taken into account to reach a better conclusion. For example, while BMD is an integral measurement of bone metabolism over a long period of time, it would be better to measure biochemical bone markers and then have a more accurate suggestion of bone metabolism. In fact, HHCY is often accompanied by several medical conditions that could influence collagen cross-links as a bone marker also. Although some studies did not support the causal relationship between HCY and bone health, there is the possibility that high HCY level may adversely affect bone strength through mechanisms not related to bone turnover and mass. The prevalence of *MTHFR* C677T polymorphism in the study population is another important factor that must be considered in future studies.

Regarding the compliance of patients, it should not be forgotten that in some of these studies, the study subjects were healthier older people, as the more vulnerable subjects could not collaborate well and incorporate into some measurements. The time of HCY-concentration measurement is another important factor, because blood samples from fasting subjects tend to have higher total HCY concentrations. While measuring folate in plasma or serum represents the circulatory folate, measuring folate in RBCs illuminates the intracellular folate reservoir, which represents the amount of folate available as a cofactor in HCY metabolism.

Table 2. Effects of homocysteine on bone: cell culture and animal studies.

Study	Hypothesis	Results	Ref.
Kim *et al.* (2006)	Apoptotic effect of HCY on osteoblasts	HCY → apoptosis in hBMSCs via ROS	[32]
Koh *et al.* (2006)	Examinig the direct effect of HCY on osteoclast formation	HCY directly activates osteoclast formation and activity	[33]
Herrmann *et al.* (2007)	HHCY decreases osteoblast activity	HHCY does not affect osteoblast activity	[34]
Herrmann *et al.* (2007)	HHCY as a causal osteoporotic factor in rats	HHCY reduces bone quality in rats	[35]
Ozdem *et al.* (2007)	HHCY can disturb bone metabolism	Significant modification of bone turnover in HHCY rats	[36]

hBMSC: Human bone marrow stromal cells; HCY: Homocysteine; HHCY: Hyperhomocysteinemia; ROS: Reactive oxygen species.

Further studies are needed to clarify whether measuring RBC folate could better indicate BMD [23]. In addition, cut-off points for vitamin B12 deficiency are not generally defined yet. Some findings in white men and women may not be easily extended to other race and ethnic groups.

The full mechanism of the link between HCY and osteoporosis is still unclear. Some believe that HCY interferes with cross-links of newly formed collagen [55], and therefore with bone strength and bone mineralization [56–58]. In fact, more studies are required to determine the association between HCY and the material and structural characteristics of bone in humans. If the relationship is confirmed as a cause-and-effect link, this conclusion may have a significant effect on hip-fracture prevention strategies, as total HCY concentrations can be easily regulated by dietary intake of folate and vitamin B groups. This relationship needs to be assessed in large population studies to see if the link is nationwide. Regarding the role of oxidative stress in the pathogenesis of osteoporosis, and also in relation to oxidative stress, it would be rational to design further studies to explore whether reduction of oxidative stress by use of strong antioxidants would affect HCY level and osteoporosis [59].

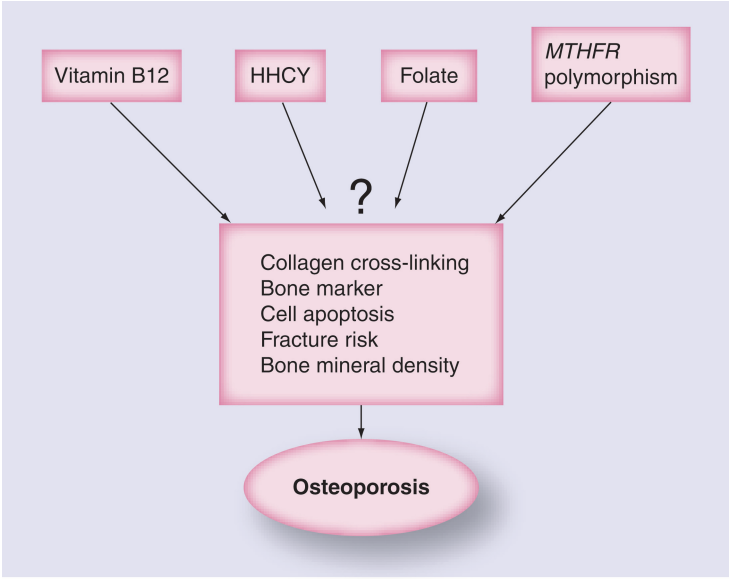
Future perspective

Future advances in healthcare and medicine will aid in the discovery of the relationship between brittle bone and cardiovascular disease.

Executive summary

- Homocysteine can affect bone quality, most likely by affecting osteoclast formation and changing bone markers.
- Animal and cell culture studies confirm the results of human studies considering change in bone quality by homocysteine.
- Some studies considered vitamin B deficiency as a major risk factor of osteoporosis, while others reported on hyperhomocysteinemia.
- Methylenetetrahydrofolate reductase polymorphism seems to interact in bone metabolism, but it has yet to be fully investigated.
- Few studies considered a difference between men and women in skeletal effects of hyperhomocysteinemia, which needs to be further evaluated.
- Vitamin B supplementation seems to affect bone marrow density, but available supporting data are limited.
- The future lies in designing randomized, controlled studies to look at the association between osteoporosis, hyperhomocysteinemia, vitamin B deficiency, methylenetetrahydrofolate reductase polymorphism and, most likely, cardiovascular disease.

Figure 1. Schematic view of unknown mechanisms of homocysteine on bone.



HHCY: Hyperhomocysteinemia; MTHFR: Methylenetetrahydrofolate reductase.

Considering the limitations of studies in this area, the design of the most reliable clinical trials would enable us to reach better conclusions.

When the different mechanisms of action of HCY and its determinant folate levels in serum or RBCs on bone are considered, it is clear that more studies need to be designed. The link between oxidative stress and HCY and osteoporosis also needs to be clarified by further studies. The simultaneous measurement of osteoblast and osteclast biomarkers and BMD in future clinical trials should be considered.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending or royalties.

No writing assistnce was utilized in the production of this manuscript.

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