



# SUMMARY

## **B-vitamins for the prevention of fractures and decline of physical function**

With the increase in life expectancy, the number of osteoporotic fractures is expected to rise. Osteoporotic fractures are associated with pain, decline in function and loss of quality of life, as well as with high costs. Circulating homocysteine has been identified as modifiable risk factor for osteoporotic fractures. Elevated homocysteine levels can effectively be reduced with vitamin B12 and folic acid supplementation.

This thesis aimed to contribute to the understanding of the association between homocysteine and increased fracture risk in older persons. The main objective was to examine the effect of homocysteine-lowering by B-vitamin supplementation on fracture incidence. Second, potential mechanisms at work were considered by examining 1) the association of homocysteine with different aspects of physical functioning and falling, and 2) the effects of homocysteine-lowering therapy on physical functioning and falling, and 3) the effect on bone characteristics, including bone mineral density and quantitative ultrasound parameters.

Data from the Longitudinal Aging Study Amsterdam (LASA) and the B-PROOF study were used. The LASA is an ongoing cohort study in the Netherlands that studies determinants, trajectories and consequences of physical, cognitive, emotional and social functioning in older persons. The LASA started in 1992-1993 with 3,107 respondents initially aged 55-85 years. Approximately every 3 years, data were collected in a main and medical interview, and a questionnaire.

B-PROOF is an acronym for 'B-vitamins in the prevention of osteoporotic fractures'. The main aim of B-PROOF was to study the effect of vitamin B12 and folic acid supplementation on fracture incidence in a general population of older adults. In Chapter 2, the rationale and design of the B-PROOF study are described in detail. A total of 2,919 persons aged 65 years or older with mildly elevated homocysteine levels (12-50  $\mu\text{mol/L}$ ) were included in this randomized controlled trial. Participants who were assigned to the intervention group received a daily tablet with 400  $\mu\text{g}$  folic acid, 500  $\mu\text{g}$  vitamin B12, and 600 IU vitamin D3; the placebo tablet contained 600 IU vitamin D3 only. Falls and fractures were recorded prospectively on a weekly calendar. Blood sampling and interviews were conducted at baseline and follow-up, with emphasis on aspects including physical functioning, cognition, cardiovascular outcomes, and quality of life.

In the next four chapters, association studies are described. Chapter 3 reports cross-sectional and longitudinal findings on homocysteine and vitamin B12 in relation to physical performance within the LASA. Higher homocysteine levels were associated with lower physical performance scores and decline in physical performance in women. The association was observed with chair stands and walking test as indicators of physical functioning, but

not with balance. The associations in men and with vitamin B12 were less consistent, and interaction with age was not observed.

Chapter 4 describes other aspects of physical functioning in relation to homocysteine, including muscle mass, muscle strength, functional limitations, and falling in the LASA study. We observed that higher homocysteine levels were associated with lower handgrip strength in men, and more functional limitations in both men and women. We did not observe associations of homocysteine with muscle mass, or with falling.

Next, we studied MTHFR polymorphism and homocysteine, as well as their interaction in relation to physical performance and muscle function, using baseline data from the B-PROOF study (Chapter 5). The inverse association of homocysteine with different indicators of physical functioning in certain groups was also observed here. Again, the association with physical performance was only observed in women. With respect to handgrip strength, a significant association was observed in woman only. An association of homocysteine with muscle mass was not observed. High homocysteine in the 677CC and 677CT genotypes, but not in the 677TT genotype, was associated with lower physical performance.

In Chapter 6, the association between serum folate and C-reactive protein (CRP), a marker of inflammation, was described within the B-PROOF study. We identified a significant U-shaped association between serum folate and CRP in the total study sample, with optimal serum folate concentrations around 20 nmol/L. In elderly with cardiovascular disease (CVD), higher folate concentrations were significantly associated with higher CRP concentrations. Below a threshold of approximately 17 nmol/L, a borderline significant inverse association between folate and CRP was observed in elderly without CVD. High serum folate concentrations might be detrimental in elderly with CVD.

The following three chapters report effects of vitamin B12 and folic acid supplementation, using follow-up data of the B-PROOF study. The effect on osteoporotic fracture incidence was described in Chapter 7. The data showed that combined vitamin B12/folic acid supplementation had no effect on osteoporotic fracture incidence in this elderly population. Exploratory subgroup analyses suggest a beneficial effect on osteoporotic fracture prevention in compliant persons >80 years. However, treatment was also associated with increased incidence of cancer.

The effect of the B-PROOF intervention on physical performance, hand grip strength, and falling was described in Chapter 8. Two-year supplementation of vitamin B12 and folic acid was neither effective in reducing the age-related decline in physical performance and handgrip strength, nor in the prevention of falling in elderly persons.

Chapter 9 reports the effect the B-PROOF intervention on bone mineral density (BMD), and quantitative ultrasound (QUS) parameters. This study showed no overall effect of treatment with vitamin B12, folic acid on BMD or QUS parameters in elderly, mildly hyperhomocysteinemic persons, but suggests a small beneficial effect on bone elasticity in persons >80 years who were compliant in taking the supplement.

In the general discussion of Chapter 10, it was concluded that there is no effect of vitamin B12 and folic acid supplementation on osteoporotic fractures among elderly with mildly elevated homocysteine levels. Some evidence for a beneficial effect on fractures was found in a subgroup of compliant persons >80 years. In additional observational studies, we furthermore observed an inverse association of plasma homocysteine levels with physical performance in women, but B-vitamin supplementation did not have an effect on physical performance in the total study sample. A small beneficial effect on bone elasticity was observed in the intervention group compared to placebo among compliant persons >80 years, while no effect was found in the total study sample. The intervention had no effect on handgrip strength, falling, bone mineral density, or speed of sound. A higher incidence of cancer was reported in the intervention group, which was most pronounced in persons >80 years. Future research should confirm our findings and balance the possible benefits of B-vitamin supplementation in older persons against the possible harms.

