# Low-dose vitamin B-6 effectively lowers fasting plasma homocysteine in healthy elderly persons who are folate and riboflavin replete[1–3]

*Michelle C McKinley, Helene McNulty, Joseph McPartlin, JJ Strain, Kristina Pentieva, Mary Ward, Donald G Weir, and John M Scott*

## ABSTRACT

**Background:** Current data suggest that physiologic doses of vitamin B-6 have no significant homocysteine-lowering effect. It is possible that an effect of vitamin B-6 was missed in previous trials because of a much greater effect of folic acid, vitamin B-12, or both.

**Objective:** The aim of this study was to investigate the effect of low-dose vitamin B-6 supplementation on fasting total homocysteine (tHcy) concentrations in healthy elderly persons who were made replete with folate and riboflavin.

**Design:** Twenty-two healthy elderly persons aged 63–80 y were supplemented with a low dose of vitamin B-6 (1.6 mg/d) for 12 wk in a randomized, double-blind, placebo-controlled trial after repletion with folic acid (400 $\mu$g/d for 6 wk) and riboflavin (1.6 mg/d for 18 wk); none of the subjects had a vitamin B-12 deficiency.

**Results:** Folic acid supplementation lowered fasting tHcy by 19.6% ($P < 0.001$). After folic acid supplementation, baseline tHcy concentrations ranged from 6.22 to 23.52 $\mu$mol/L and 10 subjects had suboptimal vitamin B-6 status (plasma pyridoxal-*P* < 20 nmol/L). Two-way analysis of variance showed that the significant improvement in vitamin B-6 status in response to vitamin B-6 supplementation (on the basis of both pyridoxal-*P* and the erythrocyte aspartate aminotransferase activation coefficient) was reflected in a significant reduction in plasma tHcy of 7.5%.

**Conclusions:** Low-dose vitamin B-6 effectively lowers fasting plasma tHcy in healthy subjects who are both folate and riboflavin replete. This suggests that any program aimed at the treatment or prevention of hyperhomocysteinemia should include vitamin B-6 supplementation.    *Am J Clin Nutr* 2001;73:759–64.

**KEY WORDS**    Fasting homocysteine, vitamin B-6, folic acid, riboflavin, plasma pyridoxal-*P*, erythrocyte aspartate aminotransferase activation coefficient, EASTAC, elderly

## INTRODUCTION

Even mild elevations in total homocysteine (tHcy) are associated with an increased risk of cerebrovascular, peripheral vascular, and cardiovascular diseases (1). Once formed, homocysteine is either remethylated to methionine, which requires vitamin B-12 (2), folate (2), and riboflavin (3) as a cofactor, cosubstrate, and prosthetic group, respectively, or undergoes a transsulfuration reaction to form cysteine. The transsulfuration pathway is catalyzed by cystathionine β-synthase, which is dependent on pyridoxal-*P* (PLP) (4).

Although vitamin B-6 has been used for many years to successfully treat homocystinuria caused by cystathionine β-synthase deficiency (5), a role for vitamin B-6 in the prevention or treatment of mild hyperhomocysteinemia is unclear. Results from 9 studies that investigated the effect of vitamin B-6 (when it was given as a treatment on its own) on fasting plasma tHcy concentrations are inconclusive. Six of the aforementioned studies found no change (6–11) in fasting tHcy and one found a significant increase (12). To our knowledge, only 2 studies in the literature found a significant reduction in fasting tHcy after vitamin B-6 supplementation (13, 14). The first of these studies, carried out in 10 young women who had clinical and biochemical vitamin B-6 deficiency, found a significant lowering of fasting tHcy of 19.7% after 15 d of treatment with 20 mg pyridoxine hydrochloride/d. The trial, however, was not blinded or placebo-controlled and had no washout period. The second study (14), a recent placebo-controlled trial, showed a significant lowering of fasting tHcy of 17% ($P < 0.011$) in response to extremely large doses (120 mg/d) of vitamin B-6 in healthy subjects ($n = 9$).

Thus, the literature to date suggests either that vitamin B-6 does not have a homocysteine-lowering effect (6–12) or that it has a lowering effect only when given in exceptionally high doses (14) or in exceptional circumstances, ie, in pyridoxine-responsive homocystinuria (5), in selective patients with severe vitamin B-6 deficiency (13), or after methionine loading (6, 15, 16). This evidence may lead to the conclusion that in apparently healthy persons, a suboptimal vitamin B-6 status does not represent a general public health problem with respect to its effect on tHcy. This is at odds with the literature, which suggests that

suboptimal vitamin B-6 status might represent a general risk for vascular disease (17–20), and with other studies that showed a significant inverse relation between tHcy and vitamin B-6 status (17–19, 21–24) and an accumulation of homocysteine in vitamin B-6 depletion-repletion studies (25, 26).

All of the above supplementation studies were carried out in volunteers aged 18–60 y and used pharmacologic doses of vitamin B-6. To date, no vitamin B-6 intervention has been carried out in elderly persons, even though it is well accepted that vitamin B-6 status declines with age (27, 28) and plasma tHcy concentrations increase with age (22, 29). Therefore, the aim of this study was to investigate the effect of low-dose vitamin B-6 supplementation on fasting tHcy concentrations in healthy elderly persons after exclusion of those deficient in vitamin B-12 and after optimization of folate and riboflavin status.

## SUBJECTS AND METHODS

### Subject recruitment

Ethical approval was granted by the Research Ethical Committee of the University of Ulster and subjects gave written, informed consent. Subjects aged ≥60 y were recruited between January and April 1998 through senior citizen groups and local folds (ie, sheltered accommodation providing no medical support for healthy elderly persons who live independently within a housing complex and take care of themselves). All potential subjects were interviewed by using a short medical questionnaire about the subjects' general health and drug and supplement use. The exclusion criteria were as follows: B vitamin supplementation, gastrointestinal disease, hematologic disorders, use of drugs known to affect vitamin B-6 or riboflavin metabolism (eg, antacids containing magnesium or ioniazid), impaired cognitive function [score < 7 on Hodkinson 10-Point Mental State Questionnaire (30)], serum creatinine concentration ≥105 μmol/L, serum vitamin B-12 concentration <111 pmol/L (150 ng/L), and vascular, hepatic, or renal disease.

### Study design

The vitamin B-6 intervention was a 12-wk, randomized, double-blind, placebo-controlled trial. Subjects were randomly assigned to 1 of 2 groups to receive either vitamin B-6 or a placebo from a laboratory technician who was not involved with the study in any other way. Before starting the vitamin B-6 intervention, all subjects received supplementation with riboflavin (1.6 mg/d) for 12 wk followed by a combination of folic acid (400 μg/d) and riboflavin (1.6 mg/d) for an additional 6 wk. Folic acid and riboflavin supplementation continued throughout the vitamin B-6 intervention. At the start of the vitamin B-6 intervention, a 20-mL blood sample was taken after the subjects had fasted overnight. Subjects were then supplied with either vitamin B-6 (1.6 mg) or a placebo tablet daily. To maximize compliance, subjects were visited in their homes every 3 wk, at which time they were supplied with additional supplements; any unused tablets from the previous 3 wk were returned and counted. Subjects were instructed to maintain their usual diets and refrain from commencing any form of vitamin supplementation during the intervention.

### Blood sampling

All blood samples were collected in the subjects' homes while they were in a sitting position. Three tubes of blood were collected from each subject: one 8-mL EDTA-treated tube for plasma and red blood cell extraction, one 4-mL EDTA-treated tube for preparation of red blood cell lysate and measurement of packed cell volume, and one 8-mL serum separation tube for serum extraction. The tube containing plasma for homocysteine, vitamin B-6 (plasma PLP), erythrocyte aspartate aminotransferase activation coefficient (EASTAC), and riboflavin (erythrocyte glutathione reductase activation coefficient, EGRAC) measurements was wrapped in foil and placed on ice immediately after collection and centrifuged within 0.5–2.5 h at $719 \times g$ (2000 rpm) for 15 min at room temperature to separate plasma and red blood cells. After centrifugation, the plasma layer was removed and stored. The remaining red blood cells were washed 3 times with phosphate-buffered saline. The saline and buffy layer were removed after each centrifugation and the resulting washed red blood cells were stored. A lysate of red blood cell folate was prepared from the 4-mL tube by diluting blood 1 to 10 with a freshly prepared 1% ascorbic acid solution. The packed cell volume (required for the calculation of red blood cell folate concentrations) was measured in the remaining whole blood by using an automated Coulter counter (Coulter Electronics, Hialeah, FL). Serum separation tubes were centrifuged at $719 \times g$ (2000 rpm) for 20 min at room temperature and the serum layer was removed. All preparations were stored at −70°C for batch analysis at the end of the study.

### Biochemical measurements

Plasma tHcy was measured by immunoassay (31), plasma PLP was determined by reversed-phase HPLC with fluorescence detection (32), and red blood cell folate (33), serum folate (33), and serum vitamin B-12 (34) concentrations were measured by microbiological assay. EASTAC (35) and EGRAC (36) were measured by enzyme assay with the Cobas Fara centrifugal analyzer (Roche Diagnostics, Welwyn Garden City, United Kingdom). For all assays, samples were analyzed blind, in duplicate (except for EGRAC, for which triplicate samples were measured), and within 6 mo after sampling. Quality control was provided by repeated analysis of stored batches of pooled erythrocytes (for EASTAC and EGRAC), plasma (for tHcy and plasma PLP), serum (for vitamin B-12 and folate), and lysates of red blood cell folate (for red blood cell folate) covering a wide range of values.

### Dietary intake and anthropometric data

Current dietary intake was assessed with use of a 4-d estimated food record. Portion sizes were estimated by the subjects using household measures. Volunteers were required to keep the food records from Saturday to Tuesday to ensure that both weekdays and weekend days were included. All volunteers received comprehensive instructions on how to complete the food records from a trained nutritionist (MCM), who also subsequently quantified portion sizes using published food portion sizes (37) and calculated nutrient intakes using the nutrient analysis program COMP-EAT (Lifeline; Nutritional Services Ltd, Grantham, United Kingdom). Body mass index (BMI) was calculated as weight (in kg)/height$^2$ (in m). The basal metabolic rate (BMR) was calculated by using the formulas of Schofield (38), which are based on sex, height, and weight for the age groups 60–74 y and >74 y. The ratio of BMR to energy intake (obtained from the 4-d estimated food record) was calculated on an individual basis to identify likely underreporters of food intake according to the statistically derived cutoff limits of Goldberg et al (39).

**TABLE 1**
General characteristics and biochemical status of subjects at baseline[1]

| Group | Placebo group ($n$ = 4 M, 7 F) | Vitamin B-6 group ($n$ = 3 M, 8 F) |
|---|---|---|
| Age (y) | 72.25 ± 5.69 | 67.36 ± 3.50 |
| BMI (kg/m$^2$) | 27.5 ± 2.9 | 25.6 ± 3.2 |
| Plasma PLP (nmol/L) | 24.6 ± 20.4 | 26.2 ± 17.6 |
| EASTAC[2] | 1.66 ± 0.13 | 1.70 ± 0.12 |
| Red blood cell folate (nmol/L) | 1680 ± 579 | 1630 ± 405 |
| Serum folate (nmol/L) | 37.3 ± 14.8 | 36.6 ± 10.9 |
| EGRAC[3] | 1.12 ± 0.07 | 1.10 ± 0.05 |
| Serum vitamin B-12 (pmol/L) | 225.0 ± 55.3 | 269.5 ± 44.0 |
| Plasma tHcy (μmol/L) | 10.44 ± 4.69 | 9.90 ± 3.03 |

[1] $\overline{x}$ ± SD. PLP, plasma pyridoxal-$P$ (pyridoxal phosphate); tHcy, total homocysteine. There were no significant differences between groups by independent-samples $t$ test.

[2] Erythrocyte aspartate aminotransferase activation coefficient, a functional indicator of vitamin B-6 status.

[3] Erythrocyte glutathione reductase activation coefficient, a functional indicator of riboflavin status.

## Statistics

All statistical analyses were performed by using the SPSS Statistical Package for the Social Sciences (version 9.0.1; SPSS UK Ltd, Chersey, United Kingdom). Correlation analysis was performed by using bivariate Pearson correlation coefficients. Baseline values for the placebo and vitamin B-6 groups were compared by using an independent-samples $t$ test. Responses to intervention within and between groups were examined by using two-way (time and treatment) ANOVA; $P$ values < 0.05 were considered significant.

## RESULTS

### Baseline characteristics and study compliance

On the basis of screening for vitamin B-12 deficiency at baseline, no individual was found to be vitamin B-12 deficient, ie, no subject had a serum vitamin B-12 concentration <111 pmol/L (150 ng/L). A total of 22 elderly volunteers with a mean age of 69.9 y participated in the vitamin B-6 intervention for ≥12 wk. There were no significant differences between the vitamin B-6 and placebo groups at baseline (**Table 1**). The compliance of all but one subject was excellent (one subject missed taking >2 tablets during the 12-wk supplementation period but was included in the analysis) and none of the subjects dropped out during the study. Baseline plasma tHcy concentrations ranged from 6.22 to 23.52 μmol/L; 20 of the 22 participants were classified as normohomocysteinemic at baseline (ie, plasma tHcy < 15 μmol/L; 40). Vitamin B-6 status was also assessed at baseline; 10 subjects were classified as deficient or suboptimal on the basis of a plasma PLP concentration <20 nmol/L and 6 were classified as deficient or suboptimal on the basis of an EASTAC value ≥1.80. There was no significant correlation between plasma PLP and EASTAC measurements. Serum vitamin B-12 ($r$ = −0.437, $P$ = 0.021) and red blood cell folate ($r$ = −0.392, $P$ = 0.035), but not serum folate, vitamin B-6 (on the basis of EASTAC or PLP), or riboflavin (on the basis of EGRAC) status, were significantly correlated with plasma tHcy at baseline (data not shown).

### Baseline dietary data

Only one individual had a ratio of EI to BMR of <1.14; this individual was classified as an underreporter (39) and was not included in the dietary analysis. The mean dietary intake of the remaining group is shown in **Table 2**. The percentages of the population with dietary intakes of folate, vitamin B-6, vitamin B-12, and riboflavin below the reference nutrient intake (41) were 38%, 4.8%, 0%, and 19%, respectively. The dietary intake of all subjects, however, was above the lower reference nutrient intake. Plasma tHcy was not significantly correlated with the dietary intake of folate, vitamin B-6, vitamin B-12, or riboflavin (data not shown).

### Intervention study

Folic acid supplementation, given before the vitamin B-6 intervention, significantly improved folate status, as indicated by a significant increase in serum folate ($P$ < 0.001) from 15.27 ± 9.00 to 36.94 ± 12.71 nmol/L and a significant decrease in fasting plasma tHcy ($P$ < 0.001) of 19.6%, from 12.65 ± 6.19 to 10.17 ± 3.86 μmol/L (data not shown). The responses of plasma tHcy and B-vitamin status to vitamin B-6 or placebo supplementation are shown in **Table 3**. Examination of individual responses to intervention showed one outlier in the placebo group (change in tHcy = −5.11 μmol/L compared with next largest change of 1.68 μmol/L). This outlier was excluded from the analysis. Two-way ANOVA showed that the significant change in vitamin B-6 status (on the basis of both PLP and EASTAC values) in the vitamin B-6 group compared with the placebo group was reflected in a significant lowering in tHcy of 7.5%. The change in serum or red blood cell folate status (as a result of continued supplementation with folic acid throughout the vitamin B-6 intervention) was not significantly different between the placebo and vitamin B-6 groups.

## DISCUSSION

The results of this study indicate that vitamin B-6 effectively lowers fasting tHcy concentrations in healthy elderly persons; however, the homocysteine-lowering effect is modest compared with that of folic acid [estimated to be ≈25% in a recent meta-analysis (42) and 19.6% in the present study]. The magnitude of the effect of folic acid compared with that of vitamin B-6 may differ because folate acts as a cosubstrate (2) whereas vitamin B-6 acts as a cofactor (4) in homocysteine metabolism. Alternatively, the magnitude of the effect may differ because the diet consumed currently by most people tends to result in a higher proportion of people with suboptimal folate rather than suboptimal vitamin B-6 status. Although both serum and red blood cell folate concentrations appeared to increase during the 12-wk vitamin B-6 supplementation period in both the placebo and vitamin B-6 groups (as a result of continued supplementation with folic acid), this increase in folate status did not affect plasma tHcy concentrations. Before intervention with vitamin B-6, plasma tHcy concentrations had reached a plateau as indicated by the lack of change in tHcy concentrations in the placebo group despite an apparent increase in red blood cell and serum folate concentrations.

The strengths of the present study were that it was double-blind and placebo-controlled and that the subjects were highly motivated and followed closely, which ensured excellent compliance. There are many possible reasons why vitamin B-6

**TABLE 2**
Energy and B-vitamin intakes in free-living healthy elderly persons aged 63–80 y assessed with use of a 4-d food record

| | Men (n = 7) | | Women (n = 14) | |
| --- | --- | --- | --- | --- |
| | Intake | UK RNI[1] | Intake | UK RNI[1] |
| BMR (MJ/d)[2] | 7.16 ± 0.74[3] | — | 5.51 ± 0.40 | — |
| EI:BMR[4] | 1.85 ± 0.26 | — | 1.52 ± 0.31 | — |
| Energy | | | | |
| (MJ/d) | 13.27 ± 2.0 | 8.77–9.93 | 8.34 ± 1.79 | 7.61–7.99 |
| (kcal/d) | 3170 ± 481 | 2100–2380 | 1989 ± 429 | 1810–1900 |
| Vitamin B-6 (mg/d) | 2.29 ± 0.27 | 1.4 | 1.54 ± 0.35 | 1.2 |
| Folate (μg/d) | 283.9 ± 45.5 | 200 | 209.7 ± 73.5 | 200 |
| Riboflavin (mg/d) | 1.91 ± 0.30 | 1.3 | 1.40 ± 0.60 | 1.1 |
| Vitamin B-12 (μg/d) | 5.20 ± 1.56 | 1.5 | 3.42 ± 1.50 | 1.5 |

[1] Reference nutrient intake in the United Kingdom for persons aged >50 y, except for energy intake, for which the estimated average requirement is given for persons aged 60–75 y (41).

[2] Basal metabolic rate calculated with the equations of Schofield (38).

[3] $\bar{x}$ ± SD; n = 21.

[4] Ratio of energy intake to BMR; a ratio <1.14 was used to exclude subjects likely to be underreporters of food intake; however, the use of this ratio in the elderly needs to be confirmed (39). Only one underreporter was identified, who was excluded from the dietary analysis.

intervention successfully lowered plasma tHcy in the present study but not in the other trials mentioned previously. First, all subjects in the present study were riboflavin replete, having taken riboflavin (1.6 mg/d) for a total of 18 wk before the vitamin B-6 intervention began. This may have been an important factor because the flavin mononucleotide–dependent conversion of vitamin B-6 to PLP by pyridoxamine-phosphate oxidase appears to be the limiting step in vitamin B-6 metabolism (43, 44). Therefore, in theory, vitamin B-6 supplementation in persons with suboptimal riboflavin status would be ineffective because adequate conversion of vitamin B-6 to the active form PLP would not occur and, thus, the transsulfuration pathway of homocysteine metabolism would not be stimulated. Second, all subjects in the present study were folate replete, having taken folic acid (400 μg/d) for ≥6 wk before the vitamin B-6 intervention began. This may have been an important factor in the activation of the transsulfuration pathway because high S-adenosylmethionine concentrations (as a result of enhanced remethylation resulting from folic acid supplementation) might activate the vitamin B-6–dependent transsulfuration pathway while inhibiting the remethylation pathway (45). A third possible explanation is that other studies may have missed a significant effect of vitamin B-6 because most of the ele-

vation in homocysteine was due to suboptimal folate status. This being the case, the effect of vitamin B-6 supplementation may be significant only after all the homocysteine lowering in response to folic acid supplementation is accounted for. A final possible reason is that the present vitamin B-6 intervention was conducted in elderly subjects, whereas the studies that failed to show a homocysteine-lowering effect were not. Thus, we may have observed a significant homocysteine-lowering effect in response to vitamin B-6 even if we had not ensured the optimization of folate and riboflavin status in our subjects.

The effect of tHcy on vascular disease is graded (21, 23). Reductions in tHcy concentrations, therefore, are likely to be beneficial, even if tHcy concentrations are within reference ranges. If fortification with B vitamins is to be part of a public health strategy aimed at vascular disease prevention in a population, it is vital that the lowest effective dose and combination of B vitamins that will result in the greatest reductions in tHcy is known so that the risk of overexposure in those with the highest intakes of the fortified food is limited. Therefore, interventions undertaken to investigate the effect of very low doses of B vitamins on fasting tHcy concentrations, such as the present study, are vital.

**TABLE 3**
Response of plasma total homocysteine (tHcy) and B-vitamin status to 12 wk of supplementation with 1.6 mg vitamin B-6/d in free-living healthy elderly persons[1]

| | Placebo group (n = 10)[2] | | Vitamin B-6 group (n = 11) | | |
| --- | --- | --- | --- | --- | --- |
| | Before (week 0) | After (week 12) | Before (week 0) | After (week 12) | P[3] |
| Plasma tHcy (μmol/L) | 10.33 ± 4.93 | 10.68 ± 4.85 | 9.90 ± 3.03 | 9.16 ± 2.34 | 0.008 |
| Plasma PLP (nmol/L) | 28.1 ± 19.9 | 29.7 ± 21.8 | 26.2 ± 17.6 | 47.4 ± 29.7 | 0.046 |
| EASTAC[4] | 1.68 ± 0.13 | 1.64 ± 0.11 | 1.70 ± 0.12 | 1.57 ± 0.15 | 0.048 |
| Red blood cell folate (nmol/L) | 1691 ± 609 | 2335 ± 943 | 1630 ± 405 | 2237 ± 620 | 0.859 |
| Serum folate (nmol/L) | 35.89 ± 14.84 | 49.84 ± 22.31 | 36.6 ± 10.9 | 45.9 ± 20.2 | 0.598 |
| EGRAC[5] | 1.26 ± 0.06 | 1.24 ± 0.06 | 1.10 ± 0.05 | 1.10 ± 0.05[5] | 0.239 |
| Serum vitamin B-12 (pmol/L) | 219.2 ± 54.7 | 243.8 ± 37.6 | 269.5 ± 44.0 | 267.2 ± 51.5 | 0.088 |

[1] $\bar{x}$ ± SD; n = 21. tHcy, total homocysteine; PLP, plasma pyridoxal-P (pyridoxal phosphate).

[2] Examination of individual responses to the intervention showed one outlier (in the placebo group), who was excluded from the analysis.

[3] Response to intervention within and between groups by using two-way ANOVA.

[4] Erythrocyte aspartate aminotransferase activation coefficient, a functional indicator of vitamin B-6 status.

[5] Erythrocyte glutathione reductase activation coefficient, a functional indicator of riboflavin status.

Until the results of this study, vitamin B-6 appeared to lower homocysteine only in exceptional circumstances; therefore, many investigators involved in the design of secondary prevention trials (1) did not include vitamin B-6 supplementation in an effort to reduce the number of subjects required for these trials. Although the results of the present study need to be confirmed and investigated in other subgroups of the population, we suggest that it may be pertinent to include vitamin B-6 in any program aimed at the prevention or treatment of hyperhomocysteinemia. Additional work is also necessary to find the combination of B vitamins that most effectively lowers tHcy concentrations and to investigate the homocysteine-lowering capacity of riboflavin, which is intimately involved in homocysteine metabolism but has thus far received little attention. In conclusion, the results of the present study indicate that low-dose vitamin B-6 supplementation for 12 wk in highly compliant elderly subjects with optimal riboflavin and folate statuses effectively lowers fasting plasma tHcy concentrations.

## REFERENCES

1. Eikelboom JW, Lonn E, Genest J, Hankey G, Yusuf S. Homocyst(e)ine and cardiovascular disease: a critical review of the epidemiologic evidence. Ann Intern Med 1999;131:363–75.
2. Finkelstein JD. Methionine metabolism in mammals. J Nutr Biochem 1990;1:228–37.
3. Bates CJ, Fuller NJ. The effect of riboflavin deficiency on methylenetetrahydrofolate reductase (NADPH) (EC 1.5.1.20) and folate metabolism in the rat. Br J Nutr 1986;55:455–64.
4. Mudd SH, Levy HL, Skovby F. Disorders of transsulfuration. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The metabolic basis of inherited disease. 7th ed. New York: McGraw-Hill, 1995:1279–327.
5. Mudd SH, Skovby F, Levy HL, et al. The natural history of homocystinuria due to cystathionine β-synthase deficiency. Am J Hum Genet 1985;37:1–31.
6. Bostom AG, Gohh RY, Beaulieu AJ, et al. Treatment of hyperhomocysteinemia in renal transplant recipients: a randomised placebo-controlled trial. Ann Intern Med 1997;127:1089–92.
7. Wilcken DEL, Gupta VJ, Betts AK. Homocysteine in the plasma of renal transplant recipients: effects of cofactors for methionine metabolism. Clin Sci 1981;61:743–9.
8. Brattstrom LE, Israelsson B, Jepsson JO, Hultberg BL. Folic acid—an innocuous means to reduce plasma homocysteine. Scand J Clin Lab Invest 1988;48:215–21.
9. Ubbink JB, Vermaak WJH, van der Merwe A, Becker PJ, Delport R, Potgeiter HC. Vitamin requirements for the treatment of hyperhomocysteinemia in humans. J Nutr 1994;124:1927–33.
10. Dierkes J, Kroesen M, Pietrzik K. Folic acid and vitamin B6 supplementation and plasma homocysteine concentrations in healthy young women. Int J Vitam Nutr Res 1998;68:98–103.
11. Brattstrom L, Israelsson B, Norrving B, et al. Impaired homocysteine metabolism in early-onset cerebral and peripheral occlusive arterial disease. Effects of pyridoxine and folic acid treatment. Atherosclerosis 1990;81:51–60.
12. Arnadottir M, Brattstrom L, Simonsen O, et al. The effect of high-dose pyridoxine and folic acid supplementation on serum lipid and plasma homocysteine concentrations in dialysis patients. Clin Nephrol 1993;40:236–40.
13. Lakshmi AV, Ramalakshmi BA. Effect of pyridoxine or riboflavin supplementation on plasma homocysteine levels in women with oral lesions. Natl Med J India 1998;11:171–2.
14. Mansoor MA, Kristensen O, Hervig T, et al. Plasma total homocysteine response to oral doses of folic acid and pyridoxine hydrochloride (vitamin B$_6$) in healthy individuals. Oral doses of vitamin B$_6$ reduce concentrations of serum folate. Scand J Clin Lab Invest 1999;59:139–46.
15. Franken DG, Boers GHJ, Blom HJ, Trijbels FJM, Kloppenborg PWC. Treatment of mild hyperhomocysteinemia in vascular disease patients. Arterioscler Thromb 1994;14:456–70.
16. Dudman NPB, Wilcken DEL, Wang J, Lynch JF, Macey D, Lundberg P. Disordered methionine/homocysteine metabolism in premature vascular disease: its occurrence, cofactor therapy, and enzymology. Arterioscler Thromb 1993;13:1253–60.
17. Verhoef P, Stampfer MJ, Buring JE, et al. Homocysteine metabolism and risk of myocardial infarction—relation with vitamin B-6, vitamin B-12, and folate. Am J Epidemiol 1996;143:845–59.
18. Robinson K, Mayer EL, Miller DP, et al. Hyperhomocysteinemia and low pyridoxal phosphate: common and independent reversible risk factors for coronary artery disease. Circulation 1995;92:2825–30.
19. Robinson K, Arheart K, Refsum H, et al. Low circulating folate and vitamin B6 concentrations—risk factors for stroke, peripheral vascular disease, and coronary artery disease. Circulation 1998;97:437–43.
20. Rimm EB, Willett WC, Hu FB, et al. Folate and vitamin B6 from diet and supplements in relation to risk of coronary heart disease among women. JAMA 1998;279:359–64.
21. Stampfer MJ, Malinow MR, Willett WC, et al. A prospective study of plasma homocyst(e)ine and risk of myocardial infarction in US physicians. JAMA 1992;268:877–81.
22. Selhub J, Jacques PF, Wilson PWF, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. JAMA 1993;270:2693–8.
23. Graham IM, Daly LE, Refsum HM, et al. Plasma homocysteine as a risk factor for vascular disease: The European Concerted Action Project. JAMA 1997;277:1775–81.
24. Osganian SK, Stampfer MJ, Spiegelman D, et al. Distribution of and factors associated with serum homocysteine levels in children. JAMA 1999;281:1189–96.
25. Smolin LA, Crenshaw TD, Kurtycz D, Benevenga NJ. Homocyst(e)ine accumulation in pigs fed diets deficient in vitamin B6: relationship to atherosclerosis. J Nutr 1983;113:2122–33.
26. Shultz TD, Hansen CM. Plasma homocysteine concentration changes with vitamin B-6 depletion and repletion in young women. Nutr Res 1998;18:975–8.
27. Hamfelt A, Soderhjelm L. Vitamin B-6 and aging. In: Leklem JE, Reynolds RD, eds. Clinical and physiological applications of vitamin B-6. New York: Alan R Liss, Inc, 1988:95–107.
28. Reynolds RD, Mosser-Veillon PB, Kant AK. Effect of age on status and metabolism of vitamin B-6 in men. In: Leklem JE, Reynolds RD, eds. Clinical and physiological applications of vitamin B-6. New York: Alan R Liss, Inc, 1988:109–25.
29. Andersson A, Brattstrom L, Israelsson B, Isaksson A, Hamfelt A, Hultberg B. Plasma homocysteine before and after methionine loading with regard to age, gender, and menopausal status. Eur J Clin Invest 1992;22:79–87.
30. Quereshi KN, Hodkinson HM. Evaluation of a ten-question mental test in the institutionalised elderly. Age Ageing 1974;3:152–7.
31. Leino A. Fully automated measurement of total homocysteine in plasma and serum on the Abbott IMx analyzer. Clin Chem 1999;45:569–71.
32. Bates CJ, Pentieva KD, Mathews N, MacDonald A. A simple, sensitive and reproducible assay for pyridoxal phosphate in human plasma. Clin Chim Acta 1999;280:101–11.
33. Molloy AM, Scott JM. Microbiological assay for serum, plasma and red cell folate using cryopreserved, microtiter plate method. Methods Enzymol 1997;281:43–53.

34. Kelleher BP, O'Broin SD. Microbiological assay for vitamin B12 performed in 96-well microtitre plates. J Clin Pathol 1991;44:592–5.

35. Mount JN, Heduan E, Herd C, Jupp R, Kearney E, Marsh A. Adaptation of coenzyme stimulation assays for the nutritional assessment of vitamins $B_1$, $B_2$, and $B_6$ using the Cobas Bio centrifugal analyser. Ann Clin Biochem 1987;24:41–6.

36. Powers HJ, Bates CJ, Prentice AM, Lamb WH, Jepson M, Bowman H. The relative effectiveness of iron and iron with riboflavin in correcting a microcytic anaemia in men and children in rural Gambia. Hum Nutr Clin Nutr 1983;37C:413–25.

37. Crawley H. Food portion sizes. 3rd ed. London: Her Majesty's Stationery Office, 1988.

38. Schofield WN. Predicting basal metabolic rate, new standards and review of previous work. Hum Nutr Clin Nutr 1985;39C(suppl): 5–41.

39. Goldberg GR, Black AE, Jebb SA, et al. Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. Eur J Clin Nutr 1991;45:569–81.

40. Kang S-S, Wong PWK, Malinow MR. Hyperhomocyst(e)inemia as a risk factor for occlusive vascular disease. Annu Rev Nutr 1992;12: 279–98.

41. Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy. Dietary reference values for food energy and nutrients for the United Kingdom. London: Her Majesty's Stationery Office, 1991.

42. Homocysteine Lowering Trialists' Collaboration. Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. BMJ 1998;316:894–6.

43. McCormick DB. Two interconnected B vitamins: riboflavin and pyridoxine. Physiol Rev 1989;69:1170–98.

44. Madigan SM, Tracey F, McNulty H, et al. Riboflavin and vitamin B-6 intakes and status and biochemical response to riboflavin supplementation in free-living elderly people. Am J Clin Nutr 1998;68:389–95.

45. Selhub J, Miller JW. The pathogenesis of homocysteinemia: interruption of the coordinate regulation by *S*-adenosylmethionine of the remethylation and transsulfuration of homocysteine. Am J Clin Nutr 1992;55:131–8.