Available online at www.sciencedirect.com

SCIENCE DIRECT

Metabolism Clinical and Experimental 54 (2005) 1362–1367

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Comparative effects of hydroxocobalamin and cyanocobalamin on plasma homocysteine concentrations in end-stage renal disease

L. John Hoffer*, Orchidee Djahangirian, Paul E. Bourgouin, Johana Eid, Farhad Saboohi

Lady Davis Institute for Medical Research, Sir Mortimer B Davis–Jewish General Hospital, Montreal, Quebec, Canada H3T 1E2
Division of Nephrology, Department of Medicine, St Mary's Hospital Centre, Montreal, Quebec, Canada HT3 1M5

Received 26 January 2005; accepted 30 April 2005

Abstract

End-stage renal disease (ESRD) is associated with marked hyperhomocysteinemia which is only partially corrected by folic acid and pyridoxine supplementation. We and others have reported that various forms of parenteral cobalamin reduce plasma total homocysteine (tHcy) concentrations of patients with ESRD substantially below the lowest levels attainable with folic acid. We here report a 16-week randomized controlled crossover trial which directly compared the Hcy-lowering effect of intravenous hydroxocobalamin (HC) with that of cyanocobalamin (CC). Folic acid– and vitamin B_{12}–replete maintenance hemodialysis patients were randomly assigned to receive either 1 mg intravenous HC weekly for 8 weeks followed by CC for a further 8 weeks, or CC for 8 weeks followed by HC for 8 weeks. Hydroxocobalamin increased serum cobalamin concentrations 40-fold, whereas CC increased them only 10-fold, but both treatments reduced plasma tHcy concentrations similarly by 33% ($P < .001$). Crossover to the alternate form of the vitamin greatly affected the serum cobalamin concentration but was without further effect on the plasma tHcy concentration. These results confirm that weekly cobalamin injections lower plasma tHcy concentrations of hemodialysis patients well below the level attainable with folic acid. Hydroxocobalamin and CC are equipotent despite producing very different serum cobalamin concentrations.
© 2005 Elsevier Inc. All rights reserved.

1. Introduction

End-stage renal disease (ESRD) is associated with markedly increased plasma total homocysteine (tHcy) concentrations [1,2] which predict the high risk of cardiovascular disease in this patient population [3-7]. Folic acid supplementation reduces plasma total homocysteine (tHcy) concentrations by approximately one third [8-15], but dosages greater than 1 mg/d, or different forms of the vitamin, are no more effective than 1 mg/d of ordinary folic acid [13,14,16-18]. As the majority of folate- and pyridoxine-supplemented patients with ESRD remain hyperhomocysteinemic [15,16,19-21], efforts to identify better Hcy-lowering treatments continue to be sought [22-24].

Three randomized prospective clinical trials [25-27], 2 of them by us [25,27], indicate that the addition of parenteral hydroxocobalamin (HC) or methylcobalamin to an oral regimen of folic acid and other B vitamins can reduce plasma tHcy concentrations of maintenance hemodialysis patients 30% to 50% below the level attained with the oral vitamins alone. Owing to its limited bioavailability [28], even high-dose daily oral cyanocobalamin (CC) increases serum cobalamin concentrations of patients with ESRD by 3-fold at most [15,19,29] and reduces plasma tHcy concentrations very modestly [15] if at all [23,30]. By contrast, parenteral HC or methylcobalamin increases serum cobalamin concentrations of patients with ESRD 30- to more than 100-fold [25,26,31-33]. It would be natural to attribute the potency of parenteral cobalamin to the very high serum concentrations uniquely attainable with this mode of administration. We were recently surprised to find that an 8-week course of weekly intravenous CC increased serum cobalamin concentrations only to approximately 7 times the upper normal value [27]. This raised the possibility that different forms of cobalamin might reduce plasma tHcy with different potency. We here report the results of a head-to-head comparison of HC and CC for plasma tHcy lowering in maintenance hemodialysis patients.

* Corresponding author. Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, Quebec, Canada H3T 1E2. Tel.: +1 514 340 8222x5276; fax: +1 514 340 7502.
E-mail address: l.hoffer@mcgill.ca (L.J. Hoffer).

0026-0495/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2005.04.027

2. Materials and methods

The study was carried out in the hemodialysis unit of St Mary's Hospital Center, Montreal, with the approval of the hospital's research ethics committee. All English- or French-speaking patients on the 3-times-per-week maintenance hemodialysis schedule were approached to participate if they were clinically stable, had a blood hemoglobin concentration of at least 100 g/L and serum albumin concentration of at least 30 g/L, absence of macrocytosis, and serum cobalamin concentration not lower than 200 pmol/L. Participants were randomized either to receive 1 mg IV HC each week for 8 weeks followed by the same dose of CC for a further 8 weeks (group HC-CC), or CC for 8 weeks followed by HC for 8 weeks (group CC-HC). The clinical trial was scheduled to coincide with the routine drawing of the monthly biochemical profile. Serum cobalamin, folic acid, and plasma tHcy were measured at baseline and after 4, 8, 12, and 16 weeks. All patients consumed a daily multiple B vitamin tablet (Replavite, WN Pharmaceuticals, Ltd, Burnaby, BC, Canada) which provides 1 mg folic acid, 6 mg pyridoxine, and 10 μg vitamin B_{12}, and continued this throughout the study.

Blood for plasma tHcy was collected in potassium ethylenediamine tetraacetic acid–lined tubes and kept on ice until the plasma was separated and stored at −30°C. The analysis was by high-performance liquid chromatography with fluorescence detection as described earlier [25]. Serum cobalamin and folate concentrations were analyzed after appropriate dilution by automated chemiluminescent immunoassay (Immulite, Diagnostic Products, Los Angeles, Calif) as in our earlier studies [25,27]. The normal ranges for this assay are 180 to 660 pmol/L for cobalamin and 6 to 40 nmol/L for folate.

All variables were normally distributed (Kolmogorov-Smirnov test), so changes over time were compared using 2-way repeated measures analysis of variance. The source of significant differences was identified using the Student-Newman-Keuls post hoc multiple comparison test. Statistical significance was based on a 2-tailed $P < .05$. Statistical analysis was done using SigmaStat version 2.0 (San Rafael, Calif). The results are expressed as mean ± SEM.

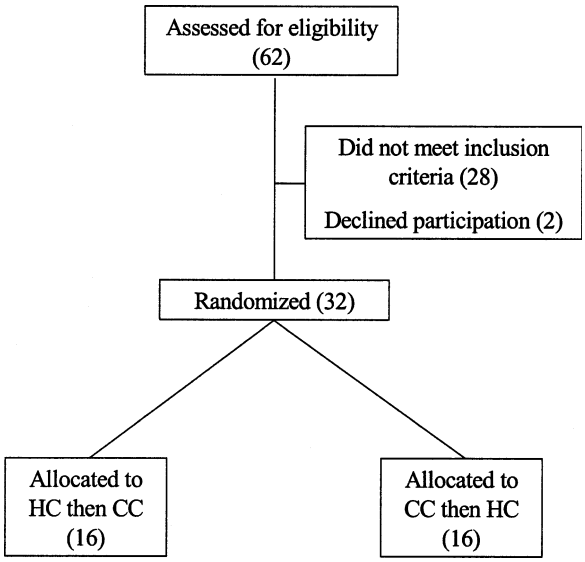

Fig. 1. Trial profile.

Table 1
Baseline patient characteristics

Characteristic	Group HC-CC (n = 16)	Group CC-HC (n = 16)	*P*
Age (y)	63 ± 4	71 ± 4	.18
Sex (% male)	37.5	62.5	.17
Dry weight (kg)	69.7 ± 4.1	68.9 ± 3.7	.88
Blood hemoglobin (g/L)	119 ± 3	125 ± 3	.87
Mean cell volume (fL)	95.6 ± 1.6	98.1 ± 1.2	.58
Serum albumin (g/L)	34.6 ± 0.6	35.4 ± 0.7	.35
Serum urea (mmol/L)	20.4 ± 1.4	18.5 ± 1.0	.26
Serum creatinine (imol/L)	742 ± 50	749 ± 35	.91
Serum cobalamin (pmol/L)	625 ± 70	531 ± 56	.30
Serum folate (nmol/L)	165 ± 45	153 ± 23	.81
Kt/V	1.73 ± 0.11	1.72 ± 0.05	.45
Plasma tHcy (imol/L)	19.0 ± 1.0	22.2 ± 1.3	.07

Participants in group HC-CC received weekly intravenous HC for 8 weeks, then switched to CC for 8 weeks. Participants in group CC-HC received the vitamins in the reverse order. The normal range for serum folate is 6 to 40 nmol/L; the normal range for serum cobalamin is 180 to 660 pmol/L; plasma tHcy concentrations are normally less than 12 µmol/L. *Kt/V*, a measure of dialysis adequacy, represents the volume of body water apparently cleared of urea during a dialysis session divided by the patient's estimated total body water. *Kt/V* that is 1.2 or greater is considered adequate hemodialysis. Values are expressed as mean ± SEM.

3. Results

The details of the trial profile are shown in Fig. 1. Of the 62 patients assessed, 28 did not meet the inclusion criteria and 2 eligible patients declined, leaving 32 participants who were randomly allocated to group HC-CC or group CC-HC. All the participants tolerated intravenous cobalamin without side effects. Two participants in group CC-HC left the study. One participant moved to another city, and the other withdrew because of elective cardiac surgery. Because these withdrawals occurred after the 8-week time point, the data acquired for both participants were included in the analysis.

Randomization resulted in comparison groups with similar physical and biochemical parameters (Table 1). All participants received a multiple vitamin supplement that provided 1 mg folic acid per day, and the serum folate concentrations of both groups were approximately 4 times the normal upper limit for the assay and did not change significantly over the course of the 16-week study (data not shown). On average, baseline serum cobalamin concentrations were in the upper part of the normal range. The lowest baseline cobalamin concentration in group HC-CC was 238 pmol/L, and the lowest in group CC-HC was 283 pmol/L. As shown in Table 2, weekly CC for 8 weeks increased the serum cobalamin concentration 10-fold, whereas HC increased it 40-fold. Switching to the alternate

Table 2
Serum cobalamin and plasma total homocysteine concentrations

	0 wk	4 wk	8 wk	12 wk	16 wk
Serum cobalamin (pmol/L)					
Group HC-CC	625 ± 70[a]	17600 ± 1470[b]	25900 ±1600[c]	13200 ± 1000[d]	11600 ± 1100[d]
Group CC-HC	531 ± 56[a]	4900 ± 540[e]	5600 ± 600[e]	27900 ± 1600[f]	33300 ± 2000[g]
Plasma tHcy (μmol/L)					
Group HC-CC	19.0 ± 1.0[a]	13.1 ± 0.9[b]*	12.8 ± 1.0[b]	13.4 ± 0.7[b]	13.0 ± 0.7[b]
Group CC-HC	22.2 ± 1.3[c]	15.8 ± 0.8[b]*	14.5 ± 0.7[b]	15.2 ± 0.8[b]	14.8 ± 0.7[b]

Participants received HC for the first 8 weeks followed by CC for 8 weeks (HC-CC), or the same vitamins in the reverse order (CC-HC).
Values are expressed as mean ± SEM. Differing superscripts in any row or column indicate statistically significant differences ($P < .05$).
* Significantly different from the comparison group at this time point ($P < .05$).

form of cobalamin reduced serum cobalamin for group HC-CC and increased it for group CC-HC. These changes are shown graphically in Fig. 2.

The plasma tHcy concentration is normally 12 μmol/L or less. Baseline predialysis plasma tHcy concentrations were 19.0 ± 1.0 μmol/L in group HC-CC and 22.2 ± 1.3 μmol/L in group CC-HC (Table 1). As shown in Table 2, weekly HC for 8 weeks reduced the average plasma tHcy concentration in group HC-CC by 33% ($P < .001$), and weekly CC reduced it in group CC-HC by 34% ($P < .001$). Despite the great changes in serum cobalamin concentration caused by switching to the alternate form of cobalamin, there was no further change in the plasma tHcy concentration. These results are shown graphically in Fig. 3.

4. Discussion

Having recently discovered that CC raises serum cobalamin concentrations of hemodialysis patients far less than HC [27], we carried out the present study to determine whether the higher serum cobalamin concentration associated with HC translates into more potent Hcy reduction. Our results confirm that intravenous HC raises serum cobalamin concentrations approximately 5 times higher than CC does in patients with ESRD. Our previous experience with HC [25] and the trend in Fig. 2 suggest that this difference would have widened if therapy had continued for longer than 8 weeks. Despite this important difference in serum cobalamin concentrations, weekly 1 mg IV injections of CC or HC reduced plasma tHcy concentrations of folic acid–supplemented maintenance hemodialysis patients with equivalent potency. The percentage reduction observed, 33%, is in close agreement with our previous observational and clinical trial findings [25,27,34] and in keeping with Koyama et al [26], who, in a prospective clinical trial, found that a regimen of 0.5 mg IV methylcobalamin 3 times per week reduced plasma tHcy concentrations of hemodialysis patients by 50%.

On the other hand, Trimarchi et al [33] have reported that a regimen of 10 mg per day folic acid plus intravenous methylcobalamin 0.5 mg twice weekly was no more effective in reducing plasma tHcy concentrations of hemodialysis patients than 10 mg folic acid alone. It is noteworthy, however, that the average serum cobalamin concentration in their group treated with folic acid alone increased to more than 7 times the upper normal value during the course of the clinical trial. This suggests that some patients in the folic acid–only group were inadvertently administered methylcobalamin, which might have reduced average plasma tHcy concentrations for the group as a whole.

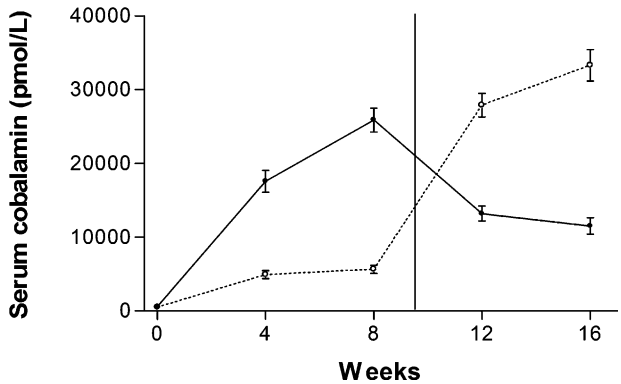

Fig. 2. Group HC-CC received hydroxocobalamin for 8 weeks, followed by cyanocobalamin for 8 weeks (closed circles); group CC-HC received cyanocobalamin for 8 weeks followed by hydroxocobalamin for 8 weeks (open circles). The vertical line indicates crossover to the alternate form of the vitamin. Indicated are the means ± SEM.

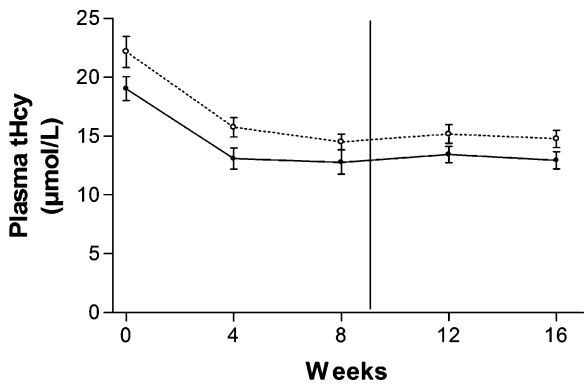

Fig. 3. Group HC-CC received hydroxocobalamin for 8 weeks, followed by cyanocobalamin for 8 weeks (closed circles); group CC-HC received cyanocobalamin for 8 weeks followed by hydroxocobalamin for 8 weeks (open circles). The vertical line indicates crossover to the alternate form of the vitamin. Indicated are the means ± SEM.

Parenteral cobalamin can reduce plasma tHcy only when folic acid status is normal [31,33]. Furthermore, low-frequency treatment such as 1 mg or less every month [35,36] may be incorrectly interpreted as ineffective unless continued for the many months that may be needed to accumulate sufficiently high cobalamin tissue stores.

The pharmacokinetics of HC and CC is known to differ in persons with normal renal function. After intramuscular injection, HC produces much higher circulating cobalamin concentrations and has a longer circulating half-life [37,38]. Approximately 25% of a 1-mg dose of HC is excreted in the urine over the subsequent 24 hours, as compared with 80% of a similar dose of CC [39]. These differences are attributed to extensive nonspecific plasma protein binding by HC, but not CC, once specific cobalamin binding sites are saturated [40,41]. This is the first study to compare the effects of these 2 forms of vitamin B_{12} in patients lacking the capacity to excrete it in their urine. It is of interest that HC has been reported to penetrate cellophane membranes more slowly than CC [39]. The dialyzer membrane used in this hemodialysis unit was polysulfone. Whereas the in vitro CC clearance of this dialyzer is routinely determined by its manufacturer (Fresenius, Medical Care, Lexington, Mass), no information could be obtained from the manufacturer regarding HC clearance. In conclusion, it appears that higher serum cobalamin concentrations associated with the administration of parenteral HC (and presumably methylcobalamin) to patients with ESRD are due, firstly, to nonspecific plasma protein binding, which protects it from clearance during dialysis, and, secondly, perhaps, to less efficient dialysis.

It should be noted that despite random allocation, there was a statistically significant difference between the treatment groups in their baseline and subsequent plasma tHcy concentrations (Table 2). The crossover design of our study, which assured that all participants were treated with both forms of cobalamin, and the clearcut nature of the results provide assurance that imperfect randomization did not confound our conclusion about the effects of the 2 forms of cobalamin.

It is also of interest that the average baseline plasma tHcy concentration of the participants in the study, approximately 21 μmol/L, was less than the average value of approximately 26 μmol/L observed in our previous study of hemodialysis patients naive to parenteral cobalamin therapy [25]. We have no good explanation for this. Perhaps it is relevant that the predialysis serum urea concentrations of the participants in the present study were 20% to 40% lower than observed in our earlier studies, which took place in hemodialysis units that service larger patient populations and where the average duration of dialysis is somewhat shorter [25,27]. Could differences in dialysis adequacy account for differing plasma tHcy concentrations? Average dialysis adequacy, calculated as Kt/V, was 1.73 ± 0.06 for the participants in the present study, but somewhat lower (1.54 ± 0.04; $P = .02$) in 24 randomly selected patients in the unit where our earlier clinical trial took place [25]. The literature is unclear as to the extent to which variations in dialysis adequacy affect plasma tHcy concentrations. In a study of peritoneal dialysis patients, Ducloux et al [42] found more adequate dialysis was associated with lower plasma tHcy, but Arnadottir et al [43] found no such evidence in hemodialysis patients. Notwithstanding the lower average baseline plasma tHcy concentration observed in the present study than in our previous ones, parenteral CC and HC reduced plasma tHcy concentrations by precisely the same fraction, 33%, as previously observed. In the present study, this therapy reduced the average plasma tHcy concentration nearly to normal.

The novel conclusion of this study is that HC and CC reduce plasma tHcy concentrations equivalently in hemodialysis patients despite producing very different serum concentrations. Large doses of cobalamin in any form penetrate and accumulate within the tissues [40,44], so we speculate that following their injection postdialysis, HC and CC accumulate nearly equally in the tissues, reducing plasma tHcy with comparable potency, although serum concentrations of CC are lowered more than HC by subsequent hemodialysis treatments before the next vitamin injection. The equipotency of HC and CC shown here represents an important advantage. Cyanocobalamin for injection costs only pennies and is stocked by all Canadian and American pharmacies, whereas HC for injection is much more expensive and can be difficult to obtain in these countries. A potential theoretical advantage of HC is that, unlike CC, its predialysis serum concentration appears to provide an approximate index of its potency.

It is possible that HC could prove useful for patients with chronic renal failure in the predialysis phase of their disease. These people have plasma tHcy concentrations nearly as high as in ESRD [45,46]. People with normal renal function clear parenteral CC into their urine so completely it has been used to measure the glomerular filtration rate [47]. It therefore seems likely that people with moderately severely chronic renal failure could clear parenteral CC fast enough to mitigate its pharmacological plasma Hcy–lowering effect. In this specific setting, HC might be sufficiently protected from rapid renal clearance to preserve an important Hcy-lowering effect. A study to test this possibility is currently underway.

Acknowledgment

Supported by grant MOP-53072 from the Canadian Institutes of Health Research.

References

[1] Friedman AN, Bostom AG, Selhub J, Levey AS, Rosenberg IH. The kidney and homocysteine metabolism. J Am Soc Nephrol 2001;12: 2181-9.

[2] Blom HJ, DeVriese AS. Why are homocysteine levels increased in kidney failure? A metabolic approach. J Lab Clin Med 2002;139: 262-8.
[3] Robinson K, Gupta A, Dennis V, Arheart K, Chaudhary D, Green R, et al. Hyperhomocysteinemia confers an independent increased risk of atherosclerosis in end-stage renal disease and is closely linked to plasma folate and pyridoxine concentrations. Circulation 1996;94: 2743-8.
[4] Bostom AG, Shemin D, Verhoef P, Nadeau MR, Jacques PF, Selhub J, et al. Elevated fasting total plasma homocysteine levels and cardiovascular disease outcomes in maintenance dialysis patients. A prospective study. Arterioscler Thromb Vasc Biol 1997;17:2554-8.
[5] Moustapha A, Naso A, Nahlawi M, Gupta A, Arheart KL, Jacobsen DW, et al. Prospective study of hyperhomocysteinemia as an adverse cardiovascular risk factor in end-stage renal disease. Circulation 1998;97:138-41.
[6] Manns BJ, Burgess ED, Hyndman ME, Parsons HG, Schaefer JP, Scott-Douglas NW. Hyperhomocyst(e)inemia and the prevalence of atherosclerotic vascular disease in patients with end-stage renal disease. Am J Kidney Dis 1999;34:669-77.
[7] Clarke R, Lewington S, Landray M. Homocysteine, renal function, and risk of cardiovascular disease. Kidney Int 2003;63(Suppl 84): S131-3.
[8] Spence JD, Cordy P, Kortas C, Freeman D. Effect of usual doses of folate supplementation on elevated plasma homocyst(e)ine in hemodialysis patients: no difference between 1 and 5 mg daily. Am J Nephrol 1999;19:405-10.
[9] Dierkes J, Domrose U, Ambrosch A, Bosselmann HP, Neumann KH, Luley C. Response of hyperhomocysteinemia to folic acid supplementation in patients with end-stage renal disease. Clin Nephrol 1999;51:108-15.
[10] Arnadottir M, Gudnason V, Hultberg B. Treatment with different doses of folic acid in haemodialysis patients: effects on folate distribution and aminothiol concentrations. Nephrol Dial Transplant 2000;15:524-8.
[11] Sunder-Plassmann G, Fodinger M, Buchmayer H, Papagiannopoulos M, Wojcik J, Kletzmayr J, et al. Effect of high dose folic acid therapy on hyperhomocysteinemia in hemodialysis patients: results of the Vienna multicenter study. J Am Soc Nephrol 2000;11:1106-16.
[12] Tremblay R, Bonnardeaux A, Geadah D, Busque L, LeBrun M, Ouimet D, et al. Hyperhomocysteinemia in hemodialysis patients: effects of 12-month supplementation with hydrosoluble vitamins. Kidney Int 2000;58:851-8.
[13] Hauser AC, Hagen W, Rehak PH, Buchmayer H, Fodinger M, Papagiannopoulos M, et al. Efficacy of folinic versus folic acid for the correction of hyperhomocysteinemia in hemodialysis patients. Am J Kidney Dis 2001;37:758-65.
[14] Bostom AG, Culleton BF. Hyperhomocysteinemia in chronic renal disease. J Am Soc Nephrol 1999;10:891-900.
[15] Manns B, Hyndman E, Burgess E, Parsons H, Schaefer J, Snyder F, et al. Oral vitamin B_{12} and high-dose folic acid in hemodialysis patients with hyper-homocyst(e)inemia. Kidney Int 2001;59:1103-9.
[16] Bostom AG, Shemin D, Bagley P, Massy ZA, Zanabli A, Christopher K, et al. Controlled comparison of L-5-methyltetrahydrofolate versus folic acid for the treatment of hyperhomocysteinemia in hemodialysis patients. Circulation 2000;101:2829-32.
[17] Ghandour H, Bagley PJ, Shemin D, Hsu N, Jacques PF, Dworkin L, et al. Distribution of plasma folate forms in hemodialysis patients receiving high daily doses of L-folinic or folic acid. Kidney Int 2002;62:2246-9.
[18] Ducloux D, Aboubakr A, Motte G, Toubin G, Fournier V, Chalopin JM, et al. Hyperhomocysteinaemia therapy in haemodialysis patients: folinic versus folic acid in combination with vitamin B_6 and vitamin B_{12}. Nephrol Dial Transplant 2002;17:865-70.
[19] Bostom AG, Shemin D, Lapane KL, Hume AL, Yoburn D, Nadeau MR, et al. High dose–B-vitamin treatment of hyperhomocysteinemia in dialysis patients. Kidney Int 1996;49:147-52.
[20] Moustapha A, Gupta A, Robinson K, Arheart K, Jacobsen DW, Schreiber MJ, et al. Prevalence and determinants of hyperhomocysteinemia in hemodialysis and peritoneal dialysis. Kidney Int 1999; 55:1470-5.
[21] van Tellingen A, Grooteman MC, Bartels PM, van Limbeek J, van Guldener C, Ter Wee PM, et al. Long-term reduction of plasma homocysteine levels by super-flux dialyzers in hemodialysis patients. Kidney Int 2001;59:342-7.
[22] Massy ZA. Potential strategies to normalize the levels of homocysteine in chronic renal failure patients. Kidney Int 2003;63(Suppl 84): S134-6.
[23] Arnadottir M, Hultberg B. The effect of vitamin B_{12} on total plasma homocysteine concentration in folate-replete hemodialysis patients. Clin Nephrol 2003;59:186-9.
[24] Friedman AN, Bostom AG, Laliberty P, Selhub J, Shemin D. The effect of *N*-acetylcysteine on plasma total homocysteine levels in hemodialysis: a randomized, controlled study. Am J Kidney Dis 2003;41:442-6.
[25] Elian KM, Hoffer LJ. Hydroxocobalamin reduces hyperhomocysteinemia in end-stage renal disease. Metabolism 2002;51:881-6.
[26] Koyama K, Usami T, Takeuchi O, Morozumi K, Kimura G. Efficacy of methylcobalamin on lowering total homocysteine plasma concentrations in haemodialysis patients receiving high-dose folic acid supplementation. Nephrol Dial Transplant 2002;17:916-22.
[27] Hoffer LJ, Saboohi F, Golden M, Barre PE. Cobalamin dose regimen for maximum homocysteine reduction in end-stage renal disease. Metabolism 2005;54:835-40.
[28] Scott JM. Bioavailability of vitamin B_{12}. Eur J Clin Nutr 1997; 51(Suppl 1):S49-S53.
[29] Hong SY, Yang DH, Chang SK. Plasma homocysteine, vitamin B_6, vitamin B_{12} and folic acid in end-stage renal disease during low-dose supplementation with folic acid. Am J Nephrol 1998;18: 367-72.
[30] Billion S, Tribout B, Cadet E, Queinnec C, Rochette J, Wheatley P, et al. Hyperhomocysteinaemia, folate and vitamin B_{12} in unsupplemented haemodialysis patients: effect of oral therapy with folic acid and vitamin B_{12}. Nephrol Dial Transplant 2002;17: 455-61.
[31] Henning BF, Zidek W, Riezler R, Graefe U, Tepel M. Homocyst(e)ine metabolism in hemodialysis patients treated with vitamins B_6, B_{12} and folate. Res Exp Med 2001;200:155-68.
[32] Sombolos K, Fragia T, Natse T, Bartholomatos G, Karagianni A, Katsaris G, et al. The effect of long-term intravenous high dose B-complex vitamins with or without folic acid on serum homocysteine in hemodialysis patients. J Nephrol 2002;15:671-5.
[33] Trimarchi H, Schiel A, Freixas E, Diaz M. Randomized trial of methylcobalamin and folate effects on homocysteine in hemodialysis patients. Nephron 2002;91:58-63.
[34] Hoffer LJ, Bank I, Hongsprabhas P, Shrier I, Saboohi F, Davidman M, et al. A tale of two homocysteines—and two hemodialysis units. Metabolism 2000;49:215-9.
[35] Polkinghorne KR, Zoungas S, Branley P, Villanueva E, McNeil JJ, Atkins RC, et al. Randomized, placebo-controlled trial of intramuscular vitamin B_{12} for the treatment of hyperhomocysteinaemia in dialysis patients. Intern Med J 2003;33:489-94.
[36] Nakhoul F, Abassi Z, Plawner M, Khankin E, Ramadan R, Lanir N, et al. Comparative study of response to treatment with supraphysiologic doses of B-vitamins in hyperhomocysteinemic hemodialysis patients. Isr Med Assoc J 2004;6:213-7.
[37] Glass GB, Lee DH, Skeggs HR, Stanely JL. Hydroxocobalamin: III. Long-acting effects of massive parenteral doses on vitamin B_{12} blood levels in man. JAMA 1963;183:425-9.
[38] Skouby AP. Retention and distribution of B_{12} distribution activity, and requirement for B12, following parenteral administration of hydroxocobalamin (Vibeden). Acta Med Scand 1966;180:95-105.
[39] Hertz H, Kristensen HP, Hoff-Jorgensen E. Studies on vitamin B_{12} retention. Comparison of retention following intramuscular injection

of cyanocobalamin and hydroxocobalamin. Scand J Haematol 1964; 17:5-15.
[40] Chanarin I. The megaloblastic anaemias. 2nd ed. Oxford: Blackwell Scientific Publications; 1979.
[41] Hall CA, Begley JA, Green-Colligan PD. The availability of therapeutic hydroxocobalamin to cells. Blood 1984;63:335-41.
[42] Ducloux D, Heuze-Lecornu L, Gibey R, Bresson-Vautrin C, Vautrin P, Chalopin JM. Dialysis adequacy and homocyst(e)ine concentrations in peritoneal dialysis patients. Nephrol Dial Transplant 1999;14:728-31.
[43] Arnadottir M, Berg AL, Hegbrant J, Hultberg B. Influence of haemodialysis on plasma total homocysteine concentration. Nephrol Dial Transplant 1999;14:142-6.
[44] Birn H, Nexo E, Christensen EI, Nielsen R. Diversity in rat tissue accumulation of vitamin B_{12} supports a distinct role for the kidney in vitamin B_{12} homeostasis. Nephrol Dial Transplant 2003;18:1095-100.
[45] Arnadottir M, Hultberg B, Nilsson-Ehle P, Thysell H. The effect of reduced glomerular filtration rate on plasma total homocysteine concentration. Scand J Clin Lab Invest 1996;56:41-6.
[46] Soria C, Chadefaux B, Coude M, Gaillard O, Kamoun P. Concentrations of total homocysteine in plasma in chronic renal failure. Clin Chem 1990;36:2137-8.
[47] Hattori K, Shiigai T, Minato Y, Iwamoto H. Measurement of glomerular filtration rate by free vitamin B_{12} clearance. Intern Med 1993;32:194-6.