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Association Between Adherence to Folic Acid Supplements and Serum Folate, and Plasma Homocysteine Among Hemodialysis Patients

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Abstract

Objective—To examine the relationship between adherence to prescribed folic acid supplements and folic acid intake, serum folate and plasma homocysteine in hemodialysis patients. The effects of change in adherence patterns from enrollment to one year later on changes in these same measures were also assessed.

Design—Secondary data analysis

Participants—Eighty six hemodialysis patients who participated in the Hemodialysis (HEMO) Study's Homocysteine ancillary study.

Main Outcome Measures—Folic acid supplement intake, serum folate and plasma homocysteine.

Results—Eighty-eight percent of patients at enrollment and 91% one year later were adherent to prescribed folic acid supplements. Non-adherers had lower intakes of folic acid at both enrollment

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and one year later and lower serum folate levels at enrollment. Percent change was significantly different between the 3 adherence change groups for folic acid intake ($p=0.001$) and plasma homocysteine ($p<0.001$) from enrollment to one year later. The non-adherent group at enrollment had the lowest intakes and serum folate levels, and the highest plasma homocysteine levels. When they became adherent one year later, they had the greatest change in folic acid intake (5461%; $p=0.03$), coupled with a 69% increase in serum folate ($p=0.04$) and a 29% decrease in plasma homocysteine ($p=0.03$).

Conclusions—Hemodialysis patients who were non-adherent to folic acid supplement prescriptions had low folic acid intakes, low serum folates and high homocysteine levels. When their adherence improved, folic acid intakes rose, serum folates increased and plasma homocysteine levels decreased, although mild hyperhomocysteinemia persisted.

Keywords

Adherence; folic acid; supplements; hemodialysis; homocysteine

Introduction

Altered micronutrient status in hemodialysis patients is due to chronic kidney disease (CKD) itself, common comorbidities and iatrogenic factors including dialysis, restricted diets, drug-nutrient interactions and uremic toxins^{1–3}. Altered micronutrient status is reflected by abnormal metabolism, body stores, and serum and plasma levels of many vitamins and minerals^{1, 2, 4}. CKD patients are at high risk for cardiovascular, cerebrovascular, dietary deficiency and other chronic diseases owing to changes in intakes of vitamins and minerals, altered metabolism and the disease itself². Almost half of all deaths in hemodialysis patients are due to cardiovascular disease, and its mortality rates are up to 15 times higher among patients with CKD than in the general population^{5, 6}. Hyperhomocysteinemia, due to elevated circulating levels of homocysteine (a sulfur containing amino acid that may be a vascular toxin), is an independent risk factor for coronary, cerebral, and peripheral atherosclerosis in both CKD and the general population^{7–13}. Although severe hyperhomocysteinemia (≥ 100 $\mu\text{mol/L}$) is rare, mild to moderate hyperhomocysteinemia (12–99 $\mu\text{mol/L}$) occurs in 5–7% of the general population^{14–15}. More than 90% of CKD patients have homocysteine levels in the range of 20–80 $\mu\text{mol/L}$ ^{7, 16–19}.

Hemodialysis patients are often supplemented with specially formulated renal multi-vitamins in order to replenish dialytic losses of the water soluble B vitamins that are likely to be removed by dialysis and to address the other factors, mentioned previously, affecting micronutrient status alterations found in this population^{1–3}. The renal multi-vitamins that hemodialysis patients take typically contain folic acid (0.8–5 mg/day), B6 (10–50 mg/day), and B12 (6 μg –2 mg/day). These vitamins are also sometimes prescribed at high single doses to decrease homocysteine, in an attempt to decrease the risk of cardiovascular disease. High doses of folic acid (5–60 mg/day), vitamin B6 (up to 100 mg/day), and vitamin B12 (up to 1 mg/day) reduce homocysteine levels by 30–50% in CKD patients, including those on hemodialysis, but even with such treatment, less than 10% of them achieve homocysteine levels in the normal range^{20–25}.

While patient adherence studies in CKD patients have focused mainly on dialysis treatment regimens, fluid restriction, and medication use, it is evident that non-adherence to prescribed of dietary supplements such as calcium salts (used as phosphate binders) is also highly variable, ranging from 3–80%^{26–27}. Only a few studies have reported adherence to vitamin supplement prescriptions among hemodialysis patients^{19, 28}. Psychosocial, physical and economic constraints may all cause inadequate intakes of both supplements and foods^{29–30}.

These barriers include lack of understanding in the importance of supplement use, lack of motivation, poor self care patterns, inability to remember medication schedules, and lack of financial resources (supplement purchases are not covered by the Medicare End Stage Renal Disease Program and the supplements may be unaffordable)^{29–30}. Removing financial barriers to supplement use by providing them free of charge to patients or educating and motivating patients on why they should use supplements appear to increase adherence^{19, 28}.

Little is known about the impact of vitamin supplement adherence on clinical outcomes. To address this question, we used data from the Hemodialysis (HEMO) Study, in which after randomization into one of four dialysis treatments, all patients received free of charge a high folic acid (1000 µg) renal formulated B-complex vitamin with vitamin C. We examined patient adherence to physician prescriptions of folic acid containing supplements at enrollment and one year later and the association of adherence on folic acid intake from these vitamin supplements, serum folate and plasma homocysteine levels. We also evaluated the relationship between the change in adherence patterns from enrollment, when patients were provided with the free renal multi-vitamin containing folic acid, to one year later on changes in intakes, serum folate and plasma homocysteine levels.

Methods

HEMO Study Design and Population

The design, methods, and primary outcomes of the HEMO Study are described elsewhere³¹. The prospective, multi-center, randomized clinical trial, which was sponsored by the National Institutes of Health's National Institute of Diabetes, Digestive and Kidney Diseases from 1995 through 2001, compared effects of two dialysis doses and two dialysis membrane fluxes on morbidity and mortality in 1846 randomized thrice weekly in center hemodialysis patients between the ages of 18–80. This secondary analysis included 291 subjects who participated in the Homocysteine ancillary study. One hundred and eighty one patients were excluded because complete data on serum folate and plasma homocysteine (n=176) and folic acid supplements (n=5) were not available at both enrollment and one year later. An additional 24 patients were excluded because folic acid containing supplements were not prescribed by physicians at either enrollment or a year later or at both time periods. The total number of patients in this analysis was 86.

Prescription and Use of Vitamin and Mineral Supplements in the HEMO Study

After randomization, all study patients were provided free with “Nephro-Vite Rx” (R&D Laboratories) tablets, a high potency, high folic acid (1000 µg) renal formulated B-complex vitamin with vitamin C. Patients were encouraged, but were not required, to use the “Nephro-Vite RX” supplement, and some chose not to do so. They also were permitted to use over-the-counter or other prescribed vitamin and mineral supplements. Physician prescriptions of supplements were collected by medical chart review once at enrollment and one year later. Reported intakes of “Nephro-Vite RX”, other physician prescribed supplements, and other over-the-counter supplements that patients chose to take on their own were ascertained once at enrollment and one year later by the HEMO Study dietitian during patient interviews. The name, dosage and unit (ie. mg, µg, ml), amount, frequency (day, week, month), and form (tablet, liquid, intravenous) of vitamin and mineral supplements that were prescribed and consumed by the patient were recorded onto a HEMO Study Diet Prescription and Supplement Documentation Form once at enrollment and one year later. Nutrient values for each supplement were calculated based on the physician prescribed dose, as determined by review of the patient's medical chart, and also by the patient's reported consumption. Values were imputed for missing folic acid dosages using a proportions based median imputation method developed for use in the HEMO Study. This

procedure determined the proportions and medians of the available folic acid doses and randomly imputed values to replace the missing dosages. Total folic acid from vitamin supplements was derived by summing folic acid from all vitamins containing folic acid that were prescribed or taken. Percent change in folic acid intake consumed by patients from enrollment to one year later was also calculated.

Adherence to Prescriptions of Folic Acid Containing Supplements

Adherence at enrollment and one year later was determined using the total dose of folic acid containing supplements prescribed by the physician and actual folic acid supplement consumption reported by the patient. Adherence was defined as consumption equal to or greater than the total prescribed dosage, and non-adherence as consumption of less than the prescribed total dosage. We assumed that those who improved in their adherence over the year would increase their folic acid intakes and serum folate levels, and decrease their homocysteine levels. To examine the change in adherence to prescribed supplements from enrollment to one year later, 3 groups were formed as follows: 1) adherent at both enrollment and one year later (n=71), 2) non-adherent at enrollment but adherent one year later (n=7); and 3) non-adherent one year after enrollment (n=8), regardless of whether they were adherent (n=3) or non-adherent (n=5) at enrollment. In order to increase the small group size for carrying out a meaningful statistical analysis, we combined patients who differed in adherence at enrollment but who were non-adherent one year later.

Total Folate Intake from Food and Oral Nutritional Supplements

Two-day diet diary assisted recalls were collected on patients once at enrollment prior to randomization into the HEMO Study and one year later. All foods, beverages, and oral nutritional supplements consumed by the patients in a two day period (one dialysis and one non-dialysis day) were recorded in the diet diary and reviewed with the patients by the HEMO Study dietitian to probe for portion sizes and other details. The diet diaries were then analyzed using customized software versions of the nutrient analysis programs Nutritionist IV 4.0a (March 1995 through July 1999) and Nutritionist Five 2.0h–2.1.1h (August 1999 through the end of the study) from First DataBank, Inc., San Bruno, CA. Mean folate intake was calculated from the two day diet diaries.

In 1996, the US Food and Drug Administration required that all enriched breads, rice, pasta, cornmeal, and other grain products be fortified with folic acid by January 1998 and many manufacturers voluntarily began doing so after March 1996³². The nutrient analysis database was updated once in August 1999 after folic acid fortification was implemented. Therefore, for this study, folate values from food records analyzed between March 1996 and August 1999 were adjusted to reflect folic acid fortification in the food supply³³.

Biochemical Indicators of Folate, Vitamins B6 and B12, and Homocysteine

Predialysis serum folate, pyridoxal-5'-phosphate (PLP) (vitamin B6), vitamin B12, and plasma homocysteine at enrollment and one year later were obtained from the HEMO Study's Homocysteine ancillary study. Ten ml of blood was collected in an EDTA tube (10 ml lavender top tubes) and placed on ice or in a refrigerator at 4°C until centrifugation within three hours. Buffy coat was pipetted and saved in an additional cryo tube. Plasma and red blood cell pellets were stored at -70°C (or -20°C if -70°C freezer is not available) and mailed in dry ice monthly to the Homocysteine ancillary study central center for central laboratory analyses at the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University. Total homocysteine levels were measured by the method of Araki and Sako³⁴. Serum folate was measured by a microbial assay with a 96 well plate and manganese supplementation. Vitamin B12 was measured with a radioassay kit (Magic, Ciba-Corning, Medfield, MA) and PLP was ascertained using the tyrosine decarboxylase

method³⁵. Percent change in serum folate and plasma homocysteine from enrollment to one year later were calculated.

Clinical and Demographic Characteristics

Patient clinical and demographic characteristics at enrollment that were examined included age, gender, race, intervention effects (dose of dialysis and membrane flux), total serum cholesterol (measured at each individual laboratory of the clinical centers), and serum albumin. Pre-dialysis albumin using nephelometry was measured at Spectra East, Rockleigh, NJ. Additionally, presence of diabetes and enrollment scores for the Index of Co-Existing Disease (ICED), a comorbidity index based on presence and severity of diseases and impact on physical function, were abstracted from the patient's medical record³⁶.

Statistical Analyses

Student's t-tests for continuous variables and Fisher's exact test for categorical variables were used to compare enrollment demographic and clinical characteristics, folic acid prescription dosages, vitamin B12, and PLP of adherent and non-adherent patients to prescribed folic acid supplements. Descriptive statistics for intakes from folic acid of adherers and non-adherers at enrollment and one year later were also calculated. Fisher's exact test was used to examine folic acid intake < 1000 µg among adherers and non-adherers at enrollment and one year later. Student's t-tests were used to examine the difference in intakes of folic acid from supplements, total folate from food and oral nutritional supplements, serum folate, and plasma homocysteine among adherers and non-adherers at enrollment and one year later. Risk ratios for the difference in mean folic acid intakes between adherers and non-adherers were calculated.

ANOVA (continuous variables) and Fisher's exact test (categorical variables) were used to examine the clinical and demographic characteristics at enrollment as well as mean folic acid supplement intake, serum folate, vitamin B12, PLP and plasma homocysteine among the 3 adherence change groups at enrollment and one year later. Changes in total folic acid supplement intake, serum folate, and plasma homocysteine from enrollment to one year later among the 3 adherence change groups were examined using ANOVA. Paired t-tests were used to assess differences from enrollment to one year later within adherence groups.

Imputed values for missing folic acid supplement dosages were utilized. Data for folic acid intake, total folate intake, serum folate and plasma homocysteine were not normally distributed and therefore were log transformed for statistical analyses. All calculations were performed using the statistical software package SAS version 9.1 (SAS Institute, INC., Cary, NC).

Results

Patients in the HEMO study were adherent to physician prescriptions of folic acid containing supplements with 88% adherent at enrollment and 91% one year later. Table 1 presents their clinical and demographic characteristics at enrollment. Those who were adherent were older (58.7 ± 14.3 years, $p=0.04$) and were more likely to have diabetes mellitus (54%, $p=0.01$) than non-adherers (49.1 ± 10.4 years and 10% with diabetes mellitus), but adherers and non-adherers did not differ in other respects. Four (40%) non-adherers compared to 10 (13%) adherers had low plasma PLP levels, defined as < 20 nmol/L ($p=0.05$). No subjects had low vitamin B12 levels, defined as <100 pg/mL.

Table 2 presents differences between adherers and non-adherers in folic acid prescription dosages and intakes, serum folate, and plasma homocysteine levels at enrollment and one year later. The distribution of prescribed amounts of folic acid was significantly different

between adherers and non-adherers at both enrollment and one year later ($p < 0.001$). Seven (70%) non-adherers were prescribed dosages of folic acid $> 1000 \mu\text{g}$ compared to 4 (5%) adherers at enrollment. One year later, non-adherers (37%) continued to have higher prescribed folic acid dosages $> 1000 \mu\text{g}$ compared to 2% of adherers. Non-adherers had significantly lower folic acid supplement intakes (geometric mean $32 \mu\text{g}$, 95% CI 1, 477 $p = 0.02$) than adherers at enrollment. One year later, folic acid supplement intakes also differed significantly ($p = 0.004$) for adherers, whose intakes were higher ($1001 \mu\text{g}$, 95% CI 952, 1053) than non-adherers ($32 \mu\text{g}$, 95% CI 1, 741). The effect of adherence on folic acid intake was that adherers had more than 30 times as much folic acid intake on average as non-adherers at both enrollment (30.0, 95% CI: 13.3, 69.4) and one year later (30.8, 95% CI: 13.7, 69.5). More non-adherers had intakes of $< 1000 \mu\text{g}$ folic acid from supplements than adherers at both time periods, but the difference was significant only one year later ($p < 0.001$) when 75% non-adherers compared to 11% adherers had folic acid intakes $< 1000 \mu\text{g}$. Geometric means for total folate from food and oral nutritional supplements for adherers ($198 \mu\text{g}$, 95% CI 174, 228) and non-adherers ($206 \mu\text{g}$, 95% CI 136, 314) were not significantly different at either enrollment ($p = 0.8$) or one year later ($244 \mu\text{g}$, 95% CI 215, 279 for adherers and $164 \mu\text{g}$, 95% CI 89, 295 for non-adherers; $p = 0.07$).

For serum folate, adherers had higher geometric mean levels (24.5 ng/mL , 95% CI 22.2, 30.0) compared to non-adherers (14.9 ng/mL , 95% CI 8.2, 27.1) only at enrollment ($p = 0.02$). The mean value for non-adherers was lower but adequate ($\geq 5 \text{ ng/mL}$) for serum folate, except for one non-adherent patient at enrollment. The mean plasma homocysteine level for the entire cohort was $23.5 \pm 10.8 \mu\text{mol/L}$ at enrollment; within the mild to moderate range of hyperhomocysteinemia ($20\text{--}80 \mu\text{mol/L}$) typical of this population. Four patients had normal plasma homocysteine levels ($< 11 \mu\text{mol/L}$) at enrollment, 3 of whom were adherent and 1 non-adherent to folic acid containing supplements ($p = 0.4$), although there were no significant differences in plasma homocysteine levels among adherers and non-adherers either at enrollment ($p = 0.2$) or one year later ($p = 0.5$).

We also examined the clinical and demographic characteristics of the 3 adherence change groups. Age was the only factor upon which differences were statistically significant ($p = 0.03$). Those adherent at both enrollment and one year later were the oldest (mean 59.3 ± 14.2 years) followed by those who were only adherent one year later with a mean of 51.3 ± 11.9 years and finally those who were non-adherent one year later were the youngest (mean 47.3 ± 10.2 years).

Table 3 presents the folic acid intake, total folate intake, serum folate and plasma homocysteine levels at enrollment and one year later as well as the percent changes in intake and biochemistries from enrollment to one year later among the 3 adherence change groups. Geometric means for folic acid intake between the 3 adherence change groups were significantly different at both enrollment ($p < 0.001$) and one year later (< 0.001) as was the percent change in folic acid supplement intake from enrollment to one year later ($p = 0.001$). The patients who were adherent in both years consistently had geometric mean folic acid intakes over $1000 \mu\text{g}$ at both enrollment and one year later. Those who were only adherent one year later had improved their geometric mean folic acid intakes from $18 \mu\text{g}$ at enrollment to $1001 \mu\text{g}$ one year later. This group had the highest percent change in folic acid supplement intake from enrollment to one year later (5461%, $p = 0.03$). Conversely, the group which was non-adherent one year later had lower geometric mean intake of folic acid from supplements one year later ($32 \mu\text{g}$) compared to that of enrollment ($482 \mu\text{g}$). As was expected, this group exhibited a 93% decrease in folic acid intake from enrollment to one year later ($p = 0.04$).

Serum folate levels differed significantly between the 3 adherence change groups at both enrollment ($p=0.002$) and one year later ($p=0.03$), although percent change in serum folate did not ($p=0.9$). Among the group that was only adherent one year later, serum folate levels were higher one year later (geometric mean 17.6 ng/mL, 95% CI 11.6, 26.8) than at enrollment (geometric mean 10.4 ng/mL, 95% CI 6.6, 16.4), with a 69% increase ($p=0.04$) from enrollment to one year later, reflecting adherence to folic acid supplements. One patient from this group who had an inadequate serum folate level < 5 ng/mL at enrollment had levels ≥ 5 ng/mL one year later, as did all other patients in the group. Additionally, 4 patients in this group had PLP levels < 20 nmol/L at enrollment; one year later only 2 had inadequate levels < 20 nmol/L.

Geometric mean plasma homocysteines differed significantly between the 3 adherence change groups at enrollment ($p=0.003$) with the group of patients who were only adherent one year later having the highest levels (geometric mean 33.4 $\mu\text{mol/L}$, 95% CI 24.8, 45.1). One year later, plasma homocysteine levels did not differ between the 3 groups ($p=0.6$). However, the geometric mean plasma homocysteine for the group that was only adherent one year later was lower with a mean of 23.6 $\mu\text{mol/L}$ (95% CI 18.0, 30.9) than at enrollment (33.4 $\mu\text{mol/L}$, 95% CI 24.8, 45.1), with a 29% decrease ($p=0.03$). The percent change in plasma homocysteine from enrollment to one year later was significantly different between the 3 adherence groups ($p<0.001$).

Discussion

The benefits of high potency renal formulated B-complex vitamins in protecting the nutritional status of hemodialysis patients are well known and justify use of such products ^{1–4}. Non-adherence to such vitamin supplement prescriptions is common among hemodialysis patients and may influence their health and outcomes ^{26–28, 37–38}. We found, as have others, that by providing patients with a free supplement, we removed a potential financial barrier, improved physical access to supplements, and encouraged their use and supplement adherence ^{19, 28}. Improved adherence had an effect on total folic acid intake, serum folates and plasma homocysteines. However, even when provided free of charge, adherence to the supplements did not reach 100%. In this study, the overall change in adherence was modest because of the high prevalence of adherence at enrollment. In clinical practice, the increase in adherence might be more dramatic.

As expected, non-adherers had lower intakes of folic acid from supplements and lower serum folate levels than adherers. Of note, non-adherers were more likely to have been prescribed folic acid doses >1000 μg , although neither their rates of diabetes mellitus or their ICED scores suggested that they were sicker than adherers which would have been thought to prompt prescriptions of higher folic acid doses to lower CVD risk. We suspect that perhaps patients who were prescribed folic acid dosages greater than the standard 1000 μg (typically found in a renal multi-vitamin) may have been prescribed multiple supplement pills and pill burden is known to be associated with decreased adherence ²⁶.

When we examined the impact of change in adherence, the beneficial effects of improved adherence on intakes and blood biochemistries were apparent. Among the three groups, the group that was adherent at both enrollment and one year later had consistent folic acid intakes of > 1000 μg and their serum folate remained in the normal range. These individuals appear to have substituted their intakes at enrollment with the free supplement provided to them after randomization into the HEMO Study. The patients who were non-adherent at enrollment but became adherent one year later had the most to benefit from because of their non-adherence. They exhibited the highest increase in folic acid intake, apparently because they took the free folic acid vitamin supplement provided to them after enrollment. Their

serum folate levels rose, and they had the greatest decrease in plasma homocysteine levels. While the levels of plasma homocysteine among these patients remained higher than those in the other 2 groups, the drop in the homocysteine level was consistent with previous studies that have shown a reduction of plasma homocysteine by 30–50% with folic acid vitamin supplement use^{20–25}. However, it should be noted that the homocysteine levels still were not reduced to values within the normal range. Furthermore, those who were non-adherent at enrollment but adherent one year later also included a high number of patients (n=4) with inadequate levels of PLP levels at enrollment, which could have resulted from low intakes of vitamin B6. One year later, when the adherence to folic acid containing vitamin supplements improved, there were only 2 patients with deficient PLP levels, possibly due to increased intake of renal multi-vitamins that typically contain vitamin B6 as well as vitamin B12 and folic acid. Therefore, a positive change in adherence appeared to result in a benefit for those patients with low folic acid intake, low serum folates and high plasma homocysteines at enrollment. In contrast, the group which was non-adherent one year later had the largest decrease in folic acid intake over the year.

Serum folate levels among non-adherers were higher one year later (geometric mean 24.5 ng/mL, 95% CI 14.9, 36.6) than at enrollment (geometric mean 14.9 ng/mL, 95% CI 8.2, 27.1) despite their mean folic acid intakes being low at both enrollment (geometric mean 32 µg, 95% CI 1, 477) and one year later (geometric mean 32 µg, 95% CI 1, 741). This outcome was most likely due to 4 subjects who had been prescribed a higher dosage of folic acid one year later than at enrollment. While they were non-adherent to the higher dosage, they still consumed more than they did at enrollment, as was evident in the wider intake range one year later (95% CI: 1, 741) compared to that at enrollment (95% CI: 1, 477) and therefore the elevated serum folates may have reflected these higher intakes. Serum folate may have also been confounded by the effect of folic acid fortification. Note that among adherers, folic acid intakes (geometric mean 1042 µg at enrollment and 1001 µg one year later), PLP (mean change from enrollment to one year later of 1.7 nmol/L, p=0.9) and vitamin B12 levels (mean change from enrollment to one year later of 24.2 pg/mL, p=0.5) remained constant from enrollment to one year later. However, their mean serum folates increased from 24.5 ng/mL at enrollment to 30.0 ng/mL one year later, suggesting that folic acid fortification in the food supply had an effect on serum folate levels despite a constant multi-vitamin intake. Our finding is consistent with another large study that found increased serum folate levels in the range of 4.6 to 10 ng/mL among healthy individuals who did not use vitamin supplements³⁹.

This secondary analysis is the first study which examined the association between adherence and the impact of change in adherence to folic acid supplements and plasma homocysteine levels. Most studies examining adherence to medication use biochemical measures as a marker for adherence. We also documented the physician prescription, patient reports of consumption and adherence to folic acid containing supplements as well as the biochemical markers serum folate and plasma homocysteine, which were collected pre-dialysis so that values reflected were realistic.

Our study had certain limitations. Red blood cell folate is traditionally thought of as a better reflection of tissue folate stores than serum folate but it was not available. However, some studies suggest that tissue stores correlate equally well with both serum and red blood cell folate and there are good correlations between the two assays⁴⁰.

Another limitation was the small sample size of non-adherent patients and the imbalance of the three change in adherence groups, which could introduce an unintentional bias in the results. Although, examination of the baseline clinical and demographic characteristics revealed no significant differences among the groups except for age. Additionally, the group

that included patients who were non-adherent one year later regardless of adherence at enrollment was heterogeneous in their intakes and biochemical measures at enrollment. However, they still represented those whose adherence remained poor or worsened over time and therefore were expected to exhibit poorer intakes and outcomes, and they did indeed.

Folic acid supplement intakes that were reported by patients on their enrollment and annual visit day represented intake for the period at enrollment and one year later and, as such, intakes might have been under or overestimated, particularly if it was not a typical day for the patient. In addition, patient report of intake may be unreliable in this population due to pill burden. While personal interviews for collection of supplement use information have been shown to be adequate for assessment of nutrient intakes from supplements, use of supplement inventories, such as that recommended for prescription medications, can perhaps improve reliability in this population ^{41–43}.

Furthermore, folic acid supplement intakes and serum folate and plasma homocysteine levels were obtained at two time periods separated by one year. Changes in these biochemical levels to a stable concentration after a change in folic acid supplement intake occur over a period of time. Therefore, time averaged intake prior to obtaining the biochemical levels would be important to assess the relationship between intake and observed serum folate and plasma homocysteine levels. However, such time averaged intakes were not collected in this study.

Another limitation is that we did not obtain intakes of vitamins B6 and B12 supplements. Studies have shown that folic acid alone or in combination with vitamins B6 and/or B12 can reduce plasma homocysteine levels ^{44–49}. Therefore, further studies examining folic acid supplement use and dosage should consider intakes of vitamins B6 and B12 as well as other factors that affect plasma homocysteine levels in the analyses. Finally, patient socioeconomic data to determine whether financial inability to purchase folic acid vitamin supplements was associated with non-adherence was not available.

Many cross sectional and prospective studies have indicated that treatment with folic acid alone or in conjunction with vitamins B6 and B12 can reduce homocysteine levels and that folic acid intake is inversely related to the risk of CVD leading to the hypothesis that reduction in plasma homocysteine with use of folic acid supplements can be beneficial for cardiovascular outcomes ^{44–49}. Our study demonstrated that individuals who were non-adherent to folic acid supplements reaped the greatest benefit by improving adherence and subsequently increasing intakes of folic acid vitamin supplements and serum folate and reducing plasma homocysteine. However, the implications of adherence to folic acid supplements on clinical outcomes of lowering plasma homocysteine and cardiovascular disease related morbidity and mortality are unclear. Several large randomized controlled clinical trials examining the effects of supplemental folic acid and B vitamins on reducing homocysteine and cardiovascular outcomes in both patients with normal kidney function and chronic kidney disease have shown inconsistent findings ^{22–25, 50–57}. These studies suffered limitations that fail to clarify any putative association between plasma homocysteine and cardiovascular disease risk and whether folic acid supplementation can reduce cardiovascular disease related morbidity and mortality. Several large clinical trials that address these limitations are currently underway which may provide more definitive conclusions, but at present it must be concluded that the relationship between plasma homocysteine and cardiovascular outcomes among dialysis patients remain highly controversial ^{58–62}. However, what can be concluded from our study is that there is a clear benefit for patients to adhere to and take their prescribed vitamins in avoiding micronutrient deficiencies. Mean total folate from food and oral nutritional supplements at both baseline

and one year later among adherers and non-adherers ranged from 164–244 µg, well below the US Dietary Reference Intake's Estimated Average Requirements of 320 µg set for folate intake⁶³. Therefore, adherence and intake of folic acid vitamin supplements are critical to ensure adequate intake as well as addressing the increased needs unique to this population.

It is not possible to make any cause-and-effect associations between the factors (potential financial or physical access to vitamin supplements) affecting adherence and the change in adherence experienced by these patients. Providing free supplements to these patients did appear to improve their adherence and subsequently their folic acid intakes, serum folate and plasma homocysteine levels improved. Further exploration of factors affecting adherence and examination of other types of vitamin supplements are needed as they have potential implications for improved health outcomes.

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Table 1

Clinical and demographic characteristics at enrollment of hemodialysis patients who were adherent and non-adherent to prescribed folic acid containing supplements

Characteristics	Adherent (n=76)	Non-Adherent (n=10)	P
Age (mean±SD)	58.7±14.3	49.1±10.4	0.04
Female sex (%)	62	50	0.5
Black (%)	47	50	1.0
Presence of Diabetes Mellitus (%)	54	10	0.01
Index of Coexisting Disease (ICED) scores			0.8
mild (%)	44	40	
moderate (%)	22	30	
severe (%)	34	30	
Serum albumin (mean±SD gm/dL) *	4.0±0.3	4.1±0.3	0.3
Total cholesterol (mean±SD mg/dL)	175±37	167±57	0.6
Plasma PLP (geometric mean, 95% CI nmol/L)	62.2 (47.9, 81.5)	31.5 (12.1, 83.1)	0.09
Plasma vitamin B12 (geometric mean, 95% CI pg/mL)	685 (602, 781)	539 (308, 944)	0.2
High Kt/V group (%)	50	40	0.7
High flux group (%)	46	30	0.5

* n=54 for adherers and n=7 for non-adherers

Table 2

Differences in folic acid supplement prescription and intakes, serum folate and plasma homocysteine levels between hemodialysis patients who were adherent and non-adherent to folic acid supplements at enrollment and one year later

	Enrollment			One year later		
	Adherent (n=76)	Non-Adherent (n=10)	P	Adherent (n=78)	Non-Adherent (n=8)	P
Folic acid prescription			<0.001			<0.001
800 µg (%)	32	30		13	38	
1000 µg (%)	63	0		84	25	
>1000 µg (%)	5	70		2	37	
Folic Acid Supplement Intake						
Median (µg)	1000	500	--	1000	400	--
< 1000 µg (%)	26%	50%	0.14	11%	75%	<0.001
Geometric mean (95% CI) µg	1042 (962, 1129)	32 (1, 477)	0.02	1001 (952, 1053)	32 (1, 741)	0.04
Serum folate						
Geometric mean (95% CI) ng/mL	24.5 (22.2, 30.0)	14.9 (8.2, 27.1)	0.02	30.0 (27.1, 33.1)	24.5 (14.9, 36.6)	0.3
Plasma homocysteine						
Geometric mean (95% CI) µmol/L	20.1 (18.2, 22.2)	24.5 (16.4, 36.6)	0.2	21.1 (19.5, 23.1)	19.1 (14.2, 26.3)	0.5

Table 3

Comparison of change in intake and biochemical indicators from enrollment to one year later among hemodialysis patients in 3 adherence change groups

Intake and Biochemical Values	Adherent at enrollment and one year later (n=71)	Adherent only one year later (n=7)	Non-adherent one year later (n=8)	P Between Groups
Folic acid supplement intake				
Enrollment (geometric mean [95% CI] µg)	1031 (764, 1393)	18 (6, 49)	482 (195, 1175)	<0.001
One year later (geometric mean [95% CI] µg)	1001 (772, 1312)	1001 (436, 2163)	32 (14, 70)	<0.001
Change (%) *	-3% (p=0.2)	5461% (p=0.03)	-93% (p=0.04)	0.001
Serum folate				
Enrollment (geometric mean [95% CI] ng/mL)	25.5 (22.0, 29.4)	10.4 (6.6, 16.4)	23.6 (15.3, 36.2)	0.002
One year later (geometric mean [95% CI] ng/mL)	30.0 (27.1, 35.5)	17.6 (11.6, 26.8)	23.8 (16.1, 35.2)	0.03
Change (%) *	18% (p=0.1)	69% (p=0.04)	0.8% (p=0.7)	0.9
Plasma homocysteine				
Enrollment (geometric mean [95% CI] µmol/L)	21.1 (19.3, 23.3)	33.4 (24.8, 45.1)	16.4 (12.4, 21.8)	0.003
One year later (geometric mean [95% CI] µmol/L)	21.1 (19.3, 22.9)	23.6 (18.0, 30.9)	19.3 (14.9, 34.8)	0.6
Change (%) *	0% (p=0.5)	-29% (p=0.03)	18% (p=0.2)	<0.001

* P value for comparison of change from enrollment to one year later within adherence group