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Homocysteine-Lowering and Cardiovascular Disease Outcomes in Kidney Transplant Recipients: Primary Results from the Folic Acid for Vascular Outcome Reduction in Transplantation (FAVORIT) Trial

Andrew G. Bostom, MD,
Rhode Island Hospital

Myra A. Carpenter, PhD,
University of North Carolina

John W. Kusek, PhD,
NIDDK

Andrew S. Levey, MD,
Tufts Medical Center

Lawrence Hunsicker, MD,
University of Iowa

Marc A. Pfeffer, MD, PhD,
Brigham and Women's Hospital

Jacob Selhub, PhD,
Jean Mayer USDA HNRCA

Paul F. Jacques, ScD,
Jean Mayer USDA HNRCA

Correspondence: Andrew B. Bostom, MD, Rhode Island Hospital, 110 Lockwood St., POB Rm. 242, Providence, RI 02903, Fax: 401.444.2217 Telephone: 401.444.6460, ; Email: abostom@lifespan.org

Author contributions: Dr. Myra A. Carpenter had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study Concept and Design: Bostom, Carpenter, Gravens-Mueller, Hunsicker, Jacques, Levey
Acquisition of data: Bostom, Carpenter, Gravens-Mueller, House, Hunsicker, Kew, McKenney, Pirsch, Selhub, Smith, Weir
Analysis and interpretation of data: Bostom, Carpenter, Cole, Gravens-Mueller, Jacques, Kusek, Levey, Pacheco-Silva, Pesavento, Pfeffer, Solomon
Drafting of the manuscript: Bostom, Carpenter, Gravens-Mueller, Kusek, McKenney
Critical revision of the manuscript for important intellectual content: Bostom, Carpenter, Cole, House, Hunsicker, Jacques, Kew, Kusek, Levey, Pacheco-Silva, Pesavento, Pfeffer, Pirsch, Smith, Solomon, Weir
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Others: Cole (recruited patients), Jacques (nutritional expertise), Solomon (co-chair Endpoints committee)

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Edward Cole, MD,
University of Toronto

Lisa Gravens-Mueller, MS,
University of North Carolina

Andrew A. House, MD,
London Health Sciences Center

Clifton Kew, MD,
University of Alabama

Joyce L. McKenney, MA, MPH,
Rhode Island Hospital

Alvaro Pacheco-Silva, MD, PhD,
Universidade Federal de Sao Paulo

Todd Pesavento, MD,
Ohio State University

John Pirsch, MD,
University of Wisconsin

Stephen Smith, MD,
Duke University

Scott Solomon, MD,
Brigham and Women's Hospital

Matthew Weir, MD, and
University of Maryland

For the FAVORIT Study Investigators

Abstract

Background—Kidney transplant recipients, like other patients with chronic kidney disease (CKD), experience excess risk of cardiovascular disease (CVD) and elevated total homocysteine (tHcy) concentrations. Observational studies of patients with CKD suggest increased homocysteine is a risk factor for CVD. The impact of lowering total homocysteine (tHcy) levels in kidney transplant recipients is unknown.

Methods and Results—In a double-blind controlled trial, we randomized 4110 stable kidney transplant recipients to a multivitamin that included either a high dose (n=2056) or low dose (n=2054) of folic acid, vitamin B6, and vitamin B12 to determine whether decreasing tHcy concentrations reduced the rate of the primary composite arteriosclerotic CVD outcome (myocardial infarction, stroke, CVD death, resuscitated sudden death, coronary artery or renal artery revascularization, lower extremity arterial disease, carotid endarterectomy or angioplasty, or abdominal aortic aneurysm repair). Mean follow-up was 4.0 years. Treatment with the high dose multivitamin reduced homocysteine but did not reduce the rates of the primary outcome (n= 547 total events; hazards ratio [95% confidence interval] = 0.99 [0.84–1.17]), or secondary outcomes

of all-cause mortality (n=431 deaths; 1.04 [0.86–1.26]) or dialysis-dependent kidney failure (n=343 events; 1.15 [0.93–1.43]) compared to the low dose multivitamin.

Conclusions—Treatment with a high dose folic acid, B6, and B12 multivitamin in kidney transplant recipients did not reduce a composite cardiovascular disease outcome, all-cause mortality, or dialysis-dependent kidney failure despite significant reduction in homocysteine level.

Keywords

cardiovascular disease risk factors; mortality; clinical trials; kidney

Introduction

Homozygous genetic disorders[1–3] resulting in marked elevations of plasma homocysteine (total homocysteine concentrations, 100–500 μmol/L), a sulfur amino acid by-product of methionine metabolism, are clearly associated with atherothrombotic events early in life.[4] Total homocysteine (tHcy)-lowering treatment appears to reduce the incidence of these outcomes among such patients.[4,5] In addition, pooled data from prospective observational studies have suggested that mild to moderate hyperhomocysteinemia (tHcy levels, 12–99 μmol/L)[6] may also be a significant risk factor for arteriosclerotic cardiovascular disease (CVD) among the general population.[7]

Longitudinal investigations of persons with chronic kidney disease (CKD) without kidney failure have demonstrated a relationship between higher levels of total homocysteine and CVD risk. Typically, the greatest relative risk was confined to persons with tHcy concentrations in the uppermost distribution. {[8,9] Whether mild to moderate hyperhomocysteinemia is a direct cause of arteriosclerotic outcomes in these CKD populations, or only a surrogate for clinical CVD, remains unresolved. Although a substantial number of randomized controlled clinical trials of tHcy-lowering treatment have been undertaken to evaluate efficacy for reducing CVD events among different patient populations at high risk for atherothrombotic sequelae,[10,11] including persons with CKD,[12–16] none has revealed a significant decrease in CVD outcomes.[10–16] Kidney transplant recipients are considered to have CKD, irrespective of glomerular filtration rate (GFR) or presence or absence of markers of kidney damage.[17] Although there remains great heterogeneity among causes of CKD, many of the complications of CKD in kidney transplant recipients are similar to those experienced by persons with CKD of their native kidneys.[18] In particular, kidney transplant recipients experience a high rate of both incident and recurrent CVD, {Bostom, 1999 #783;Friedman, 2002 #1240} and an excess prevalence of hyperhomocysteinemia despite the fortification of cereal grain flour with folic acid—a major determinant of plasma homocysteine concentrations.[8,9] Importantly, these patients are not treated routinely with supplemental folic acid but unlike patients with kidney failure treated by dialysis, it is possible to “normalize” their tHcy concentrations with combined folic acid, vitamin B12, and vitamin B6 treatment.[8,9]

We conducted the Folic Acid for Vascular Outcome Reduction in Transplantation (FAVORIT) trial in clinically stable kidney transplant recipients.[19] The primary objective was to determine whether decreasing tHcy levels with a multivitamin containing high doses

of folic acid, vitamin B6, and vitamin B12 would reduce their risk of CVD outcomes compared to treatment with a “low-dose” multivitamin devoid of folic acid and with estimated average requirement amounts of vitamins B6 and B12.

Methods

Trial design

Details of the study design are published elsewhere.[19] Briefly, the study was a multi-center double-blind randomized controlled clinical trial conducted to determine whether lowering homocysteine levels by vitamin therapy reduced the rate of pooled arteriosclerotic CVD outcomes. The trial received approval from the Institutional Review or Ethics boards of all 30 clinical sites. Written informed consent was obtained from all participants.

Study Participants

Men and women aged 35 to 75 years and who were at least 6 months post-kidney transplant were screened for eligibility. Key entry criteria were stable kidney function (estimated creatinine clearance[20] (Ccr) ≥ 30 mL/min through July 7, 2005; after which the cut point for women was ≥ 25 mL/min) and elevated homocysteine (≥ 11 μmol/L for women; ≥ 12 μmol/L for men). Race and ethnicity were determined through self-report using categories as defined in the National Institutes of Health Policy on Reporting Race and Ethnicity Data.[21]

Intervention

Participants were randomized to receive either a standard multivitamin with a high dose of folic acid (5.0 mg), vitamin B6 (pyridoxine; 50 mg) and vitamin B12 (cyanocobalamin; 1.0 mg) or a multivitamin with a low dose of vitamin B6 (1.4 mg) and vitamin B12 (2.0 μg) and no folic acid. Both multivitamins were formulated similar in appearance and odor to facilitate blinding. Adherence was assessed by annual pill count and semi-annual self-report.

Baseline and Follow-up

The trial enrolled study participants from August 2002 through January 2007. Follow-up contacts occurred every six months through January 31, 2010 to obtain study related outcomes through June 24, 2009. Central laboratory methods for tHcy and creatinine determinations have been described.[22] Due to resource limitations, baseline and follow-up tHcy analyses are reported on a convenience sample of 143 participants who were among the first participants enrolled. Glomerular filtration rate (eGFR) is estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[23]

Outcomes

The primary outcome was pooled incident or recurrent CVD comprised of (1) CVD death, (2) myocardial infarction, (3) resuscitated sudden death, (4) stroke, (5) coronary artery revascularization, (6) lower extremity revascularization or amputation above the ankle for severe arterial disease, (7) carotid endarterectomy or angioplasty, (8) abdominal aortic aneurysm repair, or (9) renal artery revascularization. The first four components of the primary outcome noted above were centrally reviewed and adjudicated by the Clinical

Endpoints Committee (CEC); the remaining outcomes were identified through medical record abstraction. The CEC also reviewed records for unstable angina cases and urgent coronary revascularization procedures in search of myocardial infarctions that were not identified by the clinical site staff. Details of the outcome definitions are reported in the appendix.

Secondary outcomes were all-cause mortality, dialysis-dependent kidney failure, individual and meaningful combinations of components of the primary outcome, and the number of these that occur.

Statistical Issues

A sample size of 4,000 with an average of 5 years of follow-up was estimated to provide 83% power to detect a 19% treatment effect and 87% power to detect a 20% treatment effect. Additional details on sample size estimates and power calculations are provided elsewhere.[19]

Randomization by permuted block, stratified by clinical site, was performed through the data management system. Since the need for emergency unblinding was expected to be low, unblinding codes were stored securely at the DCC, accessible only to authorized staff.

In addition to having primary and secondary outcomes, we pre-specified primary and secondary analysis strategies. The fundamental analysis plan included using Kaplan-Meier methods and comparing unadjusted treatment effects with proportional hazards models stratified by country, using proportional hazards models to adjust for other variables (age group, race, sex, smoking, systolic blood pressure, diabetes status, LDL, CKD stage based on estimated GFR[23] and country), and performing analogous subgroup analyses by age group, sex, race, diabetes status, and baseline tHcy level.[24] Due to the limited ability of vitamins to normalize elevated tHcy levels in dialysis-dependent kidney failure patients, the primary analysis strategy invoked censoring at 3-months following return to chronic dialysis for the CVD and mortality outcomes. The secondary analysis strategy based on intention-to-treat (ITT) principles was performed for all interim and final analyses. By design, the primary analysis of the secondary outcome of dialysis-dependent kidney failure is ITT. All computations were performed using SAS®, version 9.1 (SAS Institute, Cary, NC).

The Data and Safety Monitoring Board (DSMB) planned two interim efficacy analyses at accrual of 33% and 67% of the expected number of events. To provide flexibility in the number of interim analyses, a Lan-DeMets boundary[25] was used as a stopping rule. Conditional power analyses[26,27] were also planned at these time points.

Results

Participant Characteristics

Participant flow is summarized in Figure 1; baseline data were presented previously.[22] Briefly, 7,273 patients were screened, of whom 2,056 and 2,054 were randomized to high dose and low dose multivitamins, respectively; 34% did not meet eligibility cut points for tHcy and Ccr; and, 9% were not enrolled for other reasons. Baseline characteristics (Table 1)

were well-balanced between treatment groups overall and within country. Approximately 78% of participants had CKD stage 3T or 4T (68% stage 3T, 10% stage 4T), and 20% reported a CVD history at baseline.

Follow-up and Early Termination

Follow-up ranged from 0 to 82 months yielding a mean of 4.0 ± 1.5 years. Complete follow-up through June 24, 2009 was available for 2,788 participants, 493 participants were deceased, 822 had incomplete follow-up, and 7 participants had no follow-up. Slightly more participants in the high dose group withdrew consent during the study (198 high dose, 171 low dose; P=0.16); lack of interest in continuing participation and health-related reasons or side effects were the most frequently cited reasons for withdrawal.

Interim analyses were performed at four points (proportion of total expected events): May 2007 (0.31), April 2008 (0.43, conditional power only), May 2009 (0.60) and June 2009 (0.60). The DSMB considered the fourth interim analysis on June 24, 2009 and recommended an early and orderly closeout of the study since it had “conclusively answered its original hypothesis”. Conditional power{Lan, 1988 #845} at information time 0.60 was 0.19 (80% CI: 0.03 – 0.56) assuming the study design effect for the remainder of the trial and 0.004 (80% CI: 0.000 – 0.052) assuming that the trend observed to date continued for the duration of the study. The sponsor accepted this recommendation and closeout preparations commenced immediately. Final telephone contacts or clinic visits were conducted from June 25, 2009 through January 31, 2010.

Adherence, tHcy-Lowering and Blinding

Based on pill count, 84% of participants took at least 75% of the expected number of study multivitamins; 5% took less than 75% of the expected vitamins, and adherence could not be assessed for 11%. Adherence based on pill count was balanced between the treatment groups and consistent with self-reported adherence. The high dose multivitamin was effective in lowering tHcy. Based on a sample of 143 participants, the mean 4-year change from baseline tHcy was −4.6 μmol/L (SD=4.5, N=72) in the high dose group in comparison to −0.2 (SD=5.1, N=71; P<0.0001) in the low dose group, resulting in 4-year mean tHcy levels (μmol/L) of 11.8 (SD=3.8) and 15.9 (SD=5.5), respectively. Blinding was successful; 49% of participants and 49% of study coordinators provided incorrect guesses for treatment assignment.

CVD, Mortality and Kidney Failure Outcomes

A total of 547 pooled CVD events are included in the primary (censored) analysis. Treatment groups did not differ significantly in occurrence of post-randomization CVD "(269-H, 278-L, P=0.93), all-cause mortality (217-H, 214-L, P=0.67), or dialysis-dependent kidney failure (181-H, 162-L, P=0.19) outcomes. (Table 2) As shown in Figure 2, no trends in event rates over 6 years were suggested for either the primary CVD outcome or all-cause mortality. A trend for more frequent return to dialysis-dependent kidney failure in the high dose group was not statistically significant (P=0.20). Adjustment for country, age group, race, sex, smoking, systolic blood pressure, diabetes status, LDL and CKD stage had little impact on the estimated effects. The hazard ratios (95% confidence interval) were: CVD

primary outcome 1.02 (0.85–1.22), all-cause mortality 0.99 (0.81, 1.22), and dialysis-dependent kidney failure 0.90 (0.71, 1.14).

Analyses of the primary and secondary outcomes by subgroups also failed to demonstrate a statistically significant treatment effect. (Figure 3)

In the primary analyses, 37 CVD outcomes and 62 deaths were censored for occurring more than 3 months after dialysis-dependent kidney failure. Importantly, all intention-to-treat analyses yielded results very similar to the primary analyses with censoring at 3-months post-return to chronic dialysis. The ITT analysis for the pooled primary CVD outcome (290-H, 294-L, P=0.91) was not statistically significant. For all-cause mortality, the ITT analysis included an additional 34 deaths in the high dose group and 28 deaths in the low dose group, and did not appreciably modify the survival curves (P=0.50).

Adverse Events

During follow-up, 62% of the participants were hospitalized at least once (1,272-H, 1,294-L, P=0.46) and accrued a total of 7,996 hospitalizations (3,933-H, 4,063-L). There were no statistically significant differences in discharge diagnosis (based on major ICD-9CM groupings) by treatment group. Disease of the circulatory system was the most prevalent discharge diagnosis grouping (1,934-H, 1,958-L) reported across all hospitalizations. Participant-reported multivitamin side effects also did not differ by treatment group. Among the high dose group, 269 participants reported side effects in comparison to 263 participants receiving the low dose multivitamin (P=0.32). Gastrointestinal disturbance was the side effect most often reported (121-H, 114-L, P=0.69).

Discussion

Among stable kidney transplant recipients with increased levels of homocysteine and reduced kidney function, treatment with a multivitamin containing a high dose of vitamin B6, vitamin B12 and folic acid did not reduce cardiovascular disease compared to a multivitamin with low doses of these ingredients. The high dose multivitamin also did not reduce all-cause mortality or onset of dialysis-dependent kidney failure. The lack of a beneficial effect on these outcomes was observed despite a significant reduction in tHcy in a sample of the high dose group. The frequency of adverse events did not differ by treatment group assignment.

Our findings may have applicability to the broader population of persons with CKD. Over three-fourths of our study participants had estimated GFR <60 ml/min/1.73m^2 (CKD stage 3T or 4T). As is the case for persons with CKD of their native kidneys, the risk of CVD in kidney transplant recipients is increased compared to the general population. Levels of tHcy in the patients with CKD vary according to the level of GFR, ranging from >25 μmol/L in kidney failure treated by dialysis to 10–25 μmol/L for earlier stages of CKD, and higher doses of B vitamins than those provided in cereal grain fortification are required to lower homocysteine levels to the range observed in the upper quartile of the general population. Our findings in kidney transplant recipients are in accord with two clinical trials of patients with CKD stage 5 (eGFR of < 15 ml/min/1.73 m^2 or dialysis),[12] or CKD stages 4 and 5

(eGFR of <30 ml/min/1.73 m^2 or dialysis),[13] and a secondary analysis of HOPE-2 study participants with (primarily) CKD stage 3 (eGFR of 30–59 ml/min/1.73 m^2).[14] Despite sustained ~ 4.0 to 8.5 micromolar reductions in tHcy achieved by these trials comparing active to "placebo" treatment, no decrease in CVD event or mortality rates was observed.[12_14]

The lack of a beneficial effect of homocysteine lowering that we observed in kidney transplant recipients can be added to the largely negative findings of a substantial number of clinical trials in a wide range of patient populations.[28_35] Those studies effectively rule out the possibility of large effects on risk of coronary heart disease, stroke, and all-cause mortality as the pooled risk ratio for major cardiovascular disease events in those studies was 1.02 (95% CI, 0.98–1.06).[10] However, our trial shares a few limitations with these studies. Although the high dose multivitamin significantly reduced tHcy levels, mean values remained somewhat elevated. Importantly, the B-vitamin pathway for reducing tHcy may not be the optimal one for reducing CVD risk. Also, the duration of follow-up may not have been sufficient to identify a lagged impact on modification of CVD risk.

The lack of deleterious effects of homocysteine-lowering treatment on any of the major primary or secondary outcomes studied in FAVORIT is also concordant with results from trials of CKD and non-CKD populations.[10_14] In particular, previously reported trials of CKD patients,[12] found no evidence of increased overall mortality, fatal or non-fatal cancer incidence, or progression to dialysis-dependent kidney failure associated with any of the high dose folic acid-based homocysteine-lowering regimens studied, relative to placebo treatment. Moreover, the absence of an increased hazard ratio for dialysis-dependent kidney failure in the high-dose group, despite 343 total events, is important given limited findings of a higher rate of decline in renal function observed among participants on B-vitamin therapy in the much smaller Diabetic Intervention with Vitamins to Improve Nephropathy trial.[36]

Why have clinical trials of homocysteine–lowering uniformly failed to show a beneficial effect on CVD despite strong evidence for a beneficial effect seen in patients with marked hyperhomocysteinemia due to cystathionine beta synthetase (CBS) deficiency, whose risk for CVD is reduced by 90% with treatment,[4_5] and in view of the association of elevated tHcy levels with CVD seen in epidemiological studies?[37_42] It is likely that this is related to the marked difference in tHcy levels seen in patients with CBS deficiency, whose levels even after treatment remain above 100 μmol/L,[5] in comparison with the levels more typically in the ranges of 12 – 25 μmol/L seen among patients in the observational studies. At these lower levels, it appears that the tHcy levels are a surrogate for other factors associated with increased CVD risk rather than a directly causative factor.

In conclusion, treatment of stable kidney transplant recipients with a multivitamin containing a high dose folic acid, B6, and B12 lowers tHcy levels relative to standard multivitamin supplementation and in many cases to normal levels, but does not reduce CVD outcomes, or total mortality in this patient population. Our findings add to the growing body of evidence from clinical trials of the failure of homocysteine lowering to reduce CVD, stroke and all-cause mortality in a wide range of patient populations.

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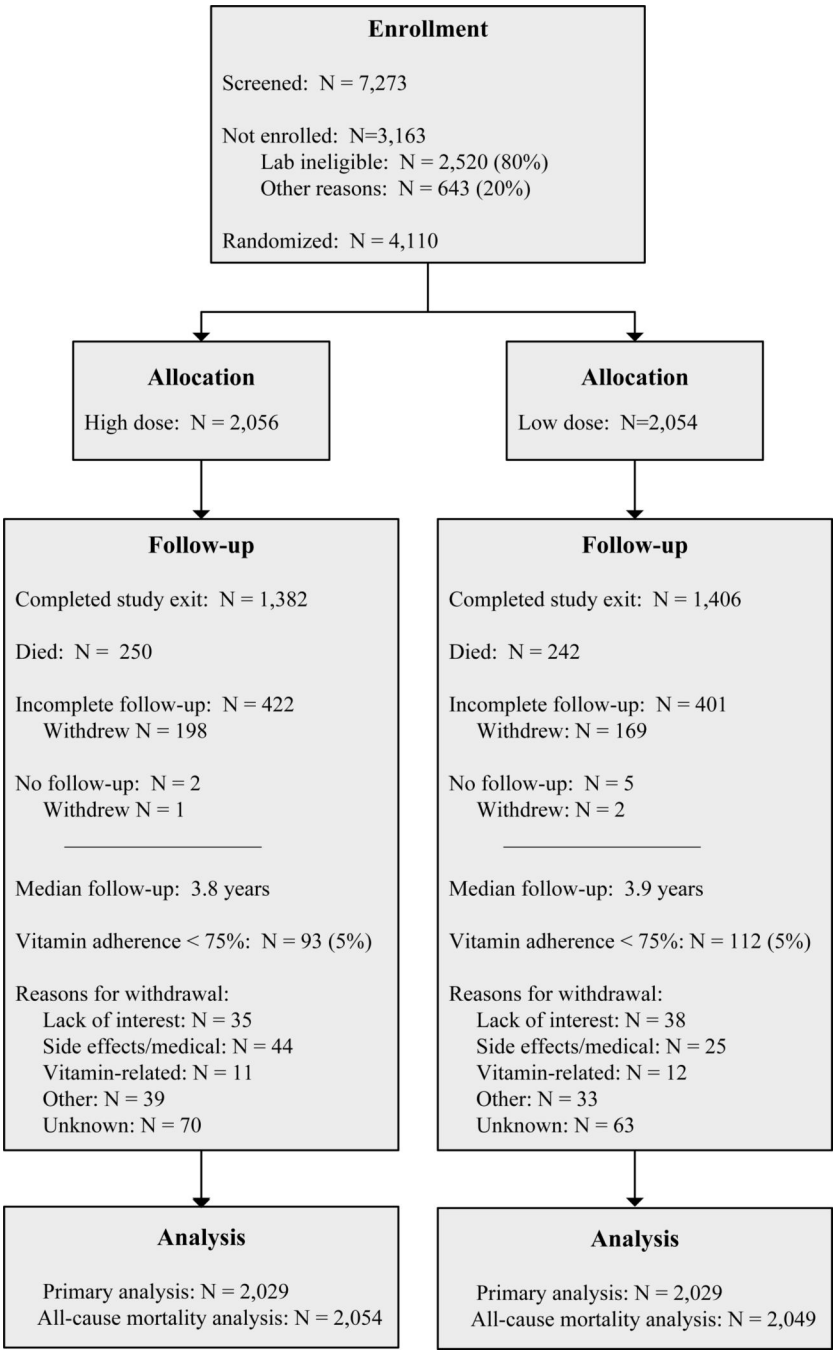

Figure 1.
Enrollment, follow-up and analysis diagram

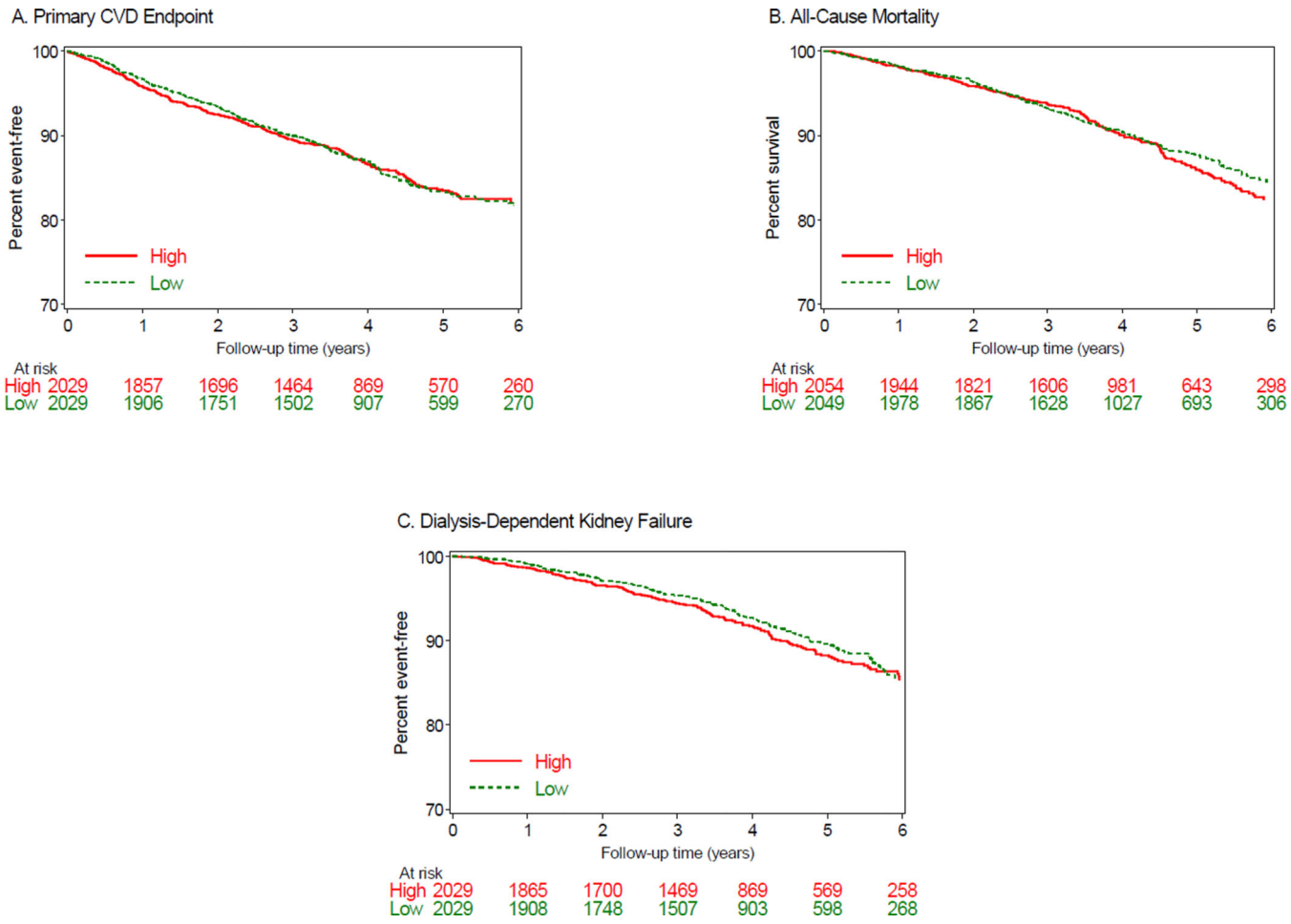

Figure 2.

Kaplan-Meier analyses for (A) Primary CVD, (B) All-cause mortality, and (C) Dialysis-dependent kidney failure outcomes

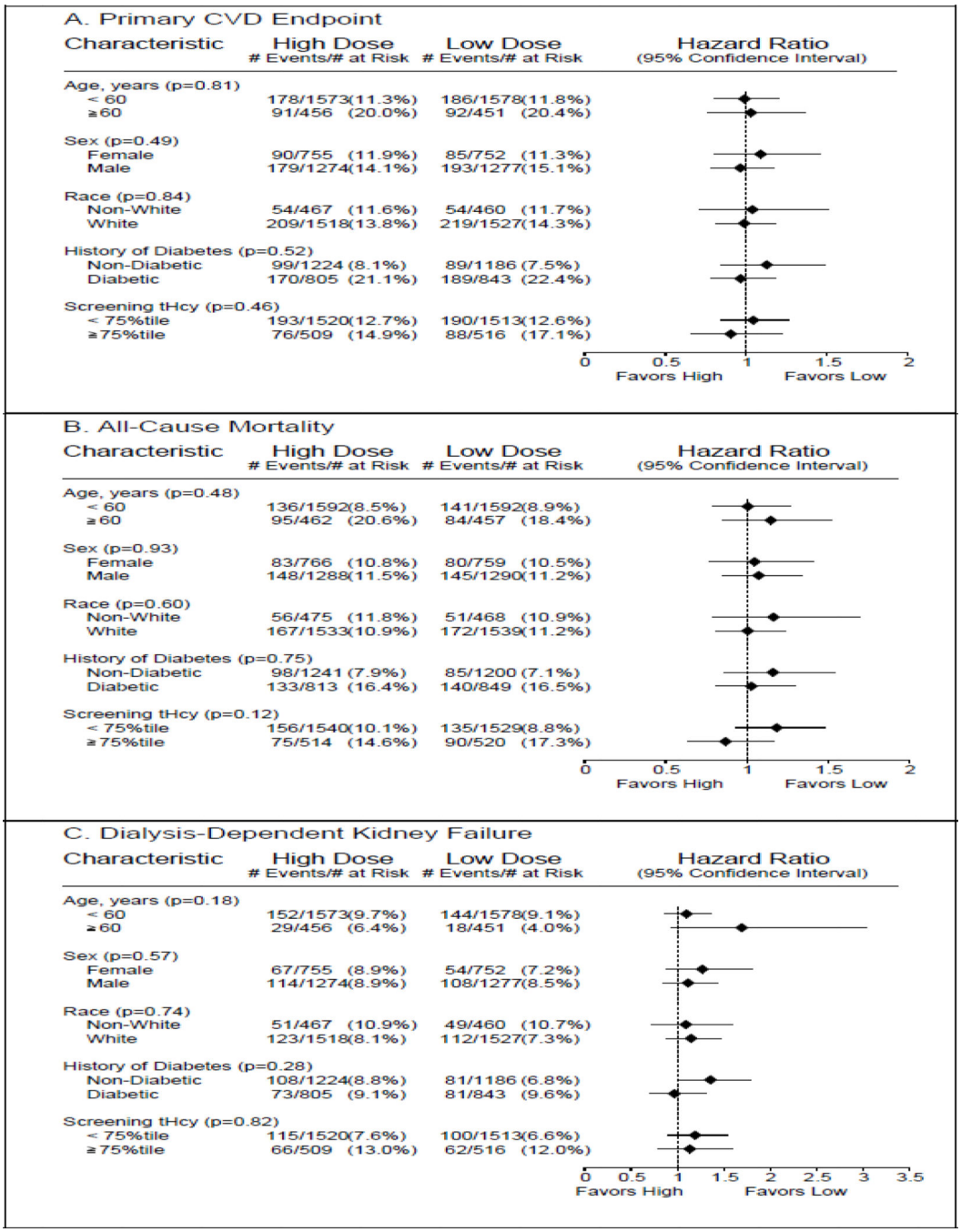

Figure 3.

Hazard ratios for treatment group comparisons from primary and secondary outcome subgroup analyses

Table 1

Baseline characteristics of study participants

Characteristics	Overall (N=4110)	High Dose (N=2056)	Low Dose (N=2054)
Age --- yr	52 ± 9.4	52 ± 9.4	52 ± 9.5
Female sex --- no. (%)	1528 (37.2)	767 (37.3)	761 (37.0)
Non-white race --- no. (%)	945 (23.5)	477 (23.7)	468 (23.3)
Location --- no. (%)			
Brazil	612 (14.9)	307 (14.9)	305 (14.8)
Canada	498 (12.1)	249 (12.1)	249 (12.1)
United States	3000 (73.0)	1500 (73.0)	1500 (73.0)
Graft Vintage --- yr	5 ± 5.0	6 ± 5.1	5 ± 5.0
History of CVD --- no. (%)	820 (20.0)	406 (19.8)	414 (20.3)
History of diabetes --- no. (%)	1663 (40.5)	813 (39.6)	850 (41.5)
Prevalent hypertension --- no. (%)	3778 (92.0)	1879 (91.5)	1899 (92.5)
Body Mass Index --- kg/m^2	29 ± 6.2	29 ± 6.2	29 ± 6.3
Current smoker --- no. (%)	451 (11.1)	230 (11.3)	221 (10.9)
Total Cholesterol --- mmol/L	4.8 ± 1.1	4.8 ± 1.2	4.8 ± 1.1
High-density lipoprotein cholesterol --- mmol/L	1.2 ± 0.4	1.2 ± 0.4	1.2 ± 0.4
Calculated or direct low-density lipoprotein cholesterol --- mmol/L	2.6 ± 0.9	2.6 ± 0.9	2.6 ± 0.9
Triglycerides --- mmol/L	2.2 ± 2.1	2.3 ± 2.5	2.2 ± 1.6
Screening homocysteine --- µmol/L	16.4 ± 1.3	16.4 ± 1.3	16.4 ± 1.3
Female	16.8 ± 1.3	17.0 ± 1.3	16.7 ± 1.3
Male	15.6 ± 1.3	15.3 ± 1.3	15.8 ± 1.3
Screening creatinine, mol/L	144.3 ± 42.1	145.0 ± 42.5	143.6 ± 41.6
Screening (eGFR, mL/min per 1.73 m^2) *	48.8 ± 16.2	48.5 ± 15.9	49.0 ± 16.5
CKD Stage, n (%) *			
Stage 1T (eGFR 90 mL/min per 1.73 m^2)	69 (1.7)	28 (1.4)	41 (2.0)
Stage 2T (eGFR 60–89 mL/min per 1.73 m^2)	819 (20.4)	405 (20.1)	414 (20.6)
Stage 3T (eGFR 30–59 mL/min per 1.73 m^2)	2738 (68.1)	1380 (68.7)	1358 (67.5)
Stage 4T (eGFR 15–29 mL/min per 1.73 m^2)	394 (9.8)	197 (9.8)	197 (9.8)
Stage 5T (eGFR 15 mL/min per 1.73 m^2)	1 (0.0)	0 (0.0)	1 (0.0)

* Based on CKD-EPI eGFR formula{Levey, 2009 #1533}

Table 2

Primary and secondary outcomes

	Censored at 3 Months After Return to Dialysis				Intention-to-Treat			
	High Dose	Low Dose	Hazard Ratio (95% CI)	p-value*	High Dose	Low Dose	Hazard Ratio (95% CI)	p-value*
Any Primary CVD Outcome	269	278	0.99 (0.84, 1.17)	0.93	290	294	1.01 (0.86, 1.19)	0.91
Component Event								
Fatal/Nonfatal MI	90	86	1.08 (0.80, 1.45)	0.61	96	94	1.05 (0.79, 1.40)	0.73
Fatal/Nonfatal Stroke	35	32	1.12 (0.69, 1.81)	0.64	38	35	1.11 (0.70, 1.75)	0.67
RSD	7	9	0.80 (0.30, 2.15)	0.66	8	10	0.82 (0.32, 2.08)	0.67
CVD death	75	91	0.84 (0.62, 1.15)	0.28	91	100	0.93 (0.70, 1.24)	0.63
Coronary Artery Revascularization	111	120	0.95 (0.73, 1.23)	0.70	116	124	0.96 (0.74, 1.24)	0.75
Lower Extremity PAD **	59	53	1.14 (0.79, 1.65)	0.49	63	54	1.19 (0.83, 1.72)	0.34
Carotid Endarterectomy or Angioplasty	10	9	1.14 (0.46, 2.80)	0.78	10	9	1.14 (0.46, 2.79)	0.78
Abdominal Aortic Aneurysm Repair	3	5	0.61 (0.15, 2.57)	0.50	3	5	0.61 (0.15, 2.57)	0.50
Renal Artery Revascularization	9	7	1.30 (0.48, 3.50)	0.60	9	7	1.30 (0.48, 3.49)	0.60
All-cause mortality	217	214	1.04 (0.86, 1.26)	0.67	251	242	1.06 (0.89, 1.27)	0.50
Dialysis-Dependent Kidney Failure	NA	NA	NA	NA	181	162	1.15 (0.93, 1.43)	0.19

*p-value was calculated with stratified proportional hazards models

+Lower extremity PAD includes lower extremity revascularization or amputation above the ankle for severe arterial disease.