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COMIRB Protocol

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Project Title: Phosphate Lowering to Treat Vascular Dysfunction in Chronic Kidney Disease
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Version Date: 08/14/2015

I. Hypotheses and Specific Aims:

Risk of cardiovascular diseases (CVD) is significantly elevated in patients with chronic kidney disease (CKD); however, the increased cardiovascular risk in this patient population is only partially explained by traditional cardiovascular risk factors [1-6]. In earlier stages of CKD, serum phosphorus levels are maintained within the normal range by up regulating fibroblast growth factor 23 (FGF23) and a consequent increase in the fractional excretion of phosphorus by the kidney [7-10]. However, elevated serum phosphorus **even within the normal range**, as is the typical case in CKD patients not requiring chronic hemodialysis, has emerged within the last decade as a novel, independent predictor of CVD risk and associated mortality [9, 11, 12].

CKD patients also exhibit vascular endothelial dysfunction and increased arterial stiffness [13, 14], two independent predictors of future CVD. A modest elevation in serum phosphorus contributes to this phenotype of vascular dysfunction [15-18]. Recent evidence supports that an acute increase in serum phosphorus impairs endothelium-dependent dilation (EDD) and increases vascular oxidative stress [15]. In addition, both vascular endothelial dysfunction and increased oxidative stress have been identified as important predictors of mortality and cardiovascular events in patients with CKD [19, 20]. Thus, reducing serum phosphorus burden in CKD patients not requiring chronic hemodialysis may reduce cardiovascular events by improving vascular endothelial function and reducing large elastic artery stiffness. However, it is currently **unknown** if reducing serum phosphorus to a low normal laboratory range (<2.8 mg/dL) with oral phosphate binders enhances vascular function in this population.

Our **primary goal** is to establish the efficacy of phosphate binding with lanthanum carbonate, a non-absorbed, non-calcium-containing phosphate binder, for treating vascular endothelial dysfunction and large elastic artery stiffness in patients with stage IIIb or IV CKD (estimated glomerular filtration rate (eGFR) 15-45 mL/min/1.73m^2) with baseline serum phosphorus of 2.8-5.5 mg/dL. A key **secondary goal** is to determine the integrative physiological mechanisms underlying the beneficial effects of phosphate binding with lanthanum.

Hypothesis 1: 3 months of treatment with lanthanum (starting dose of 1500-3,000 mg/day according to baseline serum phosphorus; up-titrated monthly as needed based on serum phosphorus) vs. placebo will improve endothelium-dependent dilation (EDD) and reduce large elastic artery stiffness in patients with CKD.

Specific Aim 1: To determine conduit artery (brachial artery flow-mediated dilation; FMD_{BA}) EDD and large elastic artery stiffness (aortic pulse-wave velocity; aPWV) before and after 3 months of lanthanum or placebo.

Hypothesis 2: The improvements in EDD and arterial stiffness in the lanthanum group will be associated with reduced oxidative stress.

Specific Aim 2: To measure FMD_{BA} and aPWV during normal vs. inhibited oxidative stress, under the conditions described in Specific Aim 1; to measure circulating and endothelial cell markers of oxidative stress.

II. Background and Significance:

Chronic Kidney Disease and Cardiovascular Diseases. Chronic kidney disease (CKD) is a major public health concern that has reached epidemic proportions, affecting ~20 million individuals (~13.1% of the population) in the United States alone. Patients with chronic kidney disease (CKD) are more likely to die of cardiovascular diseases (CVD) than to progress to end stage renal disease [9, 21-23], and the risk of cardiovascular mortality or a cardiovascular event is significantly increased compared to the general population

[24-26]. While patients with CKD exhibit a high presence of traditional CVD risk factors, they can only partially explain the increased incidence of CVD in this population [27]. Therefore, it is imperative to identify interventions targeting non-traditional cardiovascular risk factors to reduce CVD incidence in patients with CKD.

Vascular Function and CKD. As much as 80% of all CVD are associated with dysfunction and disorders of arteries [28].Two of the greatest contributors are the development of vascular endothelial dysfunction, most commonly assessed as impaired endothelium-dependent dilation (EDD), and stiffening of the large elastic arteries (aorta and carotid arteries) [29]. Patients with CKD demonstrate impaired EDD, as assessed by brachial artery flow-mediated dilation (**FMD_{BA}**)[30-32], as well as large elastic artery stiffening, as indicated by increased aortic pulse wave velocity (**aPWV**)[13, 33, 34]. Importantly, both measures are independent predictors of future cardiovascular events and mortality [35-38].

Integrative Physiological Mechanisms of CKD and Vascular Function. A key mechanism contributing to impaired EDD, even in earlier stages of CKD, is increased oxidative stress [39-41], defined as excessive bioavailability of reactive oxygen species (ROS) relative to antioxidant defenses. Superoxide, a major ROS in arteries, reacts with nitric oxide (NO) to form another ROS, peroxynitrite. Peroxynitrite, in turn, decreases the bioactivity of tetrahydrobiopterin (BH_4), the essential cofactor for NO synthesis by endothelial NO synthase (eNOS) [42, 43]. Together, the formation of peroxynitrite and reduced BH_4 decrease NO bioavailability [44, 45]. This reduction in NO is a key physiological mechanism in the CKD-associated impairment in EDD [46, 47]. Reduced NO bioavailability is a common mechanism contributing to both impaired EDD and increased arterial stiffness.

Phosphate, CKD and Vascular Function. It is widely accepted that phosphorus is maintained within the normal range (2.5-4.5 mg/dL) as CKD develops by a compensatory up-regulation of fibroblast growth factor 23 (FGF23) that increases fractional excretion of phosphorus, thus controlling serum phosphorus to avoid the potentially deleterious consequences of hyperphosphatemia on the vasculature [7-10]. However, elevated serum phosphorus **even within the normal range**, as is the typical case in early stage CKD, has emerged within the last decade as a novel, independent predictor of CVD risk and associated mortality [9, 11, 12]. Recent evidence suggests that the increase in CVD risk may be modulated by the adverse influence of phosphorus upon vascular function [15, 48].

Phosphate and EDD. Impaired EDD is one component of vascular dysfunction that may be modulated by elevations in serum phosphorus. Following an acute high phosphate meal (1,200 mg), FMD_{BA} is impaired in young adults. This occurs despite the fact that the elevation in serum phosphorus is only slightly above the normal range (4.56 mg/dL) [15]. Increased serum phosphorus also impairs EDD in both healthy [15] and CKD rodent models [16]. In CKD patients with baseline hyperphosphatemia (>5.5 mg/dL), lowering serum phosphorus with the phosphate binder sevelamer improves FMD_{BA} compared to calcium acetate[49, 50]. However, the efficacy of phosphorus lowering prior to CKD patients becoming overtly hyperphosphatemic, as well as the physiological mechanisms contributing to any functional improvements with phosphorus lowering, are currently unknown.

Phosphate and Arterial Stiffness. The available evidence suggests that serum phosphorus may also increase arterial stiffness. Modest increases in serum phosphorus in healthy adults and patients with moderate CKD are independently associated with an increased ankle-brachial index (an indirect measure of arterial stiffness), even when serum phosphorus is within the normal range [17].However, it is unknown if lowering serum phosphorus in CKD patients reduces arterial stiffness, as well as the mechanisms involved.

Integrative Physiological Mechanisms of CKD and Phosphate on Vascular Function. No direct evidence is available in humans as to the physiological mechanisms mediating any improvements in vascular function with phosphorus lowering. However, work in endothelial cell culture supports a role of oxidative stress and NO bioavailability, as administration of high phosphate increases production of superoxide [15, 51, 52] and reduces NO bioavailability [15, 51, 53] and eNOS activation/expression [15, 53]. In rodents, modest reductions in dietary phosphate decreases oxidative stress [54, 55]. Thus, reduced oxidative stress, which in turn increases NO bioavailability, likely contributes to any improvements in vascular function in CKD patients following phosphorus lowering.

III. Preliminary Studies/Progress Report:

We have demonstrated that there is a longitudinal relation between higher serum phosphorus and all-cause mortality and cardiovascular events (myocardial infarction, stroke and peripheral vascular disease), and a cross-sectional association between elevated serum phosphorus and worsening pulse pressure (PP). These data were obtained from the Homocysteine Study in Advanced Kidney Disease (HOST), which was a prospective, randomized, double-blind study on the effects of folate, vitamin B_6, and vitamin B_{12} replacement on all-cause mortality and cardiovascular events in patients with severe CKD and elevated homocysteine [56]. As the intervention did not have an effect on the proposed outcomes, it represented a unique opportunity to convert the data obtained from a randomized control trial into an observational study. The HOST dataset contains detailed information on demographics, cardiovascular risk factors, prevalent CVD, circulating markers (including serum calcium, phosphorus and intact PTH) and usage of cardioprotective medications in all study participants. In addition, an endpoints committee adjudicated all endpoints. For the purpose of this analysis, we included 1,099 HOST participants. The mean (±SD) age of the participants was 69±11 years, 98% were male, and 26% were black. The mean estimated Modified Diet Renal Disease (MDRD)-GFR was 18±6 mL/min/1.73 m^2 and most participants (n=718; 65%) had an estimated MDRD-GFR of 15-29 mL/min/1.73 m^2. The mean serum phosphorus concentration was 4.3±1.3 mg/dL; 36% and 12% had a serum phosphorus concentration greater than 4.5 and 5.5 mg/dL, respectively. **These descriptive data support that most of the patients that will be screened for the proposed study will have serum phosphorus < 5.5 mg/dL**.

***Higher serum phosphorus levels are independently associated with CVD events and mortality in patients with advanced CKD*:** During a median follow-up of 2.9 years, 472 (42.9%) patients died from any cause and 237 (21.6%) had a cardiovascular event. Higher serum phosphorus levels were positively associated with higher risks for composite of death and cardiovascular events. Identical results were observed when cardiovascular events were examined separately (not shown). Compared to the lowest quartile, the 2 highest quartiles of serum phosphorus were associated with a significantly elevated risk for the composite of death and cardiovascular events, after multivariate adjustment (Table 1; hazards ratios (HR)). The analysis was repeated using serum phosphorus levels as a continuous variable instead of quartiles. After multivariable adjustment (Model 3), the HR of the composite endpoint of death and cardiovascular events increased by 1.17 (95% CI; 1.02-1.33; p=0.02) per 1 mg/dL increase in serum phosphorus. These results provide important support to our aim to evaluate the efficacy of phosphorus lowering on vascular endothelial function and arterial stiffness (two independent predictors of CV events and mortality) in patients with advanced CKD.

Table 1. Association of Higher Serum Phosphorus with Risk of Cardiovascular Events & Death in the HOST Study

Phosphorus Concentrations	Q1 (≤3.5 mg/dL)	Q2 (3.6-4.1 mg/dL)	Q3 (4.2-4.8 mg/dL)	Q4 (>4.9 mg/dL)
Model 1	1.00	1.35(0.87- 2.09)	1.70 (1.12- 2.57)	1.93(1.29- 2.90)
Model 2	1.00	1.36 (0.88- 2.11)	1.79(1.18-2.73)	2.18(1.44-3.31)
Model 3	1.00	1.19 (0.76-1.86)	1.50 (1.01-2.31)	1.68 (1.05-2.68)

Model 1: Unadjusted; **Model 2:** Adjusted for age, gender and race; **Model 3:** Adjusted for covariates in Model 2 plus smoking, diabetes, hypertension, prevalent CVD, body mass index, systolic and diastolic blood pressure, treatment arm, eGFR, albumin, calcium, 25-hydroxyvitamin D, intact PTH, LDL-cholesterol, high density lipoprotein-cholesterol, triglycerides and use of angiotensin converting enzyme inhibitors, angiotensin receptor blockers and β-blockers.

In addition, in a cross-sectional analysis of the relation between serum phosphorus and PP in the HOST cohort, higher serum phosphorus levels are independently associated with a wider PP in patients with advanced CKD. The mean (±SD) PP in this population was 71 (20) mmHg. In adjusted linear regression models (Model 3), each 1 mg/dL increase in serum phosphorus was associated with a 2 mmHg increase in PP (β=2.0±6.9; p=0.005). These data support the notion that serum phosphorus lowering should reduce large elastic artery stiffness, as increased PP is an expression of arterial stiffness.

IV. Research Methods

A. Outcome Measure(s): A prospective, randomized, placebo-controlled double-blind trial of 60 patients will be performed to assess the effects of the phosphate binder lanthanum in stage IIIb and stage IV chronic kidney disease (CKD) patients with normal or modestly elevated serum phosphorus (2.8-5.5 mg/dL) on vascular endothelial function and arterial stiffness. Vascular endothelial function will be assessed as endothelium-dependent dilation via brachial artery flow-mediated dilation (FMD_{BA}). Arterial stiffness will be assessed as aortic pulse-wave velocity (aPWV) using applanation tonometry. The primary outcomes are the differences

between the active and placebo groups in change in FMD_{BA} and aPWV at week 12 compared to baseline. Secondary aims will determine whether phosphorus lowering improves vascular function through reduced oxidative stress and/or inflammation by assessing pre-/post- differences between groups for FMD_{BA} and aPWV measured during normal vs. inhibited oxidative stress and by measuring circulating and endothelial cell markers of oxidative stress and inflammation. Additional blood chemistries and vital signs will be monitored throughout the study for subject safety. All testing will take place it the laboratory in the University Physicians Incorporated (UPI) Building at the University of Colorado Anschutz Medical Campus.

B. Description of Population to be Enrolled: Study Design and Research Methods

Subjects. After obtaining their written informed consent, men and post-menopausal women aged 40-79 yrs with stage IIIb and stage IV CKD (eGFR 15-45 mL/min/1.73m^2) and normal or modestly elevated serum phosphorus (2.8-5.5 mg/dL) will serve as subjects. Patients will undergo screening for eGFR and phosphorus at the UPI Building to determine study eligibility. Estimated GFR will be calculated using the 4-variable MDRD prediction equation [57]. Major inclusion/exclusion criteria are presented below:

Inclusion Criteria:

1) Aged 40-79 yrs; women must be post-menopausal (serum phosphorus increases after menopause in women [58])
2) CKD stage IIIb or IV (eGFR with the 4-variable MDRD prediction equation: 15-45 mL/min/1.73m^2; stable renal function in the past 3 months)
3) Baseline serum phosphorus 2.8-5.5 mg/dL (stable in the past 3 months); not taking phosphate binders
4) Serum albumin concentration >3.0 g/dL
5) Mini-mental state examination (MMSE) score of $\geq$ 24 (cognitive function screening); ability to provide informed consent

Exclusion Criteria:

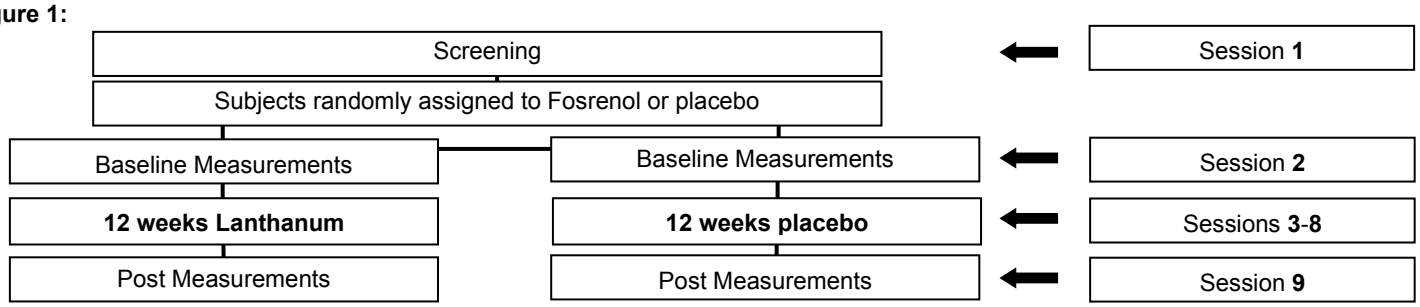
1) Patients with advanced CKD requiring chronic dialysis
2) Patients at high risk for gastrointestinal events or alterations in gastrointestinal anatomy(e.g., history of gastrointestinal surgery, colon cancer), hypomotility disorders (e.g., constipation, ileus), current bowel obstruction, or fecal impaction
3) Significant co-morbid conditions that lead the investigator to conclude that life expectancy is less than1 year
4) Expected to undergo living related transplant in next 6 months
5) History of severe congestive heart failure (i.e., EF < 35%)
6) History severe liver disease
7) Hospitalization in the past 3 months
8) BMI $\geq$40 kg/m^2 (FMD measurements can be inaccurate in severely obese patients)
9) Taking medication(s) that interact with agents administered during experimental sessions (e.g., sildenafil interacts with nitroglycerin)
10) Active vitamin D analogue use (i.e., calcitriol, paricalcitol, doxercalciferol)
11) Alcohol dependence or abuse
12) Current tobacco abuse
13) Use of prednisone and other immune suppressant medications

C. Study Design and Research Methods

Experimental Design (Figure 1). A 12-week randomized, placebo-controlled, double-blind design study with lanthanum (starting dose of 1,500-3,000 mg/day according to baseline serum phosphorus; up-titrated monthly as needed based on serum phosphorus) will be conducted. After providing informed consent, subjects will undergo screening prior to study start. Subjects who meet inclusion criteria will have baseline measurements performed as described below. Measurements will be made under supine, overnight fasted (water only) conditions. Patients will then be randomly assigned to receive either lanthanum or placebo (see dosing below). Allocation to the active drug versus placebo group will be random, and a stratification process will be used on the basis of baseline FMD_{BA} of <4% and ≥4% to protect against a baseline imbalance that would not

be well handled by other statistical procedures. With counseling and adherence monitoring from a nurse, subjects will maintain their baseline diet throughout the study, including intake of phosphate of <1,000 mg/day. Measurements will then be repeated after 12 weeks. The investigators involved in the acquisition and analysis of key outcomes will be blinded to the treatment assignment of the subjects as well as serum phosphorus levels.

Figure 1:



Subjects will come to the lab at the UPI Building on 9 separate occasions:

- **Session 1:**Screening measurements, complete 3 day diet record
- **Session 2:** Baseline measurements
  - Resting arterial blood pressure and blood sampling of circulating markers (see below)
  - Venous endothelial cell collection (for subsequent protein measurements)
  - FMD_{BA} (endothelium-dependent dilation) and aortic PWV (arterial stiffness), with and without acute inhibition of oxidative stress via systemic i.v. infusion of ascorbic acid [vitamin C])
  - Endothelium-independent dilation (EID) to sublingual nitroglycerin
  - Begin study treatment (lanthanum or placebo)
- **Sessions 3-8:** Weekly/monthly check-in (*see below*)
- **Session 9:** Identical to Session 2 (note: subjects will not take a dose the a.m. prior to testing session)
  - **Subjects will also complete a second 3 day diet record**

Lanthanum dosing. The starting dose of lanthanum will be determined from a sliding scale according to baseline serum phosphorus (Table 2). During the first 4 weeks, the dose of the phosphate binder will be titrated weekly to achieve serum phosphorus less than 2.8 mg/dL (during this period serum phosphorus will be measured weekly). After the initial 4 weeks, the dose of lanthanum will be titrated every four weeks until the end of the study to achieve serum phosphorus in the aforementioned target. This dose will be up-titrated as needed based on serum phosphorus (if not <2.8 mg/dL, dose will be increased by 1 tablet [500 mg] for each meal). If serum phosphorus decreases to <2.5 mg/dL, the dose of lanthanum will be down-titrated. Tablets will be taken with each meal (if a subject consumes <3 meals/day, the dosing will be adjusted accordingly).

Table 2:

Serum Phosphorus (mg/dL)	Starting Dose (mg)
2.8-4.0	1,500 (1 tablet with each meal)
4.0-4.5	3,000(2 tablets with each meal)
4.5-5.5	4,500(3 tablets with each meal)

Rationale of using serum phosphorus for up-titration of lanthanum:

(1) Although high FGF23 levels have also been associated with death and cardiovascular events in CKD, it is presently unclear what cutoff should be used as a treatment target.
(2) Normalization of FGF23 with use of phosphate binders is not plausible, as factors other than serum phosphorus may be involved in the regulation of FGF23.
(3) A compelling idea would be to up-titrate the lanthanum dose to lower phosphorus. Although we considered this option, we preferred the up-titration on the basis of serum phosphorus as the epidemiological data on the relation between elevated phosphorus and outcomes is significantly stronger than for changes in phosphorus. To date, a relation between $FePO_4$ and outcomes in patients with CKD has not been demonstrated[59].

(4) Finally, phosphorus and FGF23 will be followed closely in the study to obtain better understanding of how phosphate binding affects these important markers of phosphate homeostasis.

Those who drop-out/do not complete the study will undergo an end-of study visit which will include identical measurements to the ones planned for session 9. For those completing the entire study, total participation is approximately 13 hours spread over 9 visits (approximately 1 week for screening + 12 weeks the research study).

Power. An effect size of 1.2 was estimated from published data assessing the effects of sevelamer on vascular function in CKD patients with hyperphosphatemia [49, 50]. A sample size of 10 subjects/ group was calculated based on this effect size, 90% power and an alpha level of 0.05. However, to ensure that there is adequate power, as patients without overt hyperphosphatemia may not respond as strongly to the intervention, studies will be completed on **20 subjects/group**. Assuming a **dropout** rate of ~30% (**10 subjects/group**) based on our experience with similar studies, I will plan to enroll up to **~30 subjects/group** (**~60 total subjects total)**.

Study Sites

Patients for the study will be recruited from the Nephrology Clinic at the UC-Denver Anschutz Medical Campus and from the Denver VA Medical Center Renal Clinic with access to ≥950 CKD patients from all over the Denver/Boulder metro area (at least 33% with ICD 9 code 585.3 and 585.4 as diagnosis) with over 2146 visits in the past year.

Based on the preliminary screening of our database at the University of Colorado Hospital Renal Clinic, we expect at least 100 eligible patients per year. The screening evaluation, all follow-up patient visits, study measurements, and blood draws will take place at the UPI Building.

Randomization and Treatment

Once inclusion/exclusion criteria have been met and informed consent obtained patients will undergo baseline measurement of primary and secondary (exploratory) outcomes. Once baseline measurements are obtained, patients will be randomized to either lanthanum or placebo. Randomization will occur within 4 weeks of the screening evaluation and will be performed by Dr. Eugene Nuccio, a statistician, by blocks using a computer-generated procedure to ensure equal distribution. The lanthanum and placebo will be prepared and provided by Shire and will be identical. There will be no cost to the PI for the study drug and placebo

a) ***Study Drug*****:** Lanthanum carbonate (Fosrenol) is an FDA approved non-absorbed, non-calcium-containing phosphate binder to lower serum phosphate in patients requiring chronic dialysis.

b) ***Rationale for lanthanum dose:*** This dosing is indicated in patients with end-stage renal disease requiring chronic dialysis. The population being studied in this protocol will be healthier than this patient group. In addition, an IND has been obtained from the FDA.

c) ***Blinding:*** The investigators and patients will remain blind to the study intervention, as the lanthanum and placebo will be identical. A nurse at the UPI Building lab will be responsible for administering the pills, which will be stored in a locked cabinet on site. A professional research assistant will be unblinded and will manage the study drug records and interact directly with the nursing staff for administration of the study drug. The investigators will be blinded to laboratory results, and the research assistant will monitor laboratory results for safety and inform the investigator if necessary.

d) ***Drug accountability****:* Pill counts will be assessed weekly during the 1st month and then monthly to document subject adherence.

e) ***Withdrawal from study:*** Withdrawal will be done at the patient's request, IRB request, or if there is a medical rationale for discontinuation as determined by the principal investigator.

Safety monitoring

The patients will be instructed to call the PI or research coordinator with any subjective complaints, in addition, abnormal labs will be reported to the PI immediately, who will determine if the study drug needs to be discontinued. The following are reported adverse events of the study drugs and the monitoring plan:

a) Lanthanum. The safety profile of lanthanum has been studied in over 5200 subjects in completed clinical trials. The most common adverse reactions for lanthanum are gastrointestinal events, such as nausea, vomiting, and abdominal pain and they generally abate over time with continued dosing. In end-stage renal disease patients receiving 4-6 weeks of treatment with lanthanum the incidence of nausea was 11% (vs. 5% placebo), vomiting 9% (vs. 4%) and abdominal pain 5% (vs. 0%). Lanthanum may reduce calcium absorption and result in hypocalcemia (~5%, same incidence as active comparator group). The following additional adverse reactions have been identified in during post-approval use of lanthanum: constipation, dyspepsia, allergic skin reactions, hypophosphatemia, and tooth injury (incidence not known). Lanthanum may decrease bioavailability of cationic antacids, quinolone antibiotics or levothyroxine.

There have been reports of serious cases of gastrointestinal obstruction, ileus, and fecal impaction reported in association with lanthanum, some requiring surgery or hospitalization. Risk factors for gastrointestinal obstruction identified from post-marketing reports include alteration in gastrointestinal anatomy (e.g., history of gastrointestinal surgery, colon cancer), hypomotility disorders (e.g., constipation, ileus, diabetes) and concomitant medications (e.g., calcium channel blockers). Some cases were reported in patients with no history of gastrointestinal disease.

Hypophosphatemia has been reported only in post-marketing surveillance. The risk of hypophosphatemia is very low. Clinically significant (causing symptoms) hypophosphatemia associated with the use of lanthanum carbonate has not been reported.

Safety measures will include: lanthanum dose titration and close monitoring of serum phosphate (as described above) to avoid severe hypophosphatemia, excluding patients at high risk for gastrointestinal events or current bowel obstruction, ileus and fecal impaction, repeatedly emphasizing to patients the importance of chewing tablets completely to reduce the risk of serious adverse gastrointestinal events such as those described above. Patients will also be instructed to take concomitant drugs at least one hour before or four hours after lanthanum to avoid potential interactions, and blood levels of concomitant drugs with a narrow therapeutic range will be monitored if deemed appropriate by Dr. Chonchol.

Patient Stopping Criteria:

- Withdrawal of informed consent (subject's decision to withdraw for any reason)
- Any clinical adverse event (AE), laboratory abnormality or intercurrent illness which, in the opinion of the investigator, indicates that continued participation in the study is not in the best interest of the subject
- Loss of ability to freely provide consent through imprisonment or involuntary incarceration for treatment of either a psychiatric or physical (e.g., infectious disease) illness
- Inability to comply with protocol
- Use of an investigational therapy or device other than study medication
- Significant non-compliance with protocol (ie, procedures, assessments, medication, etc.)
- Hypocalcemia that does not resolve with decreased dosage of study drug
- Hypophosphatemia (<2.5 mg/dL) that does not resolve with decreased dosage of study drug
- Hospitalization for gastrointestinal obstruction, ileus, and fecal impaction
- Serious acute hypersensitivity reactions to investigational drug
- Any major cardiovascular event such as myocardial infarction, stroke, unstable angina, thromboembolic event, or a clinically significant deterioration of a baseline cardiovascular condition.

Study Stopping Criteria:

The study may be stopped if:

- The data show a significantly increased risk of serious adverse effects in one of the treatment groups.
- Interim efficacy analyses show significant treatment benefits or futility in the intervention group. The interim efficacy analyses are based on pre-specified stopping boundaries for the primary endpoint of the study which preserve the study wide Type I error rate.

- It becomes clear that successful completion of the study is not feasible (e.g. there is an excess of patient dropout, missing data, lack of recruitment etc).

Standard medical treatment for all subjects/concomitant medications includes:

All subjects must be on a stable anti-hypertensive, diabetic and lipid lowering regimen for at least one month prior to inclusion.

a) Control of hypertension with anti-hypertensives with goal blood pressure less than 130/80 mm/Hg. Medication choice includes angiotensin converting enzyme inhibitor/angiotensin receptor blocker as first line agent, diuretic as second line agent, calcium channel blocker as third line agent, and beta blocker as fourth line agent (unless patient has known CVD and needs to be on a beta blocker regardless of blood pressure). b) Diabetes will be treated as needed to goal HbA1C of 7.0%. c) Control of hyperlipidemia with statins to goal of low density lipoprotein cholesterol (LDL-C) <100 mg/dL and total cholesterol levels to less than 200 mg/dL. d) Smoking cessation counseling. e) Control of anemia following the latest recommendation from the National Kidney Foundation. f) Control of hyperphosphatemia with phosphorus binders and down titration of the intervention as describe above to a goal phosphorus < 4.6 mg/dL.

Data Collection

Screening Measures and Potential Confounders:

***Medical History, Physical Examination and Blood Chemistries*.** Subjects will report fasted for assessment of serum phosphorus and a standard blood chemistry analysis including a metabolic panel (with creatinine/calcium), blood count, lipid panel and follicle stimulating hormone (women only), then undergo a medical history and physical examination.

Outcome Measures. Please see below under "**D. Description, Risks and Justification of Procedures and Data Collection Tools"**

Data management:

Study data will be collected and managed using REDCap (Research Electronic Data Capture). REDCap is a secure, web application designed to support data capture for research studies, providing user-friendly web-based case report forms, real-time data entry validation (e.g. for data types and range checks), audit trails and a de-identified data export mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus). The system was developed by a multi-institutional consortium, which includes the University of Colorado Denver and was initiated at Vanderbilt University. Currently 52 institutions in 4 countries are using the software. The database is hosted at the University of Colorado Denver Development and Informatics Service Center (DISC), which will be used as a central location for data processing and management. REDCap data collection projects rely on a thorough study-specific data dictionary defined in an iterative self-documenting process by all members of the research team with planning assistance from the DISC. The iterative development and testing process results in a well-planned data collection strategy for individual studies. Both REDCap and REDCap Survey systems provide secure, web-based applications that are flexible enough to be used for a variety of types of research, provide an intuitive interface for users to enter data and have real time validation rules (with automated data type and range checks) at the time of entry. These systems offer easy data manipulation with audit trails and reporting for reporting, monitoring and querying patient records, and an automated export mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus). Both systems were found to meet or exceed all of the security audit requirements.

D. Description, Risks and Justification of Procedures and Data Collection Tools:

Primary Outcomes:

a)Conduit artery vascular endothelial dependent dilation (EDD) and endothelial independent dilation (EID): Conduit artery EDD, brachial artery flow-mediate dilation (FMD_{BA}), will be determined using high-resolution ultrasonography (GE Vivid7 Dimension) as described originally by Celermajer et al. [60] and used recently by the P.I. [61, 62]. ECG-gated end-diastolic ultrasound images and Doppler flow of the brachial artery will be acquired during baseline and FMD_{BA} conditions. For FMD_{BA}, reactive hyperemia will be produced by

inflating a pediatric BP cuff around the forearm to 250 mmHg for 5 minutes followed by rapid deflation. Brachial artery endothelium-independent dilation (EID), an index of smooth muscle sensitivity to exogenous nitric oxide, will be determined by measuring brachial artery dilation for 10 minutes after administration of sublingual nitroglycerin (0.4 mg) [63, 64]. A commercially available software package (Vascular Analysis Tools 5.8.1, Medical Imaging Applications) will be used to acquire ECG-gated brachial artery diameters. As recently recommended [65], FMD_{BA} will be determined as the mm and % Δ from baseline diameter [63-65]. Shear rate will also be calculated, and if group- or condition- differences exist, FMD_{BA} will be adjusted accordingly [65-67].

Risks of this procedure include: The assessment of FMD_{BA} is minimally-invasive. Inflating the blood pressure cuff below the elbow during this procedure may cause a mild to moderate intensity "pins and needles or numbing" sensation that goes away as soon as the cuff is deflated. In addition, patients will receive a dose of sublingual nitroglycerin to measure EID, an essential control measure without which the results cannot be properly interpreted. Sublingual nitroglycerin is a medication with a rapid vasodilator effect and is routinely used in patients with coronary artery disease to help relieve chest pain. Side effects of sublingual nitroglycerin are short-lived (30-60 minutes). Approximately 60% of people who receive nitroglycerin will experience a headache. The subject may experience symptoms such as lightheadedness, and in rare instances (1 in 1000) lowered heart rate, which could lead to fainting. However, these risks are primarily when a person is upright, and subjects will be lying supine during administration of nitroglycerin. Finally, placing the pill under the tongue could cause irritation to the area in the back of the mouth. The patients will be monitored by frequently asking about subjective complaints, as well as by frequent heart rate and blood pressure measurements (prior to and every 15 minutes after taking the medication for 1 hour). In addition, during the 10-minute period when brachial artery dilation to nitroglycerin is assessed (peak activity of drug), blood pressure and heart rate will be assessed every 2 minutes. In the rare case that blood pressure drops drastically, an intravenous catheter will be available for infusion of atropine. Still, nitroglycerin has been administered safely for over a decade in the laboratory the PI performed her doctoral training, as well as in other laboratories and in clinical situations worldwide. Nitroglycerin will not be given to any subject with a systolic blood pressure less than 100 mmHg on the day the measurements are performed, history of migraine headaches or previous adverse reaction to administration.

Justification: As discussed above in the background/significance sections, patients with CKD have an increased risk of death due to CVD, and endothelial dysfunction plays a critical role in the development of CVD. Endothelial dysfunction is evident early in the course of kidney disease. It is unknown if administration of an inhibitor of inflammation improves vascular endothelial function in CKD patients. FMD_{BA} is a non-invasive way to assess endothelial function. The other method available to study endothelial function (EDD) would include acetylcholine administration via brachial artery infusion, which is more invasive than the proposed methodology. In order to interpret improvements in brachial artery dilation in response to a shear stress (FMD_{BA}) following the drug intervention, it is necessary to assess smooth muscle sensitivity to nitric oxide using EID. If EID is unchanged in the presence of improved FMD_{BA}, this indicates the improved dilation in response to a shear stimulus is due to positive adaptations at the level of the vascular endothelium (i.e. endothelium-dependent).

b) Arterial Stiffness: Aortic pulse-wave velocity (aPWV) will be determined as described in detail previously [68, 69]. Briefly, a transcutaneous custom tonometer (Noninvasive Hemodynamics Workstation (NIHem), Cardiovascular Engineering Inc., Norwood, MA) will be positioned at the carotid, brachial, radial and femoral arteries to non-invasively assess aPWV. The tonometer sits on the surface on the skin and detects a waveform associated with the pulse at these various sites. The time delay between the R-wave of each heart beat (ECG) and waveforms at these various sites is calculated using the NIHem software, and by also measuring the distance between these landmarks, velocity can be calculated (aPWV). Increased pulse-wave velocity is indicative of increased stiffness of the aorta, a large elastic artery. In addition, the carotid artery will be imaged using an ultrasound (GE Vivid 7) equipped with a linear array transducer as previously described [51,52]. Change in carotid diameter across 10-15 cardiac cycles will be analyzed using image analysis software (Carotid Analyzer) as previously described [51,53]. Using the change in carotid pressure also provided by tonometry with the NIHem and the change in diameter obtained with carotid artery ultrasound imaging, carotid artery compliance and the ß-stiffness index will be calculated as previously described[70, 71], providing an index

of local arterial stiffness. The carotid images will also be assessed for measurement of carotid artery intimal-medial thickness (IMT), another CVD risk factor.

Risks of this procedure include: The test is minimally-invasive and the main potential discomfort is from the pressure/placement of the tonometer and ultrasound probe.

Justification: As discussed above in the background/significance sections, patients with CKD have an increased risk of death due to cardiovascular disease and large artery damage plays a critical role in CVD. It is unknown if lowering serum phosphorus improves arterial stiffness in CKD patients. These measurements will provide us with important insight into our specific aims.

Secondary (Exploratory) Outcomes:

a) Oxidative Stress-Associated Suppression of EDD and Large Elastic Artery Stiffness: The influence of oxidative stress on FMD_{BA} and aPWV will be determined by infusing a supraphysiological dose of ascorbic acid (vitamin C; American Regent Laboratories Inc.) or isovolumic saline, as described previously by the PI's doctoral training laboratory and others [63, 72-74]. A priming bolus of 0.06 g ascorbic acid/kg fat-free mass (FFM) dissolved in 100 mL of saline will be infused intravenously at 5 mL/min for 20 minutes, followed immediately by a "drip-infusion" of 0.02 g/kg FFM dissolved in 30 mL of saline administered over 60 minutes at 0.5 mL/min. Vascular measurements will be made at the end of the 20 min bolus during the "drip infusion" when peak plasma concentrations of ascorbic acid occur (34). The difference in FMD_{BA} and aPWV during ascorbic acid vs. saline infusion will be taken as a measure of the modulation of EDD/stiffness by oxidative stress [63, 72].

Risks of this procedure include: Ascorbic acid has been infused intravenously without any unfavorable side effects into young and older healthy subjects, as well as patients with chronic cardiovascular diseases. However, administration of concentrated ascorbic acid may cause irritation to the local area around the infusion. In order to reduce this risk we will dilute the infusion of ascorbic acid in sterile saline.

Justification: Infusing ascorbic acid is a safe, effective and established method of acutely inhibiting oxidative stress. As the difference in our primary outcome measures (FMD_{BA} and aPWV) in the presence of ascorbic acid vs. saline is an index of a modulation of EDD/stiffness by oxidative stress, we will be provided with mechanistic insight into the physiological adaptations contributing to any improvements in vascular function with phosphorus lowering. Specifically, less of an improvement in EDD/stiffness with ascorbic acid post- vs. pre- treatment, in the presence of improved EDD/stiffness during saline infusion post- vs. pre- treatment, would indicate that reduced oxidative stress contributed to the improvements in vascular function with phosphorus lowering.

b) Vascular endothelial cell protein expression: These procedures have been described in detail previously [61, 75-77]. A trained nurse, nurse practitioner or physician will collect endothelial cells from the intima of the antecubital vein of the non-dominant arm using up to 3 sterile 0.021 inch J-wires briefly advanced through a catheter. The distal portion of the wire will be transferred to a 50-ml conical tube containing a buffer solution. Cells will be recovered by centrifugation, fixed with formaldehyde, and slides will be prepared and frozen for later staining for the primary antibody of interest, a complementary fluorescent secondary Alexafluor 555 antibody (Invitrogen), VE cadherin for positive identification as endothelial cells and DAPI for nuclear integrity. The endothelial cells count per slide averages ~55 (range 25-92, estimated total of 200-736 endothelial cells per subject). More than 90% of the endothelial cells retain cellular and nuclear integrity (as determined by DAPI staining). Images will be digitally captured and analyzed using Metamorph Software (Universal Imaging Corp). Values will be reported as ratios of subject endothelial cell protein expression to human umbilical vein endothelial cell protein expression, removing the possible confound of differences in staining intensity between sessions. Using quantitative immunofluorescence, protein expression of the following proteins will be assessed:

- nitrotyrosine, as a marker of oxidative stress (Abcam) [75, 77, 78]
- NADPH $p47^{phox}$, manganese superoxide dismutase (MnSOD; Chemicon Intl) and copper-zinc SOD (CuZnSOD; Upstate) [75, 77, 78] as markers of pro and anti-oxidant signaling
- total eNOS (BD Biosciences) and eNOS phosphorylated at Ser 1177 (BD Biosciences) [79]

Note: It is well establish that expression of proteins in endothelial cells obtained from venous sampling reflect individual and group differences observed in cells obtained from brachial artery sampling [80].

Risks of this procedure include: The risks associated with this procedure are those that occur with the placement of the venous catheters including small risk of pain, phlebitis, infection and blood clots (i.e., the same risks associated with venous catheterization).The risk of blood clots and infection may be slightly greater than those of an intravenous catheter due to increased manipulation of the blood vessel when compared with catheterization alone. There is also a very low likelihood that the J-shaped wire could get stuck in the vein and/or that the vein could be damaged.

Justification: This is a novel approach to evaluate the potential mechanisms (including expression of proteins modulating inflammation) by which phosphorus lowering may improve vascular function in CKD patients, with risk analogous to venous catheterization. These procedures were described originally by Colombo et al.[54] and more recently by Dr. Doug Seals' laboratory[55-58]. An alternative to understanding these mechanisms would be via obtaining arterial endothelial cells through an arterial line. This would be considerably more invasive, and in light of data supporting that venous endothelial cells are a good surrogate marker for arterial endothelial cells, the investigators feel this risk is not justified for the purposes of this proposal.

c) Laboratory Analyses: The majority of the laboratory measurements will be performed at the University of Colorado Clinical Laboratory using standard techniques. Samples will be collected by research nurses at the UPI Building laboratory facilities and sent to the clinical laboratory or core laboratory.

- *Serum phosphorus* (**Visits 1-9**): Serum phosphorus (1 mL serum) will be measured using standard methodology for study inclusion, drug titration and to determine the degree of phosphate lowering with lanthanum.
- *Follicle stimulating hormone (FSH)* (**Visit 1**): FSH will be measured in all women <57 years of age to confirm post-menopausal status for study inclusion (1 mL serum).
- *Comprehensive metabolic panel* (**Visits 1, 2, 9**): A comprehensive metabolic panel (4.5 mL plasma) will be performed for study inclusion and monthly throughout the study using standard methodology.
- *Lipid panel* (**Visits 2, 9**): A lipid panel (1 mL plasma) will be performed at baseline (Visit 2) and 12 weeks after lanthanum or placebo.
- *Fibroblast growth factor 23 (FGF23)* **(Visits 2, 9)**: FGF23 (200 μL serum) will be measured pre- and post- treatment as a marker of mineral metabolism (Kainos).
- *Intact Parathyroid hormone (iPTH)* **(Visits 2, 9)**: iPTH (4.5 mL plasma/serum) will be measured pre- and post- treatment as a marker of mineral metabolism using standard methodology.
- *25 hydroxyvitamin D (25(OH)D)* **(Visits 2, 9)**: 25(OH)D (4.5 mL serum) will be measured pre- and post-treatment as a marker of mineral metabolism by the core laboratory (Chemilluminescence, DiaSorin Liaison).
- *1,25 dihydroxyvitamin D (1,25(OH)$_2$D)* **(Visits 2, 9)**: 1,25(OH)$_2$D (1 mL serum) will be measured pre- and post- treatment as a marker of mineral metabolism by the core laboratory (EIA, Immunodiagnostic Systems).
- *Interleukin-6 (IL*-6) (**Visits 2, 5, 9**): IL-6 (1 mL plasma) will be measured pre- and post- treatment as a marker of inflammation by the CTRC core laboratory (ELISA; R&D Systems).
- *C-reactive protein (CRP)* (**Visits 2, 5, 9)**: CRP (1 mL plasma) will be measured pre- and post- treatment as a marker of inflammation by the CTRC core laboratory (Immunoturbidimetric; Beckman Coulter).
- *Vitamin C (VITC)* (**Visits 2, 9**): VITC (0.5 mL plasma) will be measured before and after infusions pre- and post- treatment to assess whether the study doses change Vitamin C levels.
- *Markers of Oxidative Damage* **(Visits 2, 5, 9)**: (1 mL serum) will be measured before and after infusions pre- and post- treatment to measure markers of oxidative damage, such as F-2 isoprostanes, by the CTRC core laboratory (ELISA, Mercodia).
- *Future studies* (**Visits 2, 5, 9**): For subjects signing the Informed Consent Addendum, an additional 5 mL of serum and 9 mL of plasma will be collected for future research.
- *Urine phosphorus* **(Visits 2, 9)**: Urine phosphorus will be measured pre- and post- treatment as a marker of phosphorus excretion.
- *Urine creatinine* **(Visits 2, 9)**: Urine creatinine will be measured pre- and post- treatment to aid in the calculation of phosphorus excretion.

Risks of this procedure include: All of the lab work will be obtained via venipuncture and the insertion of an 18 gauge cannula. The risks that are entailed in this procedure include discomfort and pain of venipuncture, failed attempt resulting in more than one attempt, bleeding upon cannula removal, and rarely thrombophlebitis (a non infectious inflammatory state of the vein typically due to prolonged cannulation). In the protocol the cannulation procedure is expected to last only for a few minutes (5-10), and the cannula will be removed immediately after obtaining the lab tests above and the endothelial cell collection. A separate cannula will then be inserted in the dorsal aspect of the hand to infuse the saline and ascorbic acid, in order to help preserve access in the arm. Subjects may be at a very small risk of anemia from the blood draws required for the assays described above. However, only the minimum amount of blood needed will be drawn and experienced research nurses/nurse practitioners will be collecting the specimens. All blood draws will be performed on the arm not designated for future vascular access for dialysis.

Justification: Please refer to the background/significance section for a detailed discussion of the importance of these serum markers with endothelial dysfunction. Patients with CKD have increased oxidative stress and inflammation compared to the general population, and lanthanum may improve vascular function by reducing oxidative stress and inflammation. These systemic blood markers will provide important insight as to the mechanisms by which phosphate lowering may improve vascular function. The other laboratory tests are necessary for subject inclusion and safety monitoring.

E. Potential Scientific Problems:

Although subject recruitment/retention are always challenging, we should be able to meet target enrollment given our location in the densely populated Denver metro area and access to the UC-Denver Nephrology Clinic at the University Hospital overseen by board-certified nephrologist, Dr. Chonchol, and at the Denver VA Medical Center Renal Clinic, staffed by Dr. Jovanovich. We expect few difficulties with the proposed experimental procedures and protocols as the co-investigator has employed them during her graduate training. In addition, there are currently three intervention protocols in patients with CKD being performed using all/the majority of the proposed methods (Drs. Jablonski, Jalal and Kendrick). We recognize that alternate phosphate binders could have been selected; however, we chose lanthanum (a) to avoid the potential side-effect of hypercalcemia that may be occur with calcium-based phosphate binders [81], (b) because calcium-based binders have not improved EDD in hyperphosphatemic CKD patients [49, 50], and (c) to control for the beneficial effects that may occur with sevelamer by mechanisms other than phosphorus lowering [82-84]. We also recognize that it would be of interest to compare to lanthanum to other phosphate binders, but given the duration and scope of the project, feel it is necessary to first compare the efficacy of lanthanum vs. placebo.

F. Data Analysis Plan:

Statistical Analysis

General: Descriptive statistics will be used to summarize continuous variables (mean, standard deviation, minima, median, and maxima) and categorical variables (N and %) for baseline characteristics by treatment group. Summaries will be provided by treatment group (lanthanum and placebo). Means and medians with variability estimates based on standard deviations or interquartile ranges will be used for graphical displays of continuous variables and histograms of percentages will be used to display the distribution of categorical variables. Highly skewed variables may be transformed prior to statistical analyses. Significance is defined with a two-sided alpha of 0.025 for the primary outcomes and 0.05 for the secondary outcomes.

Analysis Populations: The primary analysis will be intention-to-treat. The data will also be analyzed as per protocol. Subjects randomized to treatment who receive at least one drug dose and complete baseline measurements will be included in the intention-to-treat analysis, regardless of whether they adhered to the assigned intervention. In the per protocol analysis, only those subjects who were adherent to the protocol (completed all 12 weeks of drug or placebo) and completed the end of the study measurements will be included. Decisions to remove subjects from the per protocol analysis set will be made prior to breaking the treatment blind. The intention-to-treat analysis will analyze subjects as randomized; the per protocol analysis will analyze subjects as treated.

Prior to all analyses, data checks will be conducted to verify or correct assumptions about the data structure and to identify potential issues with the data being collected. This process will include range checks

to identify impossible values (e.g., age=0) and cross-tabulations identifying relations between key variables. Differences between groups at baseline will be assessed by unpaired t-tests and changes in response to lanthanum vs. placebo by ANOVA for repeated measures. In the case of a significant F-value, post hoc tests will be performed using the Newman-Keuls method. The influence of changes in 2˚ outcomes (blood pressure, mechanistic variables, etc.) on changes in 1˚ outcomes in response to lanthanum will be examined by multiple linear regression. Potential confounders, such as changes in other characteristics that are known to influence vascular function (e.g. body mass, blood pressure) will be handled using ANCOVA. Alpha for the two primary endpoints will be set at 0.025 while there will be no adjustment for secondary outcomes as they will be considered exploratory.

G. Summarize Knowledge to be gained:

The findings from the proposed research should provide important new information regarding the potential benefits of phosphorus lowering upon the reduction of vascular dysfunction in patients with CKD. It should also provide important insight into the mechanisms involved in the modulation of EDD by elevated phosphorus. Together, this information will add to the knowledge of how to prevent and treat vascular dysfunction in patients with CKD.

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Protocol #: 15-0834
Project Title: Phosphorus Balance Study
Principal Investigator: Anna Jovanovich, MD
Version Date: 06/07/2017

I. Hypotheses and Specific Aims:

Cardiovascular disease (CVD) is significantly increased in chronic kidney disease (CKD) and is an important cause of morbidity and mortality.[1-5] As much as 80% of all CVD is associated with vascular dysfunction,[6] particularly impaired endothelium-dependent dilation (EDD),[7] measured by brachial artery flow-mediated dilated (FMD_{BA}).[8,9] Not surprisingly, patients with CKD demonstrate these dysfunctional vascular phenotypes. In CKD, phosphorus remains within the normal range (2.5-4.5 mg/dL) until late in the disease.[10-13] In earlier stages of CKD, serum phosphorus levels are maintained with the normal range by up-regulating fibroblast growth factor 23 (FGF23) and a consequent increase in the fractional excretion of phosphorus by the kidney.[10-13] However, elevated serum phosphorus, even within the normal range, is associated with adverse CVD outcomes in CKD.[13-15] Elevations in serum phosphorus have also been associated with impaired EDD and with indirect measures of arterial stiffness.[16-19] Whether lowering serum phosphorus with phosphate binders in patients with CKD will change phosphorus balance and improve EDD and arterial stiffness is unknown.

This investigation is an extension of a 12-week prospective, randomized, placebo-controlled double-blind trial (Phosphorus Lowering RCT, COMIRB 13-0328) that assesses the effects of phosphorus lowering with the phosphate binder, lanthanum carbonate, in stage IIIb and IV CKD with normal or modestly elevated serum phosphorus (2.8-5.5 mg/dL) on vascular endothelial function and arterial stiffness. The primary goal of the current Phosphorus Balance protocol is to determine whether lowering serum phosphorus (accomplished during the parent Phosphorus Lowering RCT) with a low phosphorus diet and 12 weeks of treatment with lanthanum carbonate, a non-absorbed, non-calcium-containing phosphate binder, affects phosphorus balance. The key secondary goal is to determine if differences in phosphorus balance affect vascular function as measured by FMD_{BA}.

To ensure adequate enrollment, we will also recruit patients with stage IIIb and IV CKD with normal or modestly elevated serum phosphorus (2.8-5.5 mg/dL) who are *not* currently participating in the parent Phosphorus Lowering RCT (COMIRB 13-0328). Similar to the Phosphorus Lowering RCT, these patients will follow a low phosphorus diet and will be randomized to lanthanum carbonate or placebo for 12 weeks (run-in period) prior to beginning the current Phosphorus Balance protocol.

Hypothesis A. Subjects with stages IIIb and IV CKD and normal or modestly elevated serum phosphorus (2.8-5.5 mg/dL) who are treated with 12 weeks of lanthanum carbonate and a low phosphorus diet will be in negative phosphorus balance compared to subjects treated with placebo.

Hypothesis B.
Improvements in EDD will correlate with negative phosphorus balance.

Aim A. To determine phosphorus balance in subjects with stage IIIb or IV CKD after being treated with lanthanum carbonate and a low phosphorus diet or placebo and a low phosphorus diet for 12 weeks.

Aim B. To determine whether phosphorus balance in each treatment group correlates with the FMD_{BA} measurement performed at the end of the proposed Phosphorus Balance protocol.

II. **Background and Significance**:
Risk of cardiovascular diseases (CVD) is significantly elevated in patients with chronic kidney disease (CKD); however, the increased cardiovascular risk in this patient population is only partially explained by traditional cardiovascular risk factors.[1-6] In earlier stages of CKD, serum phosphorus levels are maintained within the normal range by up-regulating fibroblast growth factor 23 and a consequent increase in the fractional excretion of phosphorus by the kidney.[10-13] However, elevated serum phosphorus even within the normal range, as is the typical case in CKD patients not requiring chronic hemodialysis, has emerged within the last decade as a novel, independent predictor of CVD risk and associated mortality.[13-15] Furthermore, little is known about phosphorus balance and its association with outcomes in CKD.

CKD patients also exhibit vascular endothelial dysfunction and increased arterial stiffness,[20,21] two independent predictors of future CVD. A modest elevation in serum phosphorus contributes to this phenotype of vascular dysfunction.[16-19] Recent evidence supports that an acute increase in serum phosphorus impairs endothelium dependent dilation (EDD) and increases vascular oxidative stress.[16] In addition, both vascular endothelial dysfunction and increased oxidative stress have been identified as important predictors of mortality and cardiovascular events in patients with CKD.[22,23] Thus, reducing serum phosphorus burden in CKD patients not requiring chronic hemodialysis may reduce cardiovascular events by improving vascular endothelial function and reducing large elastic artery stiffness. However, it is currently unknown if reducing serum phosphorus to a low normal laboratory range (<2.8 mg/dL) with oral phosphate binders induces negative phosphorus balance thus, potentially enhancing vascular function in this population.

Little is known about phosphorus balance in CKD. It is assumed that CKD patients remain in neutral phosphorus balance despite decreases in kidney function. Serum phosphorus increases but remains within the normal range[10-13] until late in CKD thus making it difficult to recognize perturbations of phosphorus balance in these patients. Indeed, elevated serum phosphorus even within the normal range is associated with worse CVD outcomes.[12,14,15] In CKD subjects with normal serum phosphorus treated with six weeks of sevelamer, a non-calcium based phosphate binder, serum phosphorus did not change significantly (3.45 ± 0.65 mg/dL vs. 3.31 ± 0.64 mg/dL) but there was a significant decrease in total urinary phosphorus excretion (534 ± 137 mg/24 hours vs. 313 ± 135 mg/24 hours, $p<0.05$) as well as in parathyroid hormone (PTH) (107 [79-130] pg/mL vs. 69 [47-94] pg/mL, p 0.05) and fibroblast growth factor 23 (FGF23) (103 [62-142] pg/mL vs. 54 [38-94] pg/mL, $p<0.05$), suggesting a shift in phosphorus homeostasis.[24] However, two recent calcium balance studies performed in subjects with CKD stages III-IV, showed that subjects remained in neutral phosphorus balance despite administration of calcium-

containing phosphate binders.[25,26] There are no studies evaluating the effect of non-calcium based phosphate binders on phosphorus balance in subjects with CKD nor other studies examining the effects of changing phosphorus balance on vascular function. Whether longer-term (12 weeks) treatment with non-calcium based phosphate binders induces a negative phosphorus balance in subjects with CKD is uncertain. It is also unknown if negative phosphorus balance improves vascular function.

III. **Preliminary Studies/Progress Report:**

Serum phosphorus is related to cardiovascular disease events and mortality as well as measures of arterial stiffness

Presented below is the longitudinal relationship between higher serum phosphorus and all-cause mortality and CVD events (myocardial infarction, stroke and peripheral vascular disease), and a cross-sectional association of elevated serum phosphorus with worsening pulse pressure (PP). These data were obtained from the Homocysteine Study in Advanced Kidney Disease (HOST), which was a prospective, randomized, double-blind study on the effects of folate, vitamin B_6, and vitamin B_{12} replacement on all-cause mortality and CVD events in VA patients with severe CKD and elevated homocysteine.[27] As the intervention did not have an effect on the proposed outcomes, it represented a unique opportunity to convert the data obtained from a randomized control trial into an observational study. The HOST dataset has detailed information on demographics, CVD risk factors, prevalent CVD, circulating markers (including serum calcium, phosphorus, and intact parathyroid hormone), and usage of cardioprotective medications in all study participants. In addition, an endpoints committee adjudicated all endpoints. For the purpose of this analysis, we included 1,099 HOST participants. The mean ($\pm$SD) age of the participants was 69±11 years, 98% were male, and 26% were black. The mean estimated MDRD-GFR was 18±6 mL/min/1.73 m^2 and most participants (n=718; 65%) had an estimated MDRD-GFR of 15-29 mL/min/1.73 m^2. The mean serum phosphorus concentration was 4.3±1.3 mg/dL; 36% and 12% had a serum phosphorus concentration greater than 4.5 and 5.5 mg/dL, respectively. **These descriptive data demonstrate that poor vascular function and CVD events are associated with high serum phosphorus**.

***Higher serum phosphorus levels are independently associated with CVD events/mortality in patients with advanced CKD*:** During a median follow-up of 2.9 years, 472 (42.9%) patients died from any cause and 237 (21.6%) had a CVD event. Higher serum phosphorus levels were positively associated with higher risks for the composite of death and CVD events. Identical results were observed when CVD events were examined separately (not shown). Compared to the lowest quartile, the two highest quartiles of serum phosphorus were associated with a significantly elevated risk for the composite endpoints, after multivariate adjustment (Table 1; hazards ratios (HR)). The analysis was repeated using serum phosphorus levels as a continuous variable instead of quartiles. After multivariable adjustment (Model 3), the HR of the composite endpoint increased by 1.17 (95% CI; 1.02-1.33; p=0.02) per 1 mg/dL increase in serum phosphorus. These results provide important support to my aim to evaluate whether changes in phosphorus lowering affect vascular endothelial function and arterial stiffness (two independent predictors of CVD events/mortality) in patients with advanced CKD.

Table 1. Association of Higher Serum Phosphorus with Risk of Cardiovascular Events & Death in the HOST Study

Phosphorus Concentrations	Q1	Q2	Q3	Q4

	(≤3.5 mg/dL)	(3.6-4.1 mg/dL)	(4.2-4.8 mg/dL)	(>4.9 mg/dL)
Model 1	1.00	1.35(0.87- 2.09)	1.70 (1.12- 2.57)	1.93(1.29- 2.90)
Model 2	1.00	1.36 (0.88- 2.11)	1.79(1.18-2.73)	2.18(1.44-3.31)
Model 3	1.00	1.19 (0.76-1.86)	1.50 (1.01-2.31)	1.68 (1.05-2.68)

Model 1: Unadjusted; **Model 2:** Adjusted for age, gender and race; **Model 3:** Adjusted for covariates in Model 2 plus smoking, diabetes, hypertension, prevalent CVD, body mass index, systolic and diastolic blood pressure, treatment arm, eGFR, albumin, calcium, 25-hydroxyvitamin D, intact PTH, LDL-cholesterol, high density lipoprotein-cholesterol, triglycerides and use of angiotensin converting enzyme inhibitors, angiotensin receptor blockers and β-blockers.

In addition, we performed a cross-sectional analysis of the relationship between serum phosphorus and pulse pressure (PP) in the HOST cohort. The results confirm that higher serum phosphorus levels are independently associated with a wider PP in patients with advanced CKD. The mean (±SD) PP in this population was 71 (20) mmHg. In adjusted linear regression models (Model 3), each 1 mg/dL increase in serum phosphorus was associated with a 2 mmHg increase in PP (β=2.0±6.9; p=0.005). These data support the notion that serum phosphorus lowering should reduce large elastic artery stiffness, as increased PP is an expression of arterial stiffness.

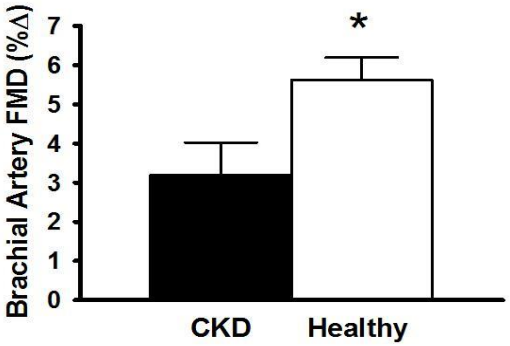
Feasibility of lanthanum carbonate treatment to lead to a sufficient separation in mean serum phosphorus between groups

Dr. Geoff Block, Clinical Assistant Professor in the Division of Renal Diseases and Hypertension at the University of Colorado Denver, recently examined the effects of phosphate binders on parameters of mineral metabolism among patients with moderate to advanced CKD. A total of 148 patients (eGFR 20-45 ml/min/1.73m^2) were randomly assigned to calcium acetate (n=30), lanthanum carbonate (n=30), sevelamer (n=30), or placebo (n=58). The primary endpoint was change in mean serum phosphorus from baseline to the average measured at months 3, 6, and 9. Serum phosphorus decreased from a baseline mean of 4.2 mg/dL (for both active and placebo arms) to 3.9 mg/dL in the active therapy arm and 4.1 mg/dL in the placebo arm (p=0.03). These results were published in a peer-reviewed journal.[28] Differences in serum phosphorus between the placebo and the lanthanum carbonate arms were further analyzed at 3 months. Serum phosphorus decreased from a baseline of 4.2 mg/dL (for both the lanthanum carbonate and placebo arms) to 3.7 mg/dL in the lanthanum carbonate arm and to 4.1 mg/dL in the placebo arm (p=0.04). The mean dose of lanthanum carbonate was 2.7 g/day. An increase in serum phosphorus of 0.3-0.5 mg/dL has been associated with a significant increase in relative risk of all-cause mortality and CVD events in various epidemiologic studies of patients with CKD. These data suggest that the treatment with lanthanum carbonate will lead to a sufficient and significant separation in the mean serum phosphorus between groups.

There is a significant difference in FMD_{BA} and aPWV between healthy individuals and those with CKD

FMD_{BA} is a well-established intermediate endpoint and validated means with which to examine CVD in subjects with and without CKD. In Figure 1 we demonstrate that among 8 men studied by our group with stage III-IV CKD (aged 63±4 years) compared to 8 healthy age-matched control subjects (aged 60±3 years), there was a significant difference in FMD_{BA} (*p<0.05). These preliminary data are additional experimental evidence supporting the testing of therapeutic interventions that improve FMD_{BA} in patients with stage III-IV CKD.

Figure 1



IV. Research Methods

A. Outcome Measure(s): The proposed investigation is an extension of a 12-week prospective, randomized, placebo-controlled double-blind trial (Phosphorus Lowering RCT) of 60 subjects that assesses the effects of the phosphate binder, lanthanum carbonate, and a low phosphorus diet in stage IIIb and IV CKD with normal or modestly elevated serum phosphorus (2.8-5.5 mg/dL) on vascular endothelial function and arterial stiffness. Vascular endothelial function is assessed as endothelium-dependent dilation via FMD_{BA}. Subjects with stage IIIb and IV CKD with normal or modestly elevated serum phosphorus (2.8-5.5 mg/dL) who are *not* participating in the Phosphorus Lowering RCT will also be enrolled and will be treated with either lanthanum carbonate or placebo and a low phosphorus diet for a 12-week run-in period prior to initiating the current Phosphorus Balance protocol in order to mimic the parent Phosphorus Lowering RCT. After completion of 12 weeks of either lanthanum carbonate and a low phosphorus diet or placebo and a low phosphorus diet, 24 research subjects (12 treated with lanthanum carbonate and 12 treated with placebo) will participate in the proposed Phosphorus Balance Study. The primary outcome is the difference in phosphorus balance between the active and placebo groups. Serum phosphorus is not expected to change during the current Phosphorus Balance protocol as subjects will remain on the same dose of active drug or placebo that they were on during either the parent Phosphorus Lowering RCT or the 12-week lanthanum carbonate or placebo run-in period. The dose of active drug or placebo was titrated during the first month of the parent Phosphorus Lowering RCT or the 12-week run-in period and is expected to be stable during months 2 and 3 of both the parent RCT and 12-week run-in period as well as during the current Phosphorus Balance protocol. Secondary outcomes include whether there is a difference in FMD_{BA} between the active and placebo groups and whether this correlates with a difference in phosphorus balance between the active and placebo groups.

B. Description of Population to be Enrolled:

Subjects. A group of 24 subjects who have completed the 12-week parent Phosphorus Lowering RCT or the 12-week run-in period described above will provide written informed consent and serve as subjects the for the current Phosphorus Balance protocol. Major inclusion/exclusion criteria are presented below:

Inclusion Criteria:

1. Completed prospective, randomized, placebo-controlled double-blind trial, Phosphorus Lowering to Treat Vascular Dysfunction in Chronic Kidney Disease (COMIRB #13-0328) or willing to participate in 12-week run-in period
2. Aged 40-79 years; women must be post-menopausal (serum phosphorus increases after menopause in women[58])
3. CKD stage IIIb or IV (eGFR by 4-variable MDRD prediction equation: 15-45 mL/min/1.73m^2); stable renal function in the past 3 months)
4. Mini-mental state examination (MMSE) score ≥24 (cognitive function screening); ability to provide informed consent

Exclusion Criteria:

1. Subjects with advanced CKD requiring chronic dialysis
2. Subjects at high risk for gastrointestinal events or alteration in gastrointestinal anatomy (e.g., history of gastrointestinal surgery, colon cancer), hypomotility disorders (e.q., constipation, ileus), current bowel obstruction, or fecal impaction
3. Significant co-morbid conditions that lead the investigator to conclude that life expectancy is less than 1 year
4. Expected to undergo living-related kidney transplant in the next 6 months
5. History of severe congestive heart failure (i.e., EF < 35%)
6. History of severe liver disease (i.e., elevated bilirubin, AST, ALT, or evidence of liver cirrhosis on biopsy or imaging)
7. Hospitalization in the past 3 months
8. BMI ≥40 kg/m^2 (FMD measurement can be inaccurate in severely obese subjects)
9. Active vitamin D analogue use (i.e., calcitriol, paricalcitol, doxercalciferol)
10. Alcohol dependence or abuse (i.e., as documented in subject's chart per PCP or mental health provider or if stipulated by subject)
11. Current tobacco abuse (i.e., current and habitual use of any tobacco product)
12. Use of prednisone and other immune suppressant medications (i.e., current and ongoing use of prednisone or other immune suppressant medication)

A Standard Operating Procedure is in place that prohibits the use of nitroglycerin (NTG) in subjects with systolic blood pressure <100 mmHg and with migraine headache. Additionally, it is part of the Standard Operating Procedure that subjects are educated regarding drugs that interact with lanthanum carbonate (levothyroxine, quinolone antibiotics, and other cationic medications) and are instructed in correct timing of lanthanum carbonate administration with respect to drugs that interact with it.

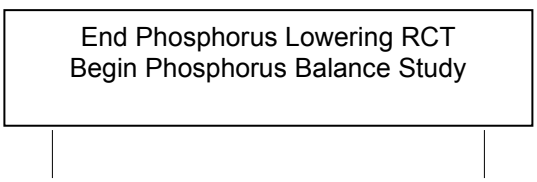
C. Study Design and Research Methods

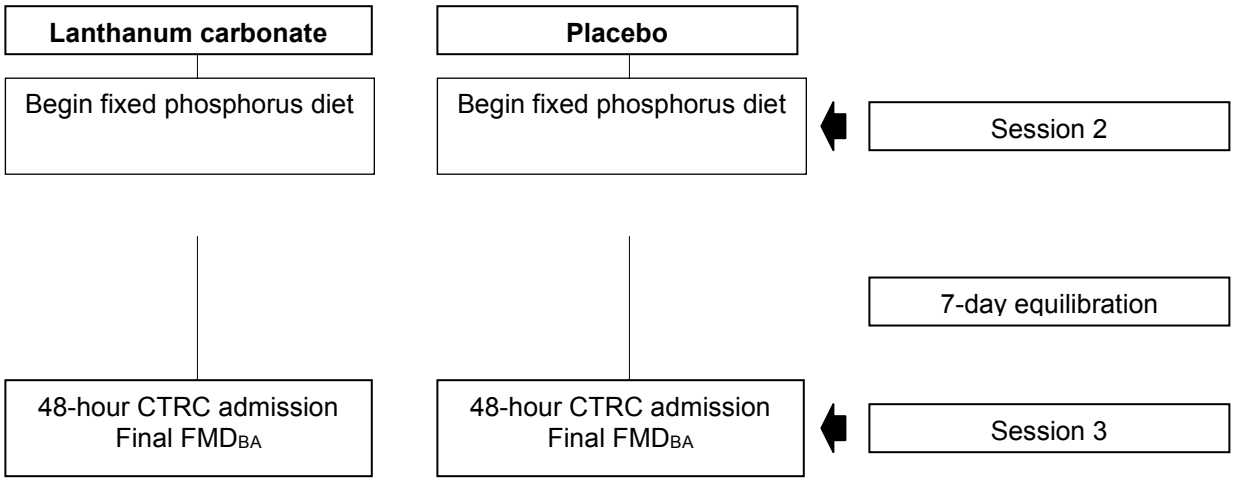
Experimental Design. After completing the 12-week Phosphorus Lowering RCT (Figure 1) or 12-week run-in period (Figure 2), a group of 24 research subjects will complete an additional 9-day Phosphorus Balance study. Subjects will provide informed consent for the proposed Balance study. Of note, all research subjects will have an FMD_{BA} measurement at the beginning and end of the parent RCT or the 12-week run-in period. Subjects will continue taking their current dose of active drug (lanthanum carbonate) or placebo from the parent RCT or the 12-week run-in period and will begin a CTRC-prepared fixed low phosphorus diet (daily breakfast, lunch, dinner, and a snack) and will be instructed to consume only deionized water, which will be provided. They will also take polyethylene glycol 1 g with each meal (totaling 3g/day) as a non-absorbable stool marker. After 7 days, subjects will be admitted to the CTRC for a 48-hour admission.

For subjects recruited from the parent Phosphorus Lowering RCT:

- **Session 1:** Recruitment and Consent
    - All subjects currently participating in the parent Phosphorus Lowering RCT will be eligible to participate in the current Phosphorus Balance protocol
- **Session 2:** Beginning of proposed Phosphorus Balance study
    - Blood sampling to monitor serum phosphorus concentration, serum creatinine for measurement of kidney function, and evaluation of markers of mineral metabolism
    - Receive CTRC-prepared fixed low phosphorus diet (daily breakfast, lunch, dinner, and a snack) including deionized water
        - Mifflin St. Joeur equation and activity factor of 1.4 will be used to determine caloric needs. Based on their caloric needs, subjects will be assigned to one of four kilocalorie diet categories: 2000-2300, 2300-2600, 2600-2900, 2900-3200. The subjects will then select their meals from four different pre-set menus.
        - Macronutrients: 15% protein/ 30% fat/ 55% CHO ± 2% for each macro
        - The diets will contain the same proportion of plant and meat/dairy proteins to control for the different rates of absorption of phosphorus from plant sources versus phosphorus from meat/dairy sources
        - Micronutrients controlled: phosphorus < 1000±50 mg/day and calcium < 800±50 mg/day
        - Each of the 4 kilocalorie group diets will be frozen and sent for exact measurement of phosphorus and calcium content. Subjects will be required to pick-up food in 3-day increments to prevent spoilage
    - Continue current dose of lanthanum or placebo
    - Receive polyethylene glycol 1 g PO TID with meals as a stool marker
    - Submit 1 stool specimen prior to initiating polyethylene glycol to be used as a comparison
- **Session 3:** End of Phosphorus Balance Study
    - 48-hour admission to CTRC
        - Blood sampling to monitor serum phosphorus concentration, serum creatinine for measurement of kidney function, and evaluation of markers of mineral metabolism
        - 24-hour urine and stool collection for phosphorus and calcium measurement (2 will be collected)
        - Subjects will continue 1 g of polyethylene glycol with each meal as a non-absorbable stool marker during the CTRC inpatient admission.
        - Continue CTRC-prepared fixed low phosphorus diet
        - Continue current dose of lanthanum or placebo
    - Final FMD_{BA} measurement to be performed at the UPI Vascular Lab

Figure 1



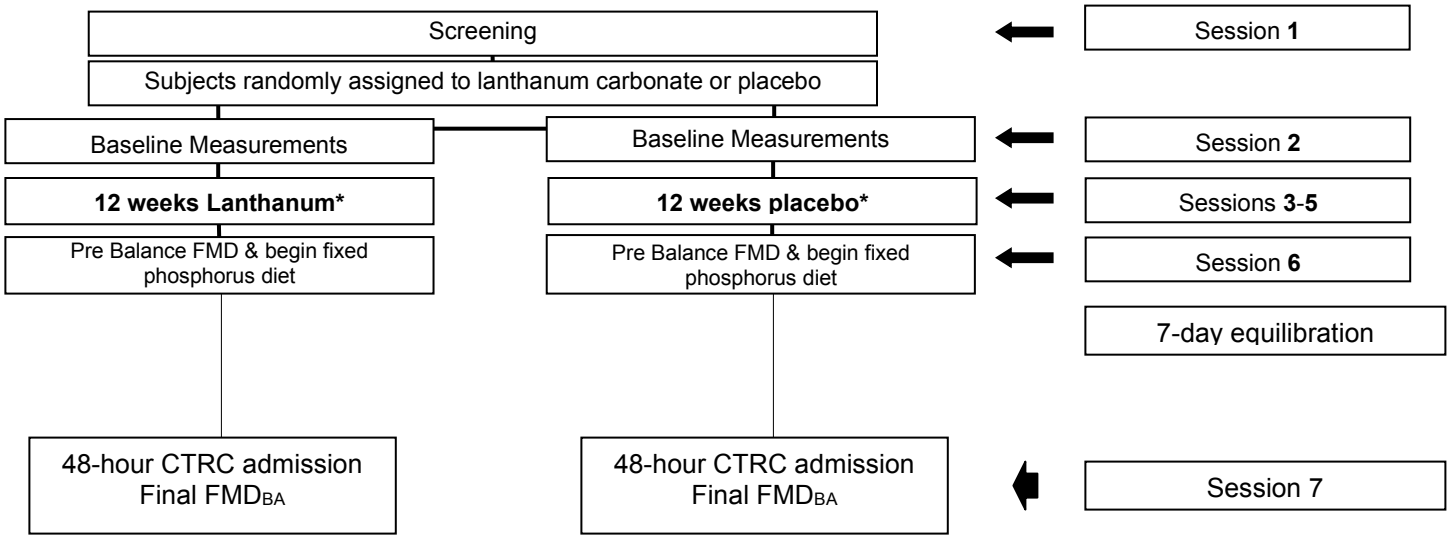


For subjects not enrolled in the parent Phosphorus Lowering RCT:

- **Session 1:** Consent and screening measurements
- **Session 2:** Baseline measurements
    - Resting arterial blood pressure
    - Blood sampling to monitor serum phosphorus concentration, serum creatinine for measurement of kidney function, and evaluation of markers of mineral metabolism
    - FMD_{BA} (endothelium-dependent dilation)
    - Endothelium-independent dilation (EID) to sublingual nitroglycerin
    - Randomization to either lanthanum carbonate or placebo
- **Session 3-5:** Biweekly/monthly check-in (see below)
- **Session 6:** Beginning of proposed Phosphorus Balance study (*same as Session 2 for those recruited from parent Phosphorus Lowering RCT*)
    - Resting arterial blood pressure
    - Blood sampling to monitor serum phosphorus concentration, serum creatinine for measurement of kidney function, and evaluation of markers of mineral metabolism
    - FMD_{BA} (endothelium-dependent dilation) and endothelium-independent dilation (EID) to sublingual nitroglycerin
    - Receive CTRC-prepared fixed low phosphorus diet (daily breakfast, lunch, dinner, and a snack) including deionized water
        - Mifflin St. Joeur equation and activity factor of 1.4 will be used to determine caloric needs. Based on their caloric needs, subjects will be assigned to one of four kilocalorie diet categories: 2000-2300, 2300-2600, 2600-2900, 2900-3200. The subjects will then select their meals from four different pre-set menus.
        - Macronutrients: 15% protein/ 30% fat/ 55% CHO ± 2% for each macro
        - The diets will contain the same proportion of plant and meat/dairy proteins to control for the different rates of absorption of phosphorus from plant sources versus phosphorus from meat/dairy sources
        - Micronutrients controlled: phosphorus < 1000±50 mg/day and calcium

< 800±50 mg/day
        - Each of the 4 kilocalorie group diets will be frozen and sent for exact measurement of phosphorus and calcium content. Subjects will be required to pick-up food in 3-day increments to prevent spoilage
    - Continue current dose of lanthanum or placebo
    - Receive polyethylene glycol 1 g PO TID with meals as a stool marker
    - Submit 1 stool specimen prior to initiating polyethylene glycol to be used as a comparison
- **Session 7:** End of Phosphorus Balance Study (*same as Session 3 for those recruited from parent Phosphorus Lowering RCT*)
    - 48-hour admission to CTRC
        - Blood sampling to monitor serum phosphorus concentration, serum creatinine for measurement of kidney function, and evaluation of markers of mineral metabolism
        - 24-hour urine and stool collection for phosphorus and calcium measurement (2 will be collected)
        - Subjects will continue 1 g of polyethylene glycol with each meal as a non-absorbable stool marker during the CTRC inpatient admission.
        - Continue CTRC-prepared fixed low phosphorus diet
        - Continue current dose of lanthanum or placebo
    - Final FMD_{BA} measurement to be performed at the UPI Vascular Lab

Figure 2



***Lanthanum dosing.** The starting dose of lanthanum will be determined from a sliding scale according to baseline serum phosphorus (Table 1). The dose of the phosphate binder will be titrated at week 2, week 4, and week 8 (Sessions 3-5) to achieve serum phosphorus less than 2.8 mg/dL. This dose will be up-titrated as needed based on serum phosphorus (if not <2.8 mg/dL, dose will be increased by 1 tablet [500 mg] for each meal). If serum phosphorus decreases to <2.5 mg/dL, the dose of lanthanum will be down-

titrated. Tablets will be taken with each meal (if a subject consumes <3 meals/day, the dosing will be adjusted accordingly).

Table 1:

Serum Phosphorus (mg/dL)	Starting Dose (mg)
2.8-4.0	1,500 (1 tablet with each meal)
4.1-4.5	3,000 (2 tablets with each meal)
4.6-5.5	4,500 (3 tablets with each meal)

For all subjects:
Phosphorus Balance. A simplified definition of balance will be used:

Balance = oral intake – urine output – stool output.

Subjects will be admitted to the CTRC to ensure adequate collection of 24-hour urine and stool collections; two 24-hour collections will be obtained during each CTRC admission. Stool phosphorus will be collected and measured according to previous protocols used at the CTRC.[25,26] The urine and stool collected during the 48-hour admissions to the CTRC will be averaged to give the best estimate of phosphorus balance for each subject. While calcium balance is not a study end-point, we are collecting urine and stool calcium as these data may be important in the interpretation of phosphorus balance. Polyethylene glycol (PEG, 3 g/day), a non-absorbable marker, will be given with meals during the 9-day study to assess steady state and will be measured in the stool using a turbidimetric assay.[26]

FMD_{BA}. Measurements will be made at the UPI Vascular Lab.

Lanthanum dosing. Subjects will continue taking the same dose of lanthanum carbonate or placebo as they are taking at the end of the phosphorus lowering RCT or during the 12-week run-in phase. There will be no dose titration unless the serum phosphorus falls below 2.5 mg/dL (the lower limit of normal), in which case the dose of lanthanum carbonate will be decreased by 1500 mg daily (equivalent to 1 tablet with each of three meals daily) at a time until the serum phosphorus concentration is above 2.5 mg/dL. Tablets will be taken with each meal.

Study Sites. Subjects will be recruited from the active prospective, randomized, placebo-controlled double-blind trial, Phosphorus Lowering to Treat Vascular Dysfunction in Chronic Kidney Disease (COMIRB #13-0328). Subjects for the parent Phosphorus Lowering RCT were recruited from the Nephrology Clinic at the University of Colorado Denver Anschutz Medical Campus and from the Denver VA Medical Center Renal Clinic with access to ≥950 CKD patients from all over the Denver/Boulder metro area (at least 33% with ICD 9 code 585.3 and 585.4 as diagnosis) with over 2146 visits in the past year. Subjects not recruited from the parent Phosphorus Lowering RCT will be recruiting from the Nephrology Clinic at the University of Colorado Denver Anschutz Medical Campus and from the Denver VA Medical Center Renal Clinic.

The recruitment and screening as well as FMD_{BA} measurements for the proposed Phosphorus Balance protocol will take place at the UPI Vascular lab, while the 48-hour admission for stool and urine collections as well as blood sampling will take place at the CTRC.

Randomization and Treatment. Once inclusion/exclusion criteria have been met and informed consent obtained, subjects will remain in the treatment group to which they were originally randomized in the Phosphorus Lowering RCT. Subjects not recruited from the parent Phosphorus Lowering RCT will be randomized to either lanthanum carbonate or placebo. Randomization will be performed by Dr. Zhiying You, a statistician, by blocks using a computer-generated procedure to ensure equal distribution. The lanthanum carbonate and placebo are prepared and provided by Shire and are identical. There is no cost to the PI for the study drug or placebo.

a) ***Study Drug*****:** Lanthanum carbonate (Fosrenol) is an FDA approved non-absorbed, non-calcium-containing phosphate binder to lower serum phosphate in patients requiring chronic dialysis.

b) ***Rationale for lanthanum dose:*** This dosing is indicated in patients with end-stage renal disease requiring chronic dialysis. The population being studied in this protocol will be healthier than this patient group. In addition, an IND exemption has been obtained from the FDA.

c) ***Blinding:*** The investigators and subjects will remain blind to the study intervention, as the lanthanum carbonate and placebo are identical. A nurse at the UPI Vascular lab will be responsible for administering the pills, which will be stored in a locked cabinet on site. A professional research assistant will be unblinded and will manage the study drug records and interact directly with the nursing staff for administration of the study drug. The investigators will be blinded to laboratory results, and the research assistant will monitor laboratory results for safety and inform the investigator if necessary.

d) ***Drug accountability****:* Pill counts will be assessed weekly at each CTRC admission.

e) ***Withdrawal from study:*** Withdrawal will be done at the patient's request, IRB request, or if there is a medical rationale for discontinuation as determined by the principal investigator.

Safety monitoring. The subjects will be instructed to call the PI or research coordinator with any subjective complaints, in addition, abnormal labs will be reported to the PI immediately, who will determine if the study drug needs to be discontinued. The following are reported adverse events of the study drug and the monitoring plan:

a) Lanthanum carbonate. The safety profile of lanthanum carbonate has been studied in over 5200 subjects in completed clinical trials. The most common adverse reactions for lanthanum carbonate are gastrointestinal events, such as nausea, vomiting, and abdominal pain and they generally abate over time with continued dosing. In end-stage renal disease patients receiving 4-6 weeks of treatment with lanthanum carbonate the incidence of nausea was 11% (vs. 5% placebo), vomiting 9% (vs. 4%), and abdominal pain 5% (vs. 0%). Lanthanum carbonate may reduce calcium absorption and result in hypocalcemia (~5%, same incidence as active comparator group). The following additional adverse reactions have been identified during post-approval use of lanthanum carbonate: constipation, dyspepsia, allergic skin reactions, hypophosphatemia, and tooth

injury (incidence not known). Lanthanum carbonate may decrease bioavailability of cationic antacids, quinolone antibiotics, or levothyroxine.

There have been reports of serious cases of gastrointestinal obstruction, ileus, and fecal impaction reported in association with lanthanum carbonate, some requiring surgery or hospitalization. Risk factors for gastrointestinal obstruction identified from post-marketing reports include alteration in gastrointestinal anatomy (e.g., history of gastrointestinal surgery, colon cancer), hypomotility disorders (e.g., constipation, ileus, diabetes), and concomitant medications (e.g., calcium channel blockers). Some cases were reported in patients with no history of gastrointestinal disease.

Hypophosphatemia has been reported only in post-marketing surveillance. The risk of hypophosphatemia is very low. Clinically significant (causing symptoms) hypophosphatemia associated with the use of lanthanum carbonate has not been reported.

Safety measures will include: lanthanum carbonate dose titration and close monitoring of serum phosphate (as described above) to avoid severe hypophosphatemia, excluding patients at high risk for gastrointestinal events or current bowel obstruction, ileus and fecal impaction, repeatedly emphasizing to subjects the importance of chewing tablets completely to reduce the risk of serious adverse gastrointestinal events such as those described above. Per standard operating procedure, subjects will also be instructed to take concomitant drugs at least one hour before or four hours after lanthanum carbonate to avoid potential interactions, and blood levels of concomitant drugs with a narrow therapeutic range will be monitored if deemed appropriate by Dr. Jovanovich.

Patient Stopping Criteria:

- Withdrawal of informed consent (subject's decision to withdraw for any reason)
- Any clinical adverse event (AE), laboratory abnormality, or intercurrent illness which, in the opinion of the investigator, indicates that continued participation in the study is not in the best interest of the subject
- Loss of ability to freely provide consent through imprisonment or involuntary incarceration for treatment of either a psychiatric or physical (e.g., infectious disease) illness
- Inability to comply with protocol
- Use of an investigational therapy or device other than study medication
- Significant non-compliance with protocol (i.e., procedures, assessments, medication, etc.)
- Hypocalcemia (<8.6 mg/dL) that does not resolve with decreased dosage of study drug
- Hypophosphatemia (<2.5 mg/dL) that does not resolve with decreased dosage of study drug
- Hospitalization for gastrointestinal obstruction, ileus, or fecal impaction
- Serious acute hypersensitivity reactions to investigational drug
- Any major cardiovascular event such as myocardial infarction, stroke, unstable angina, thromboembolic event, or a clinically significant deterioration of a baseline cardiovascular condition.

Study Stopping Criteria:
The study may be stopped if:

- The data show a significantly increased risk of serious adverse effects in one of the treatment groups.
- It becomes clear that successful completion of the study is not feasible (e.g. there is an excess of patient dropout, missing data, lack of recruitment, etc.).

Standard medical treatment for all subjects/concomitant medications includes:
All subjects must be on a stable anti-hypertensive, diabetic, and lipid lowering regimens for at least one month prior to inclusion.
a) Control of hypertension with anti-hypertensives with goal blood pressure less than 130/80 mm/Hg. Medication choice includes angiotensin-converting enzyme inhibitor/angiotensin receptor blocker as first line agent, diuretic as second line agent, calcium-channel blocker as third line agent, and beta-blocker as fourth line agent (unless patient has known CVD and needs to be on a beta-blocker regardless of blood pressure). b) Diabetes will be treated as needed to goal HbA1C of 7.0%. c) Control of hyperlipidemia with statins to goal of low-density lipoprotein cholesterol (<100 mg/dL and total cholesterol levels to less than 200 mg/dL. d) Smoking cessation counseling. e) Control of anemia following the latest recommendation from the National Kidney Foundation.

Data Collection
Medical History, Physical Examination, and Blood Chemistries. Subjects recruited for the phosphorus lowering RCT reported fasted for assessment of serum phosphorus and a standard blood chemistry analysis including a metabolic panel (with creatinine/calcium), lipid panel, and follicle stimulating hormone (women only if not post-menopausal), then underwent a medical history and physical examination. Subjects continuing in the proposed phosphorus balance study will undergo another physical examination and fasted assessment of serum phosphorus and a standard metabolic panel including creatinine and calcium.

Data management:
Study data will be collected and managed using REDCap (Research Electronic Data Capture). REDCap is a secure, web application designed to support data capture for research studies, providing user-friendly web-based case report forms, real-time data entry validation (e.g. for data types and range checks), audit trails, and a de-identified data export mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus). The system was developed by a multi-institutional consortium, which included the University of Colorado Denver and was initiated at Vanderbilt University. Currently 52 institutions in 4 countries are using the software. The database is hosted at the University of Colorado Denver Development and Informatics Service Center (DISC), which will be used as a central location for data processing and management. REDCap data collection projects rely on a thorough study-specific data dictionary defined in an iterative self-documenting process by all members of the research team with planning assistance from the DISC. The iterative development and testing process results in a well-planned data collection strategy for individual studies. Both REDCap and REDCap Survey systems provide secure, web-based applications that are flexible enough to be used for a variety of types of research, provide an intuitive interface for users to enter data and have real time validation rules (with automated data type and range checks) at the time of entry. These systems offer easy data manipulation with audit trails and reporting for reporting,

monitoring, and querying patient records, and an automated export mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus). Both systems were found to meet or exceed all of the security audit requirements.

Data Safety and Monitoring:
The Department of Veterans Affairs Clinical Science Research and Development, Central Data Monitoring Committees (DMC) is acting as the DSMB for the parent RCT (13-0328). Since this is a VA-funded study, it is expected that the Central VA DMC will act as DSMB for the current protocol as well.

D. Description, Risks and Justification of Procedures and Data Collection Tools:

a) Conduit artery vascular endothelial dependent dilation (EDD) and endothelial independent dilation (EID): Conduit artery EDD, brachial artery flow-mediate dilation (FMD_{BA}), will be determined using high-resolution ultrasonography (GE Vivid7 Dimension) as described originally by Celermajer et al.[29] and used recently by the co-mentor.[31,32] ECG-gated end-diastolic ultrasound images and Doppler flow of the brachial artery will be acquired during baseline and FMD_{BA} conditions. For FMD_{BA}, reactive hyperemia will be produced by inflating a pediatric BP cuff around the forearm to 250 mmHg for 5 minutes followed by rapid deflation. Brachial artery endothelium-independent dilation (EID), an index of smooth muscle sensitivity to exogenous nitric oxide, will be determined by measuring brachial artery dilation for 10 minutes after administration of sublingual nitroglycerin (0.4 mg).[32,33] A commercially available software package (Vascular Analysis Tools 5.8.1, Medical Imaging Applications) will be used to acquire ECG-gated brachial artery diameters. As recently recommended,[34] FMD_{BA} will be determined as the mm and % Δ from baseline diameter.[32-34] Shear rate will also be calculated, and if group- or condition- differences exist, FMD_{BA} will be adjusted accordingly.[34-36]

Risks of this procedure include: The assessment of FMD_{BA} is minimally invasive. Inflating the blood pressure cuff below the elbow during this procedure may cause a mild to moderate intensity “pins and needles or numbing” sensation that goes away as soon as the cuff is deflated. In addition, patients will receive a dose of sublingual nitroglycerin to measure EID, an essential control measure without which the results cannot be properly interpreted. Sublingual nitroglycerin is a medication with a rapid vasodilator effect and is routinely used in patients with coronary artery disease to help relieve chest pain. Side effects of sublingual nitroglycerin are short-lived (30-60 minutes). Approximately 60% of people who receive nitroglycerin will experience a headache. The subject may experience symptoms such as lightheadedness, and in rare instances (1 in 1000) lowered heart rate, which could lead to fainting. However, these risks are primarily when a person is upright, and subjects will be lying supine during administration of nitroglycerin. Finally, placing the pill under the tongue could cause irritation to the area in the back of the mouth. The subjects will be monitored by frequently asking about subjective complaints, as well as by frequent heart rate and blood pressure measurements (prior to and every 15 minutes after taking the medication for 1 hour). In addition, during the 10-minute period when brachial artery dilation to nitroglycerin is assessed (peak activity of drug), blood pressure and heart rate will be assessed every 2 minutes. In the rare case that blood pressure drops drastically, an intravenous catheter will be available for infusion of normal saline. Still, nitroglycerin has been administered safely for over a decade in the laboratory the co-mentor performed her doctoral training, as well as in other laboratories and in clinical situations worldwide. Per standard operating procedure, nitroglycerin will not be given to

any subject with a systolic blood pressure less than 100 mmHg on the day the measurements are performed, history of migraine headaches, or previous adverse reaction to administration.

Justification: As discussed above, patients with CKD have an increased risk of death due to CVD, and endothelial dysfunction plays a critical role in the development of CVD. Endothelial dysfunction is evident early in the course of kidney disease. It is unknown if lowering phosphorus improves vascular endothelial function in CKD patients. FMD_{BA} is a non-invasive way to assess endothelial function. The other method available to study endothelial function (EDD) would include acetylcholine administration via brachial artery infusion, which is more invasive than the proposed methodology. In order to interpret improvements in brachial artery dilation in response to a shear stress (FMD_{BA}) following the drug intervention, it is necessary to assess smooth muscle sensitivity to nitric oxide using EID. If EID is unchanged in the presence of improved FMD_{BA}, this indicates the improved dilation in response to a shear stimulus is due to positive adaptations at the level of the vascular endothelium (i.e. endothelium-dependent).

b) Laboratory Analyses: The majority of the laboratory measurements will be performed at the University of Colorado Clinical Laboratory using standard techniques. Samples will be collected by research nurses at the CTRC and sent to the clinical laboratory or core laboratory. Serum to measure phosphorus and the comprehensive metabolic panel will be immediately sent to the University of Colorado Clinical Laboratory for analysis. Urine will also be sent to the University of Colorado Clinical Laboratory for analysis of phosphorus, calcium, and creatitine to avoid degradation and inaccurate measurement of these substances, which may occur with prolonged storage. The rest of the serum, and feces will be collected and immediately processed for storage in a -80° C freezer until the end of the study when they will be batched and analyzed. The stored serum will be used to measure markers of mineral metabolism, including fibroblast growth factor 23 (FGF23), intact parathyroid hormone (iPTH), 25 hydroxyvitamin D (25(OH)D), 1,25 dihydroxyvitamin D ($1,25(OH)_2D$), and the stored stool will be used to measure phosphorus.

For subjects recruited from the parent Phosphorus Lowering RCT:

- *Serum phosphorus* **(Visits 2,3)**: Serum phosphorus (1 mL serum) will be measured using standard methodology for safety monitoring of serum phosphorus levels during treatment with active and placebo drugs and also to inform interpretation of data at the study end.

- *Comprehensive metabolic panel* **(Visits 2,3)**: A comprehensive metabolic panel (4.5 mL plasma) will be performed at the beginning and end of the study using standard methodology.

- *Fibroblast growth factor 23 (FGF23)* **(Visits 2,3)**: FGF23 (200 µL serum) will be measured at the beginning and end of the study as a marker of mineral metabolism.

- *Intact Parathyroid hormone (iPTH)* **(Visits 2,3)**: iPTH (4.5 mL plasma/serum) will be measured at the beginning and end of the study as a marker of mineral metabolism using standard methodology.

- *25 hydroxyvitamin D (25(OH)D)* **(Visits 2,3)**: 25(OH)D (4.5 mL serum) will be measured at the beginning and end of the study as a marker of mineral metabolism.

- *1,25 dihydroxyvitamin D (1,25(OH)$_2$D)* **(Visits 2,3)**: 1,25(OH)$_2$D (1 mL serum) will be measured at the beginning and end of the study as a marker of mineral metabolism.

- *Stool phosphorus* **(Visit 3)**: Two 24-hour stool collections will be collected during each of the CTRC admissions. Stool samples will be collected, frozen, and sent for measurement of phosphorus content.

- *Stool calcium* **(Visit 3)**: Two 24-hour stool collections will be collected during each of the CTRC admissions. Stool samples will be collected, frozen, and sent for measurement of calcium content.

- *Stool polyethylene glycol* **(Visit 1-2)**: One stool sample will be collected (this can be completed at the subjects' home) and submitted for measurement of polyethylene glycol as a baseline marker prior to initiating the balance study. Content of polyethylene glycol will also be measured in the stool collected during the 48-hour CTRC admission as a stool marker.

- *Urine phosphorus* **(Visits 3)**: Two 24-hour urine collections will be collected during each of the CTRC admissions. Urine phosphorus will be measured using standard technique.

- *Urine calcium* **(Visit 3)**: Two 24-hour urine collections will be collectd during each of the CTRC admissions. Urine calcium will be measured using standard technique.

- *Urine creatinine* **(Visit 3)**: Two 24-hour urine collections will be collected during each of the CTRC admission. Urine creatinine will be measured using standard technique and will be used to interpret and inform 24-hour urine phosphorus measurements.

<u>For subjects not enrolled in the parent Phosphorus Lowering RCT</u>:

- *Serum phosphorus* **(Visits 1-7)**
- *Comprehensive metabolic panel* **(Visits 1-7)**
- *Fibroblast growth factor 23 (FGF23)* **(Visits 2,6,7)**
- *Intact Parathyroid hormone (iPTH)* **(Visits 2,6,7)**
- *25 hydroxyvitamin D (25(OH)D)* **(Visits 2,6,7)**
- *1,25 dihydroxyvitamin D (1,25(OH)$_2$D)* **(Visits 2,6,7)**
- *Stool phosphorus* **(Visits 6,7)**
- *Stool calcium* **(Visits 6,7)**
- *Stool polyethylene glycol* **(Visits 6,7)**
- *Urine phosphorus* **(Visits 2,6,7)**
- *Urine calcium* **(Visit 6,7)**
- *Urine creatinine* **(Visit 2,6,7)**

Risks of this procedure include: All of the lab work will be obtained via venipuncture and the insertion of an 18-gauge cannula. The risks that are entailed in this procedure include discomfort and pain of venipuncture, failed attempt resulting in more than one attempt, bleeding upon cannula removal, and rarely thrombophlebitis (a non-infectious inflammatory state of the vein typically due to prolonged cannulation). A cannula will be inserted in the dorsal aspect of the hand to infuse the saline in order to help preserve access in the arm. Subjects may be at a very small risk of anemia from the blood draws required for the assays described above. However, only the minimum amount of blood needed will be drawn and experienced research nurses/nurse practitioners will be collecting the specimens. All blood draws will be performed on the arm not designated for future vascular access for dialysis.

Justification: Considered markers of mineral metabolism, FGF23, iPTH, 25(OH)D, and $1,25(OH)_2D$, are hormones that regulate the absorption of phosphorus from the gut and its excretion in the kidney. In earlier stages of CKD, serum phosphorus levels are maintained with the normal range by up-regulating FGF23 and a consequent increase in the fractional excretion of phosphorus by the kidney. FGF23 also regulates 1-α-hydroxylase and thus the conversion of nutritional vitamin D (25(OH)D) to its active form, $1,25(OH)_2D$, which in turn affects calcium and iPTH. Markers of mineral metabolism become dysregulated in CKD.[10-13] To better understand and interpret changes in phosphorus and phosphorus balance, especially in subjects with CKD, it is necessary to know the changes that occur in the levels of these markers of mineral metabolism.

E. Potential Scientific Problems:

Although subject recruitment/retention are always challenging, we should be able to meet target enrollment given that we will be recruiting from an active randomized controlled trial and we are expanding the protocol to enroll subjects in a parallel run-in phase should we be unable to achieve adequate enrollment solely from the parent RCT. We expect few difficulties with the proposed experimental procedures and protocols. Dr. Jablonski, co-investigator, has employed the measurement of FMD_{BA} during her graduate training, and numerous protocols measuring FMD_{BA} are successfully underway at the UPI Vascular Laboratory. Furthermore, a calcium balance study utilizing a similar protocol was successfully conducted in healthy and CKD subjects at the CTRC by investigators associated with our group. We recognize that alternative phosphate binders could have been selected; however, we chose lanthanum carbonate a) because calcium-containing phosphate binders have been used in other balance studies and may confound results; b) to avoid the potential for hypercalcemia as observed with calcium-containing phosphate binders; and c) because calcium-containing binders have not improved EDD in hyperphosphatemic CKD subjects;[37] and d) to control for the beneficial effects that may occur with sevelamer by mechanisms other than phosphorus lowering.[38-40] We also recognize that it would be of interest to compare lanthanum compare with other phosphate binders, but given the duration and scope of the project, feel it is necessary to first compare lanthanum carbonate with placebo. Finally, some common cardiovascular disease drugs, particularly statin therapy, have established vascular effects. To control for statin use, subjects on statin therapy at the beginning of the parent study will remain on the same dose during the parent study and during the current protocol. Subjects not on statin therapy will be asked not to initiate statin therapy until the end of both the parent

and current protocols. If there is unequal distribution of statin use in the active and placebo group, we will control for this using statistical modeling.

F. Data Analysis Plan:

We hypothesize that subjects treated with lanthanum carbonate will be in negative phosphorus balance compared to subjects treated with placebo. In addition, we expect that improvements in EDD will correlate with a negative phosphorus balance. An N = 12 from each group will be entered into the balance study (total N = 24). We will compare phosphorus balance between the two groups using an Independent Samples T-test. Data published previously[26] demonstrated that phosphorus balance was achieved on oral calcium carbonate vs. placebo (phosphorous balance of 153 vs. 95 mg/d with a SD = 82); however, the sample size of 8 in a crossover design was not enough to conclude equivalence. We propose to use N = 12 per group, which will give a two group 0.050 two-sided t-test 81.3% power with a standard deviation of 43. The results of the current protocol will be used to aid in the interpretation of the parent Phosphorus Lowering RCT. Pearson correlation coefficients will be used to determine the correlation between phosphorus balance and FMD_{BA} at the end of the 9-day diet intervention (i.e., the end of the current protocol) in each treatment group.

G. Summarize Knowledge to be Gained:

The findings from the proposed research should provide important new information regarding phosphorus balance in CKD and its effect on vascular function in this population of patients subject to a high degree of CVD morbidity and mortality. No phosphorus balance study in CKD patients treated with a non-calcium-containing phosphate binder, such as lanthanum carbonate, has ever been conducted. Moreover, the length of time subjects will be treated with active or placebo drug prior to balance measurements and the numerous measurements of stool and fecal phosphorus exceeds that of other studies. Together, this information will provide important insight into phosphorus metabolism and vascular function in CKD.

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