Effects of Dietary Phosphorus Restriction on Whole-Body Calcium and Phosphorus Balance and Kinetics in Patients with Moderate Chronic Kidney Disease

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Abbreviations

1,25D	1,25-Dihydroxyvitamin D
25D	25-Hydroxyvitamin D
AE	Adverse Event
ANOVA	Analysis of Variance Analysis
Ca	Calcium
Ca-44/^{44}Ca	Calcium-44 stable isotope
CKD	Chronic Kidney Disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CKD-MBD	Chronic Kidney Disease-Mineral Bone Disorder
CRC	Clinical Research Center
CTSI	Clinical and Translational Science Institute
DSMB	Data Safety Monitoring Board
eGFR	Estimated Glomerular Filtration Rate
FGF23	Fibroblast Growth Factor 23
HIPAA	Health Insurance Portability and Accountability Act
IRB	Institutional Review Board
IV	Intravenous
LCa/HP	Low Calcium/High Phosphorus Diet
LCa/LP	Low Calcium/Low Phosphorus Diet
NIH	National Institutes of Health
P	Phosphorus
P-33/^{33}P	Phosphorus-33 Radioisotope
PEG	Polyethylene Glycol
PI	Principal Investigator
PTH	Parathyroid Hormone
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event

1.0 Background & Rationale

Chronic kidney disease (CKD) is a common chronic disease, affecting approximately 26 million American adults (1). CKD-mineral bone disorder (CKD-MBD) is a condition of disordered bone and mineral metabolism that contributes to morbidity and mortality in patients with CKD (2). CKD-MBD is characterized by the presence of all or some of the following features: laboratory abnormalities related to calcium and phosphorus homeostasis, bone disease, and vascular calcification. These abnormalities begin to appear early in the disease process (3), and virtually all patients with moderate to advanced CKD exhibit some or all features of the disorder. The clinical consequences of CKD-MBD are dire and include increased cardiovascular events, bone fragility fractures, and mortality (4-7). In fact, cardiovascular events are the leading cause of death in patients with CKD (8, 9). Thus, prevention and management of CKD-MBD is of critical importance to reduce morbidity and mortality in these patients. Calcium (Ca) and phosphorus (P) homeostasis are central to CKD-MBD development. ***However, lack of understanding on Ca and P metabolism in CKD-MBD progression precludes the development of effective therapies.***

Ca and P are intimately related in physiology and metabolism(10). They share common hormonal regulators that include parathyroid hormone (PTH), 1,25-dihydroxyvitamin D (1,25D), and fibroblast growth factor 23 (FGF23) that act on a common tissue axis of intestine, kidney, bone, and parathyroid glands. Treatments aimed at managing CKD-MBD affect the hormonal and tissue axes of both ions and have diverse effects on Ca and P serum levels. For example, calcitriol lowers serum P while increasing serum Ca (11, 12); whereas calcimimetics lower serum P while also lowering serum Ca (13). More importantly, serum or plasma

levels of Ca or P do not reflect whole-body status of either mineral (14, 15). This challenges both clinical research on Ca and P homeostasis, as well as clinical treatment decision-making. Thus, there is a ***critical need*** for knowledge of whole-body Ca and P physiology that can only be obtained from formal metabolic balance and kinetic studies of both ions simultaneously. However, such studies are largely absent in the scientific literature. Thus, ***significant knowledge gaps*** exist regarding whole-body Ca and P physiology that include how dietary Ca and P intakes, as well as kidney disease and its treatments, influence both Ca and P whole-body retention, intestinal absorption, and the transfer of Ca and P to and from the various body pools, such as intestine, bone, blood, and soft-tissue. Dietary P restriction is a commonly prescribed therapy for patients with CKD because it is presumed to reduce P retention and serum P, but this is based on little evidence (16). It is important to determine the effectiveness of dietary P restriction on its proposed benefits, because it is a difficult diet for patients to follow and may lead to consequences such as dietary protein or calcium deficiency, which can have negative consequences for bone health and mortality risk. The ***goal*** of this project is to generate preliminary data, including estimates of effect sizes and variances, on the effects of dietary P restriction therapy on Ca and P whole-body balance and full kinetics in patients with CKD. We will use this data in a future large research grant proposal that will include an adequately powered study to test the ***hypothesis*** that a high P diet, in the context of a typical low Ca diet, leads to P retention with negative Ca balance, and that dietary P restriction modestly reduces P retention but does not alleviate negative Ca balance. This will provide a foundation for further studies examining the effects of other factors on whole-body Ca and P physiology, including common pharmaceutical agents (e.g., various P binders, calcitriol, and calcimimetics), biological factors (e.g., age, race, and sex), CKD severity, and other nutritional interventions.

Additionally, preliminary and feasibility data will be collected on magnesium (Mg) absorption using stable oral and IV Mg isotopes (similar to the use of stable Ca isotopes). Dietary Mg and absorption is of interest in CKD patients as a potential inhibitor of calcification. Yet, to our knowledge, Mg absorption has not been studied in CKD patients (though stable Mg isotopes have been used for absorption studies in human subjects ranging from infants to postmenopausal women) (17-19).

2.0 Objectives

Aim 1. Determine intestinal Ca P, and Mg fractional absorption, effect sizes and variance estimates in patients with CKD on a low Ca diet with high P diet versus dietary P restriction. We will give CKD patients oral and IV Ca P and **and Mg** isotopes during a controlled-feeding, 48-hour inpatient study. In this study, we will use two dietary conditions and a randomized 2-period, cross-over design (low Ca/high P & low Ca/low P). Fractional Ca, P and Mg intestinal absorption will be determined by kinetic modeling of serum and urine oral and IV tracer data.

Aim 2. Determine Ca, P, and Mg balance and full kinetics, effect sizes and variance estimates in patients with CKD on a low Ca diet with high P diet versus dietary P restriction. A subset of subjects from Aim 1 will be studied as inpatients for an additional 12 days (5 as inpatient) after the 48-hour inpatient absorption testing protocol and in the same randomized 2-period cross-over design with the low Ca/high P & low Ca/low P diets. Total Ca, P, and Mg data will be determined from diet, urine, and fecal samples, and will be used to determine mineral balances. Further, we will use serum, fecal, and urine tracer data to determine full kinetic modeling including rates of transfer of the three ions between body pools, such as to and from bone.

3.0 Outcome Measures/Endpoints

3.1 Primary Outcome Measures

1. **Fractional Ca Absorption**, by kinetic modeling of Ca-44 isotopic data (by ICP-MS) over 48 hour between Days 8-10
2. **Fractional P Absorption**, by kinetic modeling of P-33 isotopic data (from scintillation counting) over 48 hour between Days 8-10
3. **Calcium Balance**, calculated from diet, urine, and fecal Ca assessed by ICP-OES, throughout Days 8-14

4. **Phosphorus Balance**, calculated from diet, urine, and fecal P assessed by ICP-OES, throughout Days 8-21(the last week is outpatient follow-up)
5. **Calcium Kinetics**, by full kinetic modeling of Ca-44 isotopic data (by ICP-MS), throughout Days 8-21(the last week is outpatient follow-up)
6. **Phosphorus Kinetics** by full kinetic modeling of P-33 isotopic data (by scintillation counting), throughout Days 8-21

3.2 Secondary Outcome Measures

1. **Fractional Mg Absorption**, by kinetic modeling of Mg isotopic data (by ICP-MS) over 48 hour between Day 8-10
2. **Magnesium Balance**, calculated from diet, urine, and fecal P assessed by ICP-OES, throughout Days 8-14
3. **Magnesium Kinetics** by full kinetic modeling of Mg isotopic data (by ICP-MS), throughout Days 8-21 (the last week is outpatient follow-up)
4. **Serum & Urine Ca** by standard clinical chemistry, all time points collected
5. **Serum & Urine P** by standard clinical chemistry, all time points collected
6. **Serum & Urine Mg** by standard clinical chemistry, all timepoints collected
7. **Serum Fibroblast Growth Factor 23, intact**, by ELISA kit, Day 1, all time points on Day 8-10, Day 15
8. **Serum Parathyroid Hormone, intact**, by ELISA kit, Day 1, all time points on Day 8-10, Day 15
9. **Serum 1,25-dihydroxyvitamin D,** by ELISA kit, Day 1, all time points on Day 8-10, Day 15

4.0 Eligibility Criteria

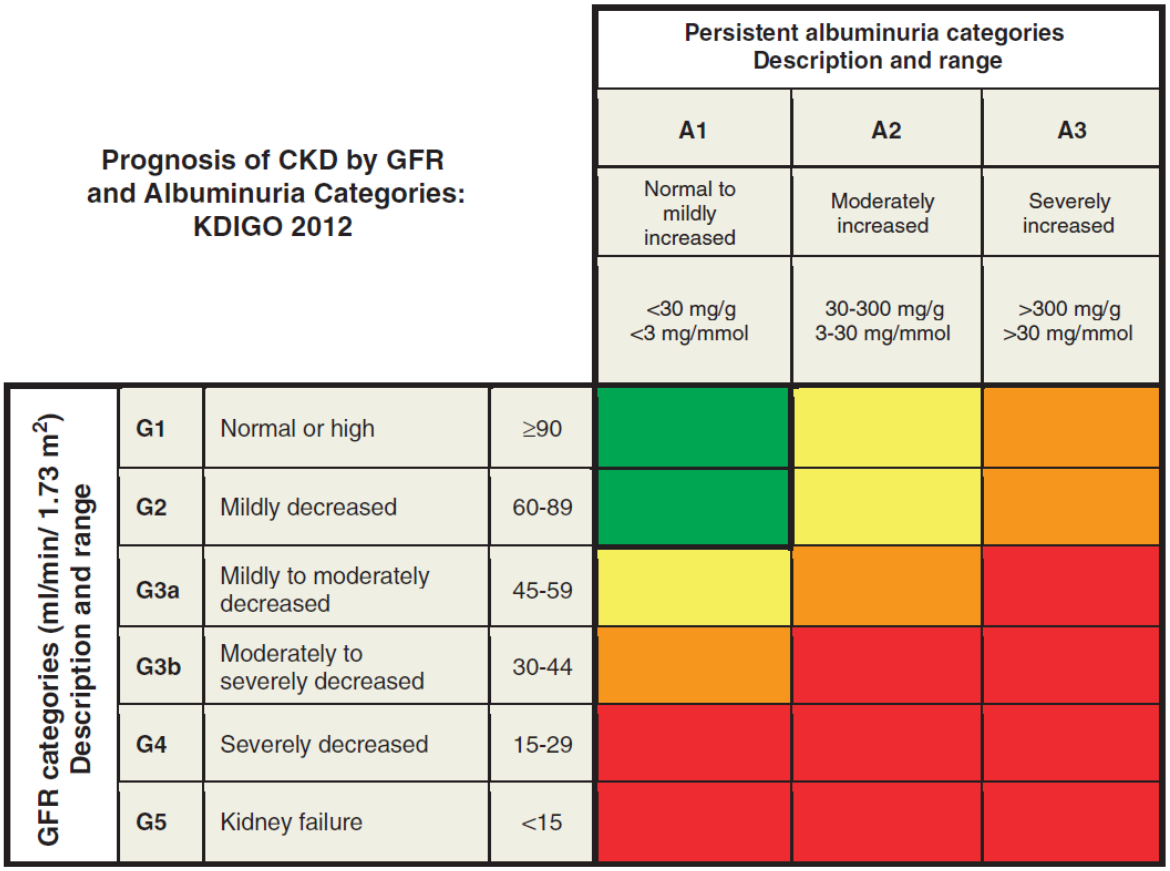
4.1 Inclusion Criteria

- Men or women, ages 30-75 years old, any race or ethnicity
- Moderate CKD, defined by KDIGO as eGFR category 3b (30-44 mL/min), with albuminuria categories A1-A3 (**Figure 1**)
- Female subjects must be postmenopausal (>12 months since last menstrual period), surgically sterile, or confirmed not pregnant by pregnancy test
- Must be on stable doses of medications (except those noted in exclusion criteria) for at least 4 weeks prior to the study
- Willing to discontinue nutritional supplements (e.g. vitamin D, calcium, multivitamin/minerals, or others) upon enrollment until completion of the study
- Adequate vitamin D status defined as serum 25D > 20 ng/mL

4.2 Exclusion Criteria

- Plans to initiate dialysis within 6 months
- Hypercalcemia defined as serum calcium >10.5 mg/dL within past 3 months
- Hyperkalemia defined as serum potassium >5.5 mg/dL within past 3 months
- Hyperphosphatemia defined as serum phosphate >5.5 mg/dL within past 3 months
- Intestinal disease that alters absorption or normal intestinal function including celiac disease, small bowel resection, or bariatric surgery
- Serious, *uncontrolled* underlying systemic disease including diabetes, lupus, hypertension (as deemed by the PI).
- Pregnant or breastfeeding
- Taking a phosphate binder medication, calcitriol, vitamin D analogs, calcimimetics, PTH analogues, and other medications that may alter Ca and P metabolism within past 30 days
- Vegan or vegetarian diet, lactose intolerance and/or milk allergy

Figure 1. Current CKD Nomenclature Used by KDIGO Guidelines



Green: low risk (if no other markers of kidney disease, no CKD); Yellow: moderately increased risk; Orange: high risk; Red, very high risk.

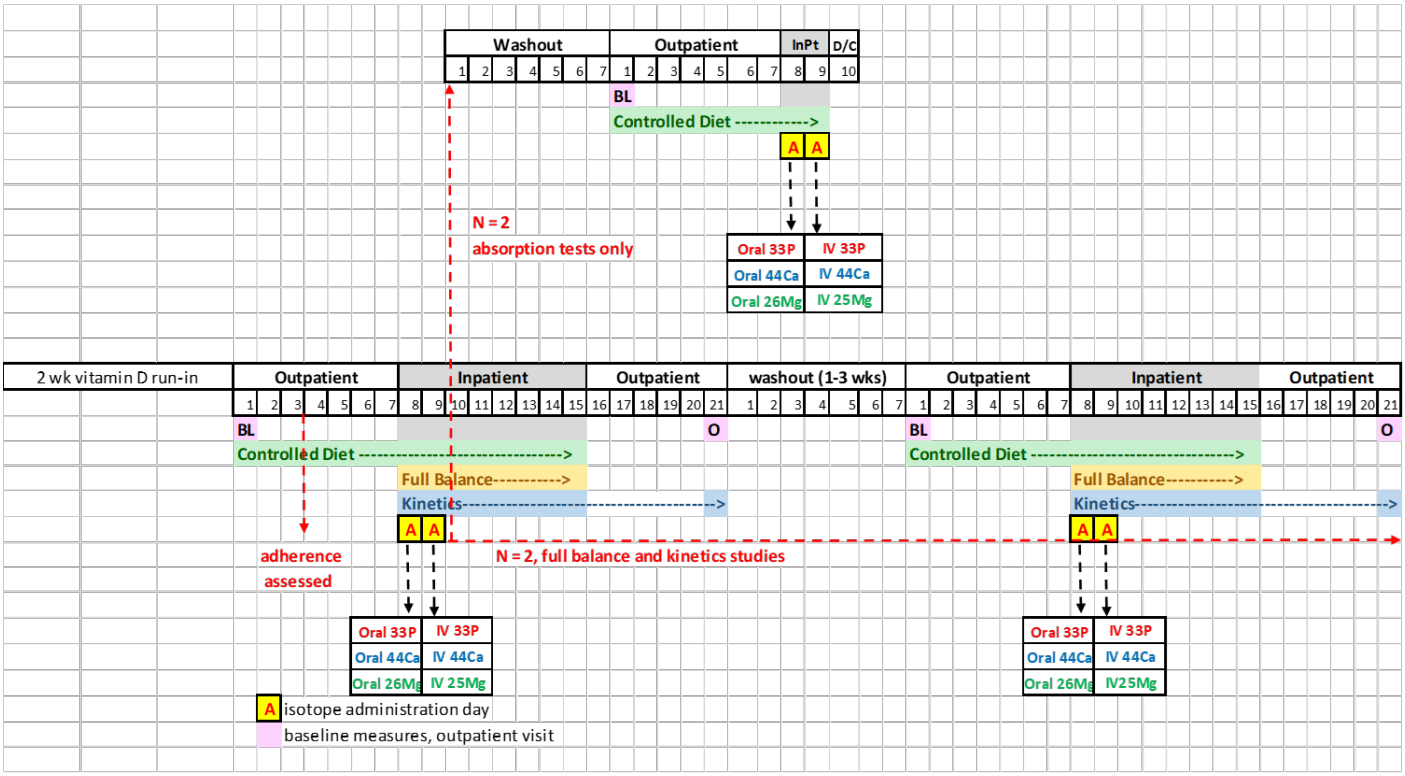
5.0 Study Design

The study design is a randomized 2-period cross-over study. **Figure 2** below describes the study timeline for participants enrolled in the study (a larger version is in attachments). All outpatient and inpatient visits will take place at the Indiana CTSI Clinical Research Center located in IU Health University Hospital in Indianapolis, IN.

For Aim 1, N = 4 subjects will be randomly assigned to controlled diets of either low Ca/high P (LCa/HP) or low Ca/low P (LCa/LP) for a 1-week, outpatient diet equilibration period, followed by a 48-hour inpatient Ca, P, and Mg absorption testing protocol. After a 1-3 week washout period, subjects only participating in Aim 1 (N = 2) will crossover to the other diet condition for another 1-week outpatient diet equilibration period, followed by another 48-hour inpatients Ca, P, and Mg absorption testing protocol.

For Aim 2, a subset of N = 2 subjects from Aim 1 will be studied as inpatients for an additional 5 days then a 7-day outpatient follow up for full Ca, P, and Mg balance and kinetic measures, after the 48-hour inpatient absorption testing protocol described under Aim 1. After a 1-3 week washout, they will crossover to the other diet condition for another 1-week outpatient diet equilibration period, followed by another 48-hour inpatient Ca, P, and Mg absorption testing protocol and additional 5 days inpatient and 7-day outpatient follow-up period for full balance and kinetics.

Figure 2. Study Timeline (larger version in Appendix)



6.0 Enrollment/Randomization

We aim to study N = 4 subjects, although seek approval enrolling up to 6 subjects due to the small sample size of 4 and the need for high adherence. Based on study diet and urine and fecal collection adherence of >90%, N = 2 patients that meet this level of adherence will be asked to continue with the full balance and kinetics protocol (Aim 2) on a first-enrolled basis. Thus 2 subjects will complete the absorption study (Aim 1), and 2 subjects will complete the full balance and kinetic studies (Aim 1 and Aim 2). It will be determined before a subject signs consent which consent they will sign (Consent A = Aim 1 or consent B= Aim 1 and Aim 2).

Recruitment will target moderate stage CKD patients aged 30-75 years old. Subjects will be recruited from patients seen in CKD clinics of the IU/Eskenazi Nephrology group who are partners of the co-investigators.

- Personnel from the IUSM Division of Nephrology will review medical charts from nephrology clinics for pre-screening to determine potential eligibility. An email will be sent to the doctors of the identified potential subjects to obtain their permission for enrollment and the potential subjects will be contacted by phone or visited by study staff during clinic.
- Information about the study may be discussed with interested potential subjects by research personnel by phone, email or in person as preferred by the subject/clinic.
- If interested, potential subjects will be either 1) scheduled for a screening visit at the Indiana Clinical Research Center, or 2) have screening information collected at the time of recruitment if subject is recruited in person at a CKD clinic by study staff.
- Prior to screening, subjects will be asked to provide written informed consent, and will be given the opportunity to have their questions about participation answered.
- Following the screening visit, all data will be evaluated by the study staff to determine eligibility of the subject. If eligible, participants will be enrolled in the study, and study visit dates will be scheduled.

Randomization Plan

At the beginning of the study, the study biostatistician will generate a randomization list for dietary intervention crossover order A or B by pairs. These orders will be A=LCa/HP to LCa/LP and B=LCa/LP to LCa/HP. Especially due to the small sample size, attention will be made to ensuring a balance of the two orders, which will be accomplished by randomizing both in pairs.

7.0 Study Procedures

All study procedures will be conducted for the purpose of research.

Screening Visit

Following informed consent, a screening visit of approximately 60 minutes to assess eligibility will include a medical history, renal panel, and urine albumin/creatine ratio (UACR). Recent lab values from the medical chart within ~3 months may be used if available. eGFR will be calculated from serum creatinine using the CKD-EPI equation (20). A urine pregnancy test of human gonadotropin will be performed for subjects of childbearing potential.

Pre-Study Washout Period and Run-In Period

Otherwise eligible subjects who take drugs that potentially alter calcium and phosphate balance or homeostasis including high dose cholecalciferol or ergocalciferol (1000 U/day or 50,000 U/wk, respectively), active vitamin D metabolites, calcimimetics, PTH analogues in the last 30 days, will undergo a 30-day pre-study washout from these drugs to be eligible for the study, with their physician approval.

All subjects will be given 600 IU/d of an oral vitamin D supplement (cholecalciferol) for 2-weeks prior to beginning the controlled feeding period and through completion of the study. This is to ensure vitamin D intake adequacy prior to the study, as was done for our previous balance study in CKD patients(14). 600 IU/d is the current recommended dietary allowance from the Institute of Medicine (18).

Dietary Interventions

The cross-over will consist of a randomly assigned order of two dietary conditions:

1) **Low Ca (500 mg/d)/high P (1500 mg/d) diet (LCa/HP)**

   The LCa/HP diet is at levels within the range observed for a typical American diet poor in Ca and high in P (18-21)

2) **Low Ca (500 mg/d)/low P (800 mg/d) diet (LCa/LP)**

   The LCa/LP diet represents coupling a typical poor Ca intake with the commonly prescribed dietary P restriction of 800 mg/d (22).

The magnesium content will be identical in both diets. Diets will be designed by a registered dietitian of the Bionutrition Center of the Indiana CTSI using nutrient analysis software, and mineral composition will be confirmed by chemical analysis of diet composites prior to and during the studies. The LCa/LP diet will be designed to contain zero-to-minimal phosphate food additives, and an equal portion of protein from animal and plant sources. The LCa/HP diet will be achieved in part through higher amounts of phosphate food additives, which is representative of a typical American diet. Diet levels will be designed to be as close to the above targets,

but final values may differ by approximately +/- 10%. Actual levels of the diets will be reported in the results of this study

All meals will be prepared in the metabolic kitchen at the Indiana CTSI CRC, with ingredients weighed with precision to 0.1 g. Outpatient meals will be provided as pack-outs for the subjects to take home; Inpatient meals will be prepared and trayed for the subjects. Diet composites will be prepared alongside subject diets, homogenized, ashed, and analyzed for accurate dietary Ca, P, and Mg intakes during the balance studies. Any leftovers not eaten will be weighed back and chemically analyzed and considered in the balance calculations (Aim 2).

A non-absorbable fecal marker, polyethelene glycol (PEG), molecular weight 3350, will be given in capsule form ~1 g with each meal (~3 g/d), to assess % fecal PEG recovery for fecal collection compliance, as well as steady-state by fecal Ca:PEG, P:PEG, and Mg:PEG ratios (23). This will be in the meal pack-outs or on the patient meal trays while inpatient.

Baseline Measures

A baseline outpatient visit of approximately 60 minutes will occur on "Day 1" of the study. This is when the controlled research diet will begin. Subjects will arrive to this appointment fasting at least 8 hours and will have blood and urine collected for baseline biochemistries. These will include a renal panel, serum 25D, and urine Ca, P, creatinine, and additional serum collected for additional measures such as baseline bone and mineral biochemistries and uremic retention solutes. eGFR will be calculated from serum creatinine using the CKD-EPI equation (17). Subjects will receive 3 days of meal pack-outs and will return on the 3rd or 4th day for the remaining meals until the inpatient stay. We will obtain meal adherence data over the outpatient period using meal checklists, PEG capsule pill counts, and leftover food weigh-backs. Anthropometrics (weight, and height) and vital signs will be taken at the baseline visit. Subjects will also be asked to bring a stool collection from home to the Day 1 visit, the stool sample should be collected within 3 days prior to the visit. Stool collection supplies will be given to the subjects at the screening visit.

Ca, P, Mg Absorption Tests (Aim 1)

Following the 7-day outpatient equilibration period on the controlled diet, participants will be asked to fast overnight at least 8 hours and will be admitted to the Indiana CTSI clinical research center (CRC) on the morning of study Day 8. Subjects will continue the controlled diet for the 2-day inpatient testing. We will collect fasting blood and urine, and then subjects will be given their assigned diet breakfast with a beverage containing 10 µCi P-33 (range 8-11 uCi) and 10 mg stable Ca-44(range ~8-11 mg), and 40-70 mg Mg-26 to drink halfway through their meal. Blood samples will be taken pre-dose, then 5, 15, 30, 60, 90, 120, 180, 240, 360 min, and 24h post-dose. All urine will be collected during the test days in 2-hour intervals until 6-hours post-dose, and then 6h-24h post-dose with collections timed and recorded by CRC staff. These urine collections will be individually measured for volume and a small aliquot taken for time point measurements, and then we will combined the values of the intervals to give a total 24h value. On Day 9, subjects will receive the same breakfast as Day 8, at the same clock time, but with no isotope; at 25h post-oral dose, an IV push of 10 µCi P-33 (range 8-11 uCi) and 6 mg stable Ca-44 (range ~5-7 mg), and 20-30 mg stable Mg-25 in sterile saline will be given over 2 minutes. Serum and urine collections post-dose will follow the same time points as during Day 8 and will continue through the 24-hour post-IV dose on Day 10, prior to discharge. The amount of isotope in the sequential blood and urine samples will be determined by liquid scintillation counting or inductively coupled plasma mass spectrometry for P-33 and Ca-44/Mg-25/Mg-26, respectively. Time points are approximate, and exact clock time of blood and urine collections will be recorded throughout the inpatient study and used in the kinetic analysis. Thus, only missed time point collections will be considered protocol deviations.

Ca, P, Mg Balance and Kinetics (Aim 2)

For the full Ca, P, Mg balance and kinetics in Aim 2, a subset of N = 2 subjects will remain inpatient for an additional 5 days following the 48-hour absorption testing protocol described under Aim 1. During this time, all urine and fecal samples will be collected. Urine will be collected in time, 24-hour pools, as well as two 2-hour fasting urine collections taken on the morning of Day 15, aliquoted for fasting biochemistries, and then data combined with the appropriate 24-hour collection. Urine Ca, P, Mg and creatinine will be measured in each 24-hour collection or 2-hour fasting collection. Blood samples will be taken each morning, Day 10-15, to use for full Ca, P, and Mg kinetic modeling. Subjects will be discharged the morning of Day 15, after the 2-hour fasting urine and fasting blood collection; at this point they will discontinue study diet and PEG, but continue taking vitamin D at home for the remainder of Day 15 through Day 21. Fasting blood biochemistries will be measured in samples collected during a return outpatient visit on Day 21. In addition to isotope analysis, total serum Ca, P, Mg and creatinine will be measured by standard clinical chemistries. All fecal samples will be collected during the inpatient periods, then homogenized, ashed, and analyzed for Ca-44, P-33, Mg-25, Mg-26, total Ca, total P, total Mg, and PEG recovery.

Other Biochemistries

Additional biochemistries will be measured in serum and urine samples collected during these studies. These will include FGF23, PTH, 1,25D at the end of the study and creatinine clearances. The sequential and timed serum and urine samples will allow for dynamic assessment of these biochemical outcomes. Data on additional minerals Na, K, and Mg will be obtained from the ICP analysis of urine, diet, and feces, and may be used in secondary analyses. Additional analyses on leftover serum, urine, or feces may include other mineral metabolism biochemistries (e.g. 25-hydoxyvitamin D or bone turnover biomarkers), metabolites considered to be gut-derived uremic retention solutes (e.g. TMAO). The fecal microbiome may be analyzed in secondary analyses.

8.0 Study Calendar

See Appendix B. "Study Calendar of Assessments"

9.0 Reportable Events

Collection of AEs will begin at the time of consent.

Prompt reportable AEs are assessed by the principal investigator (PI) as *(1) unexpected, (2) related or possibly related to study participation, AND (3) suggests that the research places subject(s) or others at greater risk of harm than was previously known.*

These will be submitted to the IRB within 5 business days of the investigators becoming aware of the event.

SAEs are any severe SE including any event leading to hospitalization, life-threatening condition, disability or incapacitation, death, or other significant hazard.

These will be reported to the IRB within 48 hours of the investigators becoming aware of the event, and in writing within 5 business days.

AEs not requiring prompt reporting will be those that are not SAEs as described above, are anticipated (i.e. described in the consent form), and do not occur any more frequently or with greater severity than expected.

These will be recorded and reported to the IRB at continuing review.

10.0 Data Safety Monitoring

The Data and Safety Monitoring Plan includes establishment of a DSMB. Members of the DSMB will include experts experienced in clinical research and patients with CKD and will consist of a Chairperson and one additional member. Please see details in the DSMP attachment.

Potential Risks

Risk of Phlebotomy and IV infusion:

The potential risks of phlebotomy are slight, but some possible risks include: excessive bleeding, fainting or feeling lightheaded, bruising, and infection (which is a slight risk any time the skin is broken). During each of the absorption test days, approximately 80 mL (11 time points of 7 mL each, approximately 5 tablespoons) of blood will be drawn over 24 hours. For comparison, the average whole blood donation is 1 pint, or 473 mL (about 32 tablespoons). Therefore, the total amount of blood drawn over the course of this study for subjects studied only under Aim 1 (absorption tests only) is 348 mL (174 mL in each crossover period (screening visit [7 mL], baseline visit [7 mL], plus the absorption test protocol [~160 mL, 22 time points of 7 mL each] which will be over ~48 hours, which is less than that drawn in a single blood donation. For subjects in Aim 2 (full balance and kinetics), they will have an additional 42 mL (6 time points of 7 mL each**)** drawn over the 12 days (5 days additional inpatient and final blood draw on Day 21 outpatient) after the 48-hour absorption testing period in each session, totaling approximately 432 mL drawn over the entire study (approximately 2 months). This is a cumulative volume of blood from sequential draws of 7 mL each, to yield approximately 3 mL of serum at each time point, which is needed for analysis of P-33, Ca-44, Mg-25, Mg-26 and other biochemistries of mineral metabolism.

The Ca-44, P-33 and Mg-25 will be administered by IV in a sterile saline "push" infusion of 1-3 mL. Potential risks are rare, but could include infection at the infusion site, sepsis, or air embolism. Steps to minimize these risks are below.

Risk of Radiation Exposure:

The risk of radiation exposure from P-33 (10 µCi in each oral and IV dose = 40 µCi total over the course of the study, effective dose of 35.6 mrem) is present for this study but is similar to some other common exposures. For example, the average radiation dose from a single round-trip cross-country flight in a commercial airplane across the United States is 4 mrem, from a single chest x-ray is 8 mrem, and from a single mammogram is 30 mrem (SC Dept of Health). People in the United States receive an average of 620 mrem of radiation per year from natural and man-made radiation sources (Source: Purdue REM website; NCRP Report #160).

Ca-44 Mg-25, and Mg-26 are stable isotopes so does not carry radioactivity risk.

Risk of Diet Intervention:

The controlled LCa/HP diet is based on a typical US diet that is low in Ca and is high in P due to its ubiquity in foods (37, 38). The LCa/LP diet changes the dietary P content to the level recommended when dietary P restrictions are prescribed. Thus, the controlled LCa/HP diet is at a level that would not be atypical in average, healthy American, and the LCa/LP diet reflects what would be commonly consumed by a CKD patient on a P restricted diet. Diets will be designed to meet the estimated energy needs of each subject to maintain current weight.

Potential risks of the controlled diet are feelings of hunger or being overly full on the research diet. A potential risk for food borne illness is present as is the case with any food consumption.

For patients with diabetes, there is risk of potential hypoglycemia with the study diet due to potential differences in amount and pattern of food consumed on study compared with at home.

Risk of Loss of Confidentiality:

As with all research studies, there is a risk of loss of confidentiality. Steps to minimize this risk are described in the following section.

Protections against Risks

Risk of Phlebotomy and IV Infusions:

To minimize risk from venipuncture and IV infusions, only trained personnel on the Indiana CTSI CRC will draw blood, place IVs, and push the IV infusions, using sterile supplies and technique. The IV infusion will be Ca-44, P-33, and Mg-25 in sterile saline. A sterile procedure for preparing these doses has been approved by the Indiana University Radioactive Drug Research Committee (RDRC) that we have used in prior studies. We send dose samples for each batch made to an independent certified lab for sterility and endotoxin testing.

Risk of Radiation Exposure:

Oral and IV doses of P-33 will be analyzed by liquid scintillation to confirm the appropriate radiation activity is given to subjects. The closest amount to 10 uCi that can be accurately measured without going over the limit of 11 uCi per dose will be given and recorded (e.g. 1.4356 mL may be needed for exactly 10 uCi, so 1.4 mL will be accurately measured, administered, and recorded). The radiotracer tests will be performed at the Indiana Clinical Research Center under medical supervision. The protocol will be approved by the Indiana University Radionuclide Radiation Safety Committee (RRSC) and the Radioactive Drug Research Committee (RDRC). These committees have approved similar P-33 use in our prior human studies.

Risk of Diet Intervention:

If a subject reports hunger or being overly full on the research diet, the diet will be modified accordingly. Research kitchen staff are trained in safe food handling, and subjects will be given explicit instructions on how to keep their pack-out meals safe to consume (including refrigeration and reheating instruction).

For diabetic subjects, we will collect usual home diet information (4-day food record), and the study physicians and dietitian will adjust the study diet and diabetes medications accordingly for the study. Diabetic subjects will be asked to perform fingersticks at home for blood glucose monitoring, and report to the study coordinator any blood sugar < 100 mg/dL. The study coordinator will alert the MD.

The study physician orders will be amended to include a specific order to the MD (first Dr. Moorthi, then Dr. Moe) for any BS < 100 mg/dL.

Risk of Loss of Confidentiality:

See **14.0 Privacy/Confidentiality Issues** below

11.0 Study Withdrawal/Discontinuation

Diet Adherence Assessment

Patients will be assessed for adherence to the controlled diet on Day 3 of the controlled diet outpatient period. Subject who do not meet a minimum 90% adherence will be withdrawn from the study. This will be determined by evaluating meal check lists and returned uneaten foods. During the 48-hour absorption testing as inpatients, subjects must show at least 90% adherence with the study diet and complete urine and fecal collections to be eligible to continue the additional 12-days (5 as inpatient) for full balance and kinetic measures. This is to ensure high quality balance data for these preliminary studies.

Participants may withdraw themselves from the study at any time by informing the study staff. The study staff may ask the participant if they are willing to talk about their reason for wanting to withdrawal from the study and if they are willing to discuss potential accommodations that might be possible that would make continuing the study possible, if appropriate. The study staff will explicitly let the participants know that they are not obligated to give a reason or discuss ways they might change their mind.

For participants who do not complete the entire study, data for the completed portions will be used for secondary/exploratory analyses, when statistically appropriate, unless the subject withdraws their consent for use of their data.

12.0 Statistical Considerations

This is a pilot study to determine effect sizes and variance estimates in fractional Ca, P, and Mg intestinal absorption (Aim 1) and Ca, P, and Mg balance (Aim 2) in patients with moderate CKD on a low Ca/High P (LCa/HP) diet vs. low Ca/low P (LCa/LP) diet. These data will be used for power calculations to determine an adequate sample size for a subsequent clinical trial to be proposed in an R01.

Four participants will be enrolled in this randomized cross-over study, 50% female, and we aim for at least ¼ black/African American and at least ¼ white race. All N = 4 will be included in Aim 1 with the primary outcomes of fractional Ca, P, and Mg absorption from a 48-hour testing periods. A subset of N = 2 subjects will be studied under Aim 2, with a week of Ca, P, and Mg balance and full kinetic model (12 days (5 as inpatient) beyond the initial 48-hour inpatient absorption test protocol).

At the beginning of the study, the study biostatistician (Dr. McCabe) will generate a randomization list for dietary intervention crossover order A or B by pairs. These orders will be [LCa/HP to LCa/LP] or [LCa/LP to LCa/HP]. Especially due to the small sample size, attention will be made to ensuring a balance of the two orders, which will be accomplished by randomizing both in pairs.

Based on our experience, we anticipate only a 15% attrition rate, which is only 0-1 enrolled subjects needing to be replaced. However, because of the small sample size of 4 and the need for high adherence due to the preliminary nature of these studies, we seek approval enrolling up to 6 subjects to complete our planned n = 4.

Statistical Analysis: A repeated measures analysis of variance (ANOVA) for cross-over designs will be sued to compare the LCa/HP diet with the LCa/LP diet on the primary outcomes:

Aim 1:	*Aim 2:*
Fractional Ca Absorption	Ca Balance
Fractional P Absorption	P Balance

Data will be examined for outliers and distributional model assumptions will be checked. Remedial measures will be taken as needed.

Power Calculations: The sample size required for the future R01 proposal will be based on the mean differences observed in the primary outcomes between the LCa/HP and LCa/LP diets, as well as the within- and among-patient variance estimates. We will determine sample sizes needed to detect a range of mean differences between diets with 80% to 90% power for a cross-over study.

13.0 Statistical Data Management

Primary data will be collected via paper, in-person and phone interviews, direct data capture from measurement instrument and stored electronically in REDCap, OnCore, and on the IU Dept of Medicine Department Server, and a Box REED (secure, HIPAA compliant) folder assigned to Dr. Hill Gallant via Purdue. The electronic storage location will be backed up automatically every day. Other data sources include outside lab data that will be stored in separate electronic files and merged with the primary data as needed. Quality assurance steps will include: 1) built in range checks and 2) testing of RedCap database by study team prior to moving to production mode. The following quality control methods will be used: 1) single entry with random checks of accuracy; and 2) extraction and cleaning of data that will be used for analysis every 6 months.

14.0 Privacy/Confidentiality Issues

All records associated with each subject's participation in the study will be managed using confidentiality standards applicable to medical records, i.e. records will only be seen by IRB-approved individuals working on the study and records will be kept in a secure area. To minimize the risk of loss of confidentiality, any hard copies of all laboratory, demographic, and health-care related information obtained, will be kept will be kept in secure locked file cabinets in the Nephrology research group offices of Dr. Moe in University Hospital, and in the Department of Nutrition Science at Purdue University in the office or locked lab spaces of Dr. Hill Gallant. Electronic data will be stored on a Purdue Box REED (secure, HIPAA compliant) folder or IU Dept of Medicine Department Network Drive, accessible only to those with IRB approval. RedCap data management (through the Indiana CTSI) will be used for this study. All personally identifiable and medical data will be kept confidential, in accordance with federal regulations. Only the Hill Gallant Lab at Purdue University will maintain the subject ID code. Codes will be stored in an electronic file separate from subject files. All specimens will be stored with coded de-identified labels. Specimens to be analyzed by other laboratories will be delivered with coded de-identified labels. All the specimens and data collected that contain protected identifiable information will be collected specifically for research purposes. The research staff is trained in privacy regulations. Only IRB-approved research staff will have access to the code key. No identifiable data are transmitted via unsecure Internet. FileLocker, Box REED, RedCap, or encrypted email will be used. The de-identified coded data will be used for statistical analysis. If publications result from this study, no reference will be made to data that can be linked to an individual participant.

The likelihood of a loss of confidentiality is deemed to be low due to these protections put in place. Every effort will be made to maintain the highest level of confidentiality.

15.0 Follow-up and Record Retention

The study duration for subjects is up to ~2 months (for those in Aim 2). We anticipate the study will be completed within 12 months of enrolling the 1st subject.

Records will be retained while the study is active and for 3 years following the closure of the study. De-identified coded data and samples will be kept for future research purposes, but the key code will be destroyed as well as other identifiable information. Electronic identifiable data will be permanently scrubbed, and any hard copy identifiable will be shredded.

16.0 References

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17.0 Appendix

A. Figure 2 – Study Timeline – larger version
B. Study Calendar of Assessments
C. DSMP (submitted to NIH)
D. Email regarding RDRC review and approval of protocol
E. Study Description Visual Aid for use with subjects for Aim 1 (2 day absorption test only)
F. Study Description Visual Aid for use with subjects for Aim 2 (14 day balance and kinetics)