COLORADO MULTIPLE INSTITUTIONAL REVIEW BOARD
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Protocol #: 16-1572
Project Title: Bicarbonate Administration in CKD
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I. Hypotheses and Specific Aims: Acid retention is a common complication of chronic kidney disease (CKD) as the diseased kidney is unable to excrete the daily dietary acid load. Lower serum bicarbonate levels, even within the normal laboratory range, are strongly linked to risks of hypertension, cardiovascular disease (CVD), CKD progression and death. Arterial dysfunction begins early in the course of kidney disease and is a key factor responsible for the development of left ventricular hypertrophy (LVH) in this population. LVH is the strongest predictor of cardiovascular mortality in CKD. Acid retention results in increased production of angiotensin II, endothelin-1 and aldosterone in order to enhance urinary acid excretion. All three of these humoral factors both directly and indirectly induce endothelial dysfunction, vascular stiffness, LVH and vascular calcification. Small interventional trials have shown that treatment with alkali therapy slows progression of kidney disease, even in patients with normal serum bicarbonate levels. Because risk factors for CVD and CKD progression often overlap, it is biologically plausible that alkali therapy in CKD patients may also result in improved cardiovascular outcomes. In our preliminary data, alkali therapy improved vascular endothelial function in 16 patients with CKD stage 3-4. Hence, treatment with alkali therapy may represent an inexpensive and novel therapeutic paradigm in CKD. We are proposing a randomized, double-blinded, placebo-controlled, 12-month trial of 108 patients with CKD stage 3-4 to examine the effect of sodium bicarbonate therapy on surrogate measures of CVD. Our overall hypothesis is that treatment with bicarbonate will improve indicators of vascular function and LVH in patients with CKD stage 3-4. Our primary goal is to determine the efficacy of alkali therapy for improving vascular endothelial function and reducing large elastic artery stiffness in patients with CKD stage 3-4 using noninvasive procedures. In Aim 1, we will compare changes over time in brachial artery flow-mediated dilation and aortic pulse wave velocity after 12 months of sodium bicarbonate therapy or placebo. In Aim 2, we will compare changes over time in LVMI, measured by gadolinium free cardiac magnetic resonance imaging, after 12 months of sodium bicarbonate therapy or placebo. In Aim 3, we will compare changes over time in humoral mediators of urinary acid excretion that promote vascular calcification (angiotensin II, endothelin-1 and aldosterone) and a novel test of calcification that provides a measure of the overall calcification propensity of serum (T50) after 12 months of sodium bicarbonate therapy or placebo. The results of this novel study have the potential to inform clinical practice by providing the necessary evidence to establish sodium bicarbonate therapy as an inexpensive and easy to administer option for the treatment of arterial dysfunction in patients with CKD stage 3-4.

II. Background and Significance:

1. Critical need for novel strategies to improve outcomes in chronic kidney disease. Chronic kidney disease (CKD) is a worldwide public health problem associated with significant morbidity and mortality. Cardiovascular complications are the leading cause of death in patients with CKD. Although patients with CKD have a high prevalence of cardiovascular risk factors, the severity and extent of cardiovascular complications is disproportionate to these risk factor profiles.23-25 It is therefore imperative to identify novel strategies targeting CKD-specific mechanisms to improve outcomes in CKD patients.

2. Cardiovascular disease (CVD) is associated with dysfunction and disorders of arteries. Two of the greatest contributors to CVD are the development of vascular endothelial dysfunction, most commonly assessed as impaired endothelium-dependent dilation (EDD) and the stiffening of the large elastic arteries (aorta and carotid arteries).26 Patients with CKD demonstrate both impaired EDD27-29 (measured by brachial artery flow mediated dilation (FMDBA)) and increased aortic pulse wave velocity (aPWV; the gold standard measure of large elastic artery stiffness).30-33 There is a stepwise increase in arterial stiffness through the CKD spectrum. Importantly, both FMDBA and aPWV are key pathophysiological antecedents to clinical CVD in patients with CKD.34-36 FMDBA and aPWV are independent predictors of all-cause and cardiovascular mortality in patients with CKD.34-36 Each 1% increase in FMDBA is associated with a 48% decreased risk of cardiovascular outcomes.36 A recent meta-analysis found that the risk of CVD mortality and all-cause mortality is two-fold higher in patients with aPWV in the highest tertile compared to the lowest tertile.37 Furthermore, every 1 m/s increase in aPWV is associated with a 14% increased risk of CV events.37 FMDBA and aPWV are important

noninvasive assessments of atherosclerosis and well recognized surrogate markers for CVD and thus constitute the primary outcomes of the current proposal. The PI and investigative team have extensive experience with these vascular function measurements and the Division of Renal Diseases and Hypertension at the University of Colorado Denver has an established Clinical Vascular Physiology Laboratory. The PI has completed a single-center randomized clinical trial of 128 patients with CKD stage 3B-4 examining the effect of vitamin D supplementation for 6 months on vascular function (K23 DK087859). The results of this study were presented at the Late-Breaking Trial session at the American Society of Nephrology Annual Meeting in San Diego, CA in November of 2015.

3. Arterial dysfunction is a key factor responsible for the development of left ventricular hypertrophy (LVH) in patients with CKD. Abnormal arterial stiffness causes an increase in myocardial oxygen consumption, a decrease in myocardial perfusion pressure, an increase in systolic blood pressure (BP) and an increase in left ventricular afterload, all of which lead to the development of LVH.38 When arterial distensibility decreases, as with increased calcification and remodeling, there is an increase in systolic BP and a fall in diastolic BP. There is a close relationship between increased arterial stiffness and increasing left ventricular mass index (LVMI) in CKD patients.39 Over 40% of CKD patients show evidence of LVH and the prevalence progressively increases with CKD progression.40 LVH is the strongest predictor of cardiovascular mortality in CKD. Unfortunately, CKD patients demonstrate progression of LV mass despite stable BP, stable kidney function and normal LV systolic function,41 suggesting a mechanism unique to CKD such as acid retention (see section 6). Failure to regress LVH over time is related to unchanged arterial stiffness.42 Aortic PWV is an important determinant of LVMI in CKD patients.43-44 Thus, therapies that can reduce arterial stiffness may have a large impact on LVH. For this reason, the objective in our study is to examine the effect of alkali therapy on both arterial dysfunction and LV mass in patients with CKD.

4. Vascular calcification (VC) induces arterial stiffness. Vascular calcification is prevalent in patients with CKD. Forty percent of patients with CKD have coronary artery calcification, compared to only 13% of age-matched controls.45 Patients with VC are at a considerably higher risk of cardiovascular events because VC induces arterial stiffness and increases aPWV.46,47 This occurs via arterial remodeling and changes in the intrinsic properties of the arterial wall (decreased elastin and increased collagen). Calcification affects both the intima and media layers of the vasculature, but medial calcification contributes the most to arterial stiffness as it has a direct effect on distensibility.48,49 In patients with CKD, studies have found that medial calcification results in higher aPWV.50 The exact pathophysiology of VC in CKD is unknown but it is an active process that involves calcium and phosphate metabolism, changes of vascular smooth muscle cells (VSMC) into an osteoblastic phenotype and an imbalance between promoters and inhibitors of calcification.51 Angiotensin II (AII), aldosterone and endothelin (ET-1) are not only important mediators of hypertension but they also induce VC (see section 7).15-18 In addition, acid retention induces the production of these factors in CKD and treatment with alkali results in decreased levels of AII, aldosterone and ET-1 (see section 6).4-8 Hence, alkali therapy may reduce arterial dysfunction by reducing VC. To date, no studies have examined the effect of alkali therapy on calcification in patients with CKD stage 3-4 (see section 7).

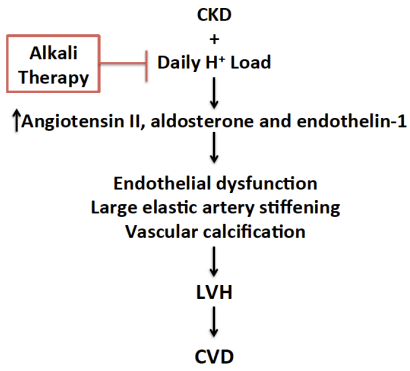
5. Acid retention is common in patients with CKD. As kidney function declines, the kidneys progressively lose the ability to synthesize ammonia and excrete hydrogen ions.1 Low bicarbonate levels are more common in patients with decreasing kidney function and approximately 19% of patients with CKD stage 4-5 have a serum bicarbonate level <22 mEq/L.2 However, over 80% of patients with CKD have normal serum bicarbonate levels.1-4 It has been proposed that CKD patients ingesting acid-inducing diets have sufficient remaining kidney function that enables them to reach a steady state of normal bicarbonate levels but at the cost of retaining hydrogen ions.52 In a recent study of 1065 patients with CKD stages 1-4, acid balance increased as glomerular filtration rate (GFR) declined eventually being significantly positive in patients with CKD stage 4 even though 90% of patients had normal serum bicarbonate.3 Another study found that acid retention was present as early as CKD stage 2.4 Human studies have found a positive acid balance of approximately 10-20 mEq/day.3,4,53 Hence, the subsequent acid retention, despite serum bicarbonate within the normal laboratory range, results in unfavorable effects that can lead to adverse outcomes.

6. Acid retention results in increased production of angiotensin II, aldosterone and endothelin-1. There are a number of humoral regulatory mechanisms that play a role in increasing urinary acidification in CKD including increased levels of AII, aldosterone and ET-1.4-8 Experimental and human studies have

shown that acid retention results in increased levels of these factors and increased expression of AII-type 1 receptors which are found in numerous tissues including the blood vessels, heart and kidney.4-8,54 Angiotensin II stimulates ammoniagenesis and increases distal nephron acidification.5,55 It also stimulates ET-1 and aldosterone.56,57 ET-1 increases both proximal and distal tubule acidification and indirectly increases acidification by stimulating aldosterone.7 In CKD stage 3 patients with bicarbonate >22 mEq/L, treatment with alkali therapy for 3 years resulted in reduced urinary angiotensinogen (an index of AII), reduced BP and preserved GFR.58 Although all subjects were on angiotensin converting enzyme inhibition, urinary angiotensinogen only decreased in those patients on alkali therapy, supporting an important role for dietary acid reduction in the decrease of AII. Alkali therapy has also been shown to reduce ET-1 and aldosterone levels in CKD.4

7. Persistent activation of angiotensin II, aldosterone and endothelin-1 may result in CVD by inducing vascular stiffness and calcification. All three of these factors can directly and indirectly induce endothelial dysfunction,9-11 arterial stiffness,9,12,13 and VC.15-18 Angiotensin II, ET-1 and mineralocorticoid receptors are found on endothelial cells and VSMC.9,10,59 Activation of these receptors results in endothelial dysfunction through inflammation and oxidative stress and arterial stiffness through functional and structural alterations in the vessel wall.9-14,59 ET-1 directly induces several processes that have been implicated in the pathogenesis of VC including: increased phosphate transport into VSMC;60 increased VSMC apoptosis;61 increased osteoblastic differentiation of VSMC;62 increases in procalcific factors (e.g. osteopontin)63 and decreases in anticalcific factors (e.g. matrix Gla protein).64 ET-1 indirectly increases VC by stimulating the production of aldosterone.65 In experimental models, endothelin receptor blockers result in decreased VC.66 Aldosterone is also known to promote VC.17 Aldosterone promotes osteoblastic differentiation of VSMC, increases expression of PIT-1 (type III sodium-phosphate transporter) and increases procalcific factors.17 Spironolactone mitigates VC in experimental models.17 Angiotensin II may also directly induce VC by increasing osteoblastic differentiation of VSMC and increasing procalcific factors such as osteopontin.16 Angiotensin II has an indirect role in VC as it stimulates the production of both ET-1 and aldosterone.56,57 Hence, correction of metabolic acidosis may result in improved arterial function through decreases in humoral mediators of VC and stiffness. The proposed mechanism of action of the cardiovascular protective effects of alkali is presented in Figure 1.

Figure 1



To elucidate the mechanisms by which alkali therapy may improve arterial function, we are proposing to measure humoral mediators of vascular calcification and stiffness and overall serum calcification propensity. We will measure T50, a novel test that measures the overall calcification propensity of serum.67 T50 is based on the transformation time of amorphous calcium phosphate-containing primary calciprotein particles (CPP) to crystalline hydroxyapatite-containing secondary CPP.67 A higher T50 represents lower calcification propensity. The balance of promoting and inhibiting factors present in serum governs the transformation time. Reduced T50 is associated with lower concentrations of fetuin-A and pyrophosphate—inhibitors of VC— and higher phosphate and calcium concentrations—promoters of VC. A reduced T50 predicts aortic stiffness and all-cause mortality in patients with CKD,21 and appears to improve prediction of calcification above and beyond single protein measurements (e.g. fetuin-A and phosphate).21 In our preliminary data (Approach section 1d), we show a positive association between serum bicarbonate levels and T50 in CKD stage 3-4, suggesting a beneficial role of alkali therapy in CKD. Finally, we will measure plasma AII, aldosterone and ET-1.

8. Serum bicarbonate levels and clinical outcomes in CKD.

a) CKD Progression: Experimental evidence suggests that metabolic acidosis contributes to CKD progression.2,52,68 Multiple observational studies have shown that higher serum bicarbonate levels, even within the normal range, are associated with a lower risk of CKD progression.69-74 The lowest risk of progression is observed with a serum bicarbonate level near 28 mEq/L.69-74 Several small interventional trials have shown that bicarbonate administration slows the rate of kidney disease progression, even in patients with normal serum bicarbonate levels.70,75,76 In this application we will not test the effects of alkali therapy on kidney disease progression, as this is the main goal of the NIH

sponsored U01 Bicarbonate Administration to Stabilize eGFR (BASE) Study. Instead we will test the effects of alkali therapy on surrogate markers of CVD.

b) CVD: In the general population, several studies have found that lower serum bicarbonate levels are associated with incident and prevalent hypertension.77-79 In a study of 695 women from the Nurses' Health Study, those with bicarbonate levels in the highest quintile (>23.8 mmol/L) had 31% lower odds of developing hypertension than the lowest quintile (<20.7 mmol/L).77 In young adults aged 20-49 without underlying kidney disease, lower bicarbonate levels were associated with lower cardiorespiratory fitness.80 In patients with CKD, metabolic acidosis is associated with insulin resistance, a well-known CVD risk factor.81-83 Human studies have also found that metabolic acidosis results in activation of the renin-angiotensin-aldosterone system.4-8 In a study of 7 healthy male subjects, the FMDBA response was significantly attenuated during metabolic acidosis such that the overall ability of the brachial artery to vasodilate relative to control conditions was impaired (FMDBA 4.11 ± 3.55% vs. 7.98 ± 2.96%, $p<0.05$).84 In a recent study of over 3,900 CKD participants from the Chronic Renal Insufficiency Cohort (CRIC), a nonlinear relationship between serum bicarbonate level and congestive heart failure was found.71 The risk of heart failure was highest in participants with serum bicarbonate levels >24 mEq/L but there was also a trend towards a higher risk of heart failure with bicarbonate levels at the lower end of the range (17-18 mEq/L).71 Thus, similar to the relationship with mortality (see below), a U-shaped relationship may exist. Hence, the optimal bicarbonate level for cardiovascular safety in patients with CKD is unknown. In our preliminary data, we show that higher serum bicarbonate levels (≥ 25 mEq/L) are associated with heart failure, higher LVMI and aortic stiffness in the general population (see Approach Section 1b). In addition, we also show that treatment of metabolic acidosis improves vascular endothelial function (FMDBA) in CKD stage 3-4 (see Approach Section 1c). To date, no interventional trials have been performed examining the effect of alkali therapy on arterial dysfunction and LVMI in patients with CKD.

c) Mortality: Patients with moderate and advanced CKD appear to have a high risk of death with both high and low serum bicarbonate (U-shaped association).73,85 In a study of 1,250 CKD patients, the risk of death increased by 43% when bicarbonate level was <22 mEq/L and by 24% with a bicarbonate level > 29 mEq/L.73

9. Bicarbonate administration in CKD. Alkali therapy is currently recommended in CKD patients when the serum bicarbonate level is <22 mEq/L to mitigate the effects of acidosis on bone and protein.86 However, >80% of CKD patients have normal serum bicarbonate levels and do not receive alkali despite the fact that they are in positive acid balance.1-4 As discussed earlier, interventional studies have found that alkali slows CKD progression in people with normal serum bicarbonate levels. Even though no interventional trials have been performed examining the effect of alkali on CVD, results from observational studies support the hypothesis that alkali replacement may also reduce cardiovascular risk, even in patients with normal bicarbonate. Hence, a major paradigm shift in the treatment of CKD would occur if alkali therapy were effective in reducing CVD in patients with normal serum bicarbonate. In the CRIC study, persistent bicarbonate >26 mEq/L was associated with an increased risk of heart failure events and death.87 Our preliminary data also show an increased risk of heart failure, higher LVMI and arterial stiffness with higher serum bicarbonate levels (≥ 25 mEq/L). The mechanism behind the increased risk of adverse outcomes with higher serum bicarbonate levels is unknown, but it is hypothesized that it may worsen vascular stiffness and calcification. An alkaline pH increases VC in cultured cells and uremic rats88,89 whereas acidosis inhibits calcification.90 However, as discussed above in section 7, data suggest that correction of metabolic acidosis may improve VC. This conflicting epidemiologic data further strengthens the need for a randomized clinical trial examining the effect of alkali therapy on surrogate endpoints of CVD and calcification in patients with CKD.

III. Preliminary Studies/Progress Report:

1. PRELIMINARY DATA

a) Feasibility of the proposed study is demonstrated by the PIs experience in the NIH sponsored Vitamin D and Arterial Function in CKD Trial (K23 DK087859). This single-center study, conducted by the PI Dr. Kendrick at the University of Colorado Denver examined the effect of oral cholecalciferol vs. calcitriol for 6 months on vascular function in 128 CKD stage 3B-4 patients. Recruitment was accomplished in 36 months with an attrition rate of 10%. The PI was responsible for all aspects of the study including overall conduct, design, implementation, administration, analysis and interpretation of FMD_{BA}. The PI presented the results at the Late-

Breaking Trial Session at the American Society of Nephrology Annual Meeting in San Diego, CA in November 2015.

b) Serum bicarbonate levels are associated with an increased risk of lower FMD_{BA}, arterial stiffness and heart failure. We tested associations of serum bicarbonate levels with major subclinical and cardiovascular outcomes in a large prospective cohort of community-living individuals, the Multi-Ethnic Study of Atherosclerosis (MESA). All MESA participants were free of CVD at study entry. We included 6,229 participants from MESA with baseline serum bicarbonate measurements. Serum bicarbonate was examined as a continuous variable and in clinically significant categories (<21, 21-22, 23-24 and ≥ 25 mEq/L). Multivariable Cox proportional hazards models and linear regression models were used to test associations of serum bicarbonate concentrations with incident heart failure, left ventricular mass (LVM) and aortic pulse pressure.

Results: The mean (SD) serum bicarbonate level and mean eGFR were 23.1 (1.8) mEq/L and 78.2 (16.2) mL/min/1.73m^2, respectively. 174 (2.8%) participants developed incident heart failure during a median (IQR) follow-up of 8.5 (7.7-8.6) years. Participants with bicarbonate levels <21 mEq/L were more likely to be younger, to be Hispanic, and to have diabetes, higher body mass index and higher eGFR. The association between bicarbonate levels and cardiovascular outcomes is shown in Table 1. Since there was a significant interaction between bicarbonate levels and diuretic use in patients with incident heart failure we stratified patients by diuretic use. After excluding patients on diuretics, we found that each 1 mEq/L increase in serum bicarbonate was associated with a 13% increased risk of incident heart failure and with 0.7 gm increase in LVM in fully adjusted analyses (Table 1). Bicarbonate levels ≥ 25 mEq/L were associated with higher aortic pulse pressure compared to levels of 23-24 mEq/L (β 1.0, 95% CI 0.4, 2.0). Additionally, each 1 mEq/L increase in bicarbonate level was associated with a 0.3 mmHg increase in aortic pulse pressure (Table 1). Among patients on diuretics, a bicarbonate level <21 mEq/L was associated with almost a two-fold increased risk of lower FMD_{BA} (β -1.77, 95% CI -2.98, -0.55). **Our data supports that lower bicarbonate may result in arterial dysfunction. The U-shaped relationship seen in this epidemiological analysis further strengthens the need for a randomized trial to determine the effect of alkali therapy on cardiovascular outcomes.**

Table 1.

Serum Bicarbonate level (mEq/L)	Brachial Artery FMD β (95% CI)	Aortic Pulse Pressure β (95% CI)	Left Ventricular Mass β (95% CI)	Incident Heart Failure HR (95% CI)
Diuretic Use				
< 21	**-1.77 (-2.98, -0.55)**	-1.2 (-5.4, 2.9)	-7.0 (-22.0, 9.0)	0.70 (0.19, 2.59)
21-22	-0.07 (-0.75, 0.61)	0.5 (-1.5, 2.5)	-1.0 (-7.0, 5.0)	1.01 (0.53, 1.95)
23-24	**REF**	**REF**	**REF**	**REF**
≥ 25	-0.2 (-0.74, 0.33)	1.2 (-0.4, 2.7)	-2.0 (-6.0, 3.0)	0.42 (0.21, 0.84)
Per 1 mEq/L increase	0.0 (0.12, 0.13)	0.2 (-0.2, 0.5)	0.0 (-1.0, 1.0)	0.90 (0.79, 1.01)
No Diuretic Use				
< 21	-0.01 (-0.4, 0.4)	-0.6 (-2.0, 0.4)	-1.0 (-5.0, 2.0)	0.61 (0.26, 1.46)
21-22	0.08 (-0.2, 0.3)	-0.08 (-0.7, 0.6)	0.4 (-1.0, 2.0)	0.83 (0.53, 1.31)
23-24	**REF**	**REF**	**REF**	**REF**
≥ 25	-0.1 (-0.4, 0.2)	**1.0 (0.4, 2.0)**	**3.0 (0.5, 5)**	1.3 (0.8, 2.11)
Per 1 mEq/L increase	-0.03 (-0.09, 0.03)	**0.3 (0.1, 0.4)**	**0.7 (0.2, 1.0)**	**1.13 (1.01, 1.26)**

Adjusted for age, sex, race, education, site, diabetes, emphysema, BMI, smoking, HDL, LDL, triglycerides, SBP, eGFR, CRP, urine albumin/creatinine ratio, antihypertensive agents, lipid-lowering agents.

We also performed a cross-sectional study examining the association between serum bicarbonate levels and arterial stiffness as measured by an ankle brachial pressure index (ABPI) > 1.3 in 1,002 participants from the Third National Health and Nutrition Examination Survey. ABPI is a non-invasive assessment of arterial stiffness and is a good indirect indicator of VC. A high ABPI indicates calcified vessels and is associated with greater LVMI.[91] Serum bicarbonate was examined as a continuous variable and in clinically significant categories (<21, 21-22, 23-24, ≥25 mEq/L). Multivariate regression analyses were used to examine the association between serum bicarbonate level and high ABPI >1.3.

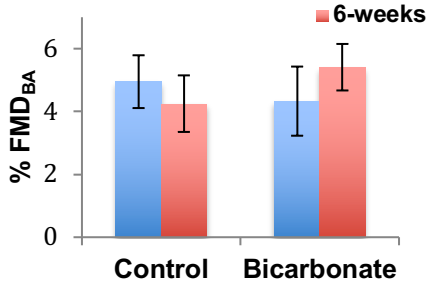
Results: The mean (SD) serum bicarbonate level and eGFR was 28.8 (4.3) mEq/L and 104 (31.4) ml/min/1.73m^2, respectively. 72 (7.2%) of the participants had a high ABPI >1.3. We found no significantly increased risk of an ABPI in unadjusted or adjusted analyses with serum bicarbonate levels <21, 21-22, or ≥ 25 mEq/L when compared to the reference group (23-24 mEq/L). When serum bicarbonate was measured as a continuous

variable, we found a U-shaped relationship in that both lower and higher bicarbonate levels compared to a level of 29.6 mEq/L were increasingly more associated with an increased risk of an ABPI >1.3 (p=0.013 each for linear and quadratic bicarbonate terms in the unadjusted model).

c) Bicarbonate administration results in improved FMD_{BA} in patients with CKD stage 3-4. We performed a pilot, prospective, open label 14-week crossover study examining the effect of treatment of metabolic acidosis (defined by a low serum bicarbonate level of 16-22 mEq/L), with oral sodium bicarbonate therapy in 16 patients with CKD stage 3B-4. The primary endpoint was change in FMD_{BA} between treatment and control conditions. All patients underwent baseline measurements of FMD_{BA} and then were randomized to either start with treatment or control. Each patient served as his or her own control. Each period was 6 weeks in duration with a two-week washout period in between. FMD_{BA} was performed at the beginning and end of each period using the methods described below in section 9a. During the treatment phase, participants received oral sodium bicarbonate tablets at a dose of 0.5 mEq/kg-lean body weight (LBW)/day. Each tablet contained 7.6 mEq of bicarbonate and 178 mg of sodium.

Results: The mean (SD) age and eGFR was 58.5 (12.8) years and 25.3 (8.3) ml/min/1.73m^2, respectively. The mean (SD) bicarbonate level increased after sodium bicarbonate administration from 19.6 (2.9) mEq/L to 22.4 (3.0) mEq/L. Blood pressure control was similar between the two conditions (mean change in systolic BP: control 0.56 mmHg, treatment -0.38 mmHg, p=0.86). FMD_{BA} significantly improved after 6 weeks of sodium bicarbonate therapy compared to control conditions (mean difference in %FMD 1.1% in the treatment arm vs. -0.70% in the control arm, p=0.027). The mean (SE) FMD_{BA} in the control and treatment arms are shown in Figure 2. Bicarbonate administration was well tolerated and there were no adverse events.

Figure 2. Percent FMD_{BA} at baseline and 6-weeks after sodium bicarbonate administration



d) **Higher bicarbonate levels are associated with higher T_{50} in CKD stage 3-4.** We performed a cross-sectional study in 128 patients with CKD stage 3B-4. We measured serum bicarbonate levels and T_{50} at baseline. Mean serum bicarbonate and T_{50} levels were 22.9 ± 2.9 mEq/L and 223.4 ± 46.8 min, respectively. Each 1 mEq/L increase in bicarbonate was associated with an increase in T_{50} of 4.83 minutes (β 4.83, 95% CI 2.19-7.48 per 1mEq/L increase in bicarbonate). The positive relationship persisted after adjustment for age, gender and race (β 5.18, 95% CI 2.45-7.90 per 1mEq/L increase in bicarbonate).

IV. Research Methods

A. Outcome Measure(s):

Subjects. After obtaining their written informed consent, 108 patients with CKD stage 3B-4 (defined as estimated GFR (eGFR) 15-44 ml/min/1.73m^2) and a serum bicarbonate level of 22-27 mEq/L, on 2 separate measurements (at least 1 day apart), will serve as subjects. **Relevant biological variables:** Men and women 21 years and older of all races/ethnicities will be included. Major inclusion/exclusion criteria are presented in the table below (Table 2). We chose a bicarbonate level of 22-27 mEq/L as this is a normal serum bicarbonate level and it is not standard of care to give alkali therapy when the bicarbonate level is >22 mEq/L.[86] Hence, we can safely administer a placebo to these patients. Only stage 3B and not 3A are included as patients with stage 3B have an increased risk of adverse outcomes including cardiovascular outcomes and death compared to 3A. Patients will undergo screening for eGFR and serum bicarbonate at the Kidney Disease Research Center (KDRC) at the University of Colorado Anschutz Medical Campus. Estimated GFR will be calculated using the 4-variable Modified Diet Renal Disease prediction equation. Patients will be recruited from nephrology clinics at the University of Colorado where the PI has access to over 2,000 CKD patients. Additionally, patients will be recruited from our CKD database, which contains over 300 CKD patients willing to participate in clinical trials. The Health Data Compass services will also be used to generate a list of potential participants. Feasibility of recruitment is demonstrated by prior recruitment experience of the PI in the Vitamin D and Arterial Function in CKD Trial (see section 1a).

Table 2

Inclusion Criteria	Exclusion Criteria

• Age ≥ 21 years • Serum bicarbonate 22-27 mEq/L on 2 separate measurements (at least 1 day apart) • CKD stage 3B or 4 at time of screening (eGFR 15-44 ml/min/1.73m^2) • Blood pressure <140/90 mm Hg prior to randomization • BMI < 40 kg/m^2 (FMD measurements can be inaccurate in severely obese patients). • Able to provide consent • Stable anti-hypertensive regimen for at least one month prior to randomization • Not taking medications that interact with agents administered during experimental sessions (e.g. sildenafil interacts with nitroglycerin).	• Significant comorbid conditions that lead the investigator to conclude that life expectancy is less than 1 year • Use of chronic daily oral alkali within the last 3 months (including sodium bicarbonate, calcium carbonate or baking soda) • Uncontrolled hypertension • Serum potassium < 3.3 or ≥ 5.5 mEq/L at screening • New York Heart Association Class 3 or 4 heart failure symptoms, known EF ≤30%, or hospital admission for heart failure within the past 3 months • Factors judged to limit adherence to interventions • Anticipated initiation of dialysis or kidney transplantation within 12 months • Current participation in another research study • Pregnancy or planning to become pregnant or currently breastfeeding • Chronic use of supplemental oxygen • Started on immunosuppression in the past 3 months • Metal implant or implanted electrical device (patient will be unable to get MRI)

Rigorous Experimental Design. A 12-month **randomized, placebo-controlled, double-blinded study** with sodium bicarbonate will be conducted. The study will include 5 phases: screening, run-in, baseline, randomization and follow-up.

- Screening: Subjects will undergo screening for inclusion/exclusion criteria during a 1-2 week period.
- Run-in: A 1-month run-in period will occur if patients do not meet the BP goal of <140/90 mmHg. The rationale for this run-in phase is to achieve a stable antihypertensive regimen and BP <140/90 mmHg, such that changes in antihypertensive agents throughout the course of the study are minimal. This will allow for better isolation of the effect of bicarbonate on FMD_{BA}, aPWV and LVMI. During the run-in phase, an up-titration of patients current angiotensin converting enzyme inhibitor or angiotensin receptor blocker to their maximum approved dose or other antihypertensive medication(s) will occur to reach a target BP of <140/90 mmHg by their primary nephrologist or PCP. The duration of the run-in phase will be 4 weeks with weekly visits to achieve the target BP. The PI will contact the primary nephrologist/PCP of the patient if the BP is not at goal and discuss the need for medication changes. A BP <140/90 mmHg is the standard of care for patients with CKD, hence we do not anticipate an issue with the primary nephrologist or PCP making changes to the BP regimen. If the patient is not able to achieve a BP of <140/90 mmHg during this period, the participant will not be included in the study. This overall approach will allow for inclusion of the typical population of patients with CKD 3-4 and increase the generalizability of our expected results.
- Baseline: During the baseline phase FMD_{BA}, aPWV, LVMI, serum basic chemistry panel, plasma AII, aldosterone, ET-1, serum T50, venous blood gas, and 24-hour urine collection measurements will be performed in all participants that are able to control their BP as described above.
- Randomization: Participants will be randomized with a 1:1 allocation ratio to treatment and placebo group using block randomization. List of randomization will be generated by the study statistician and sent to the study pharmacist (Belmar Pharmacy, Denver, CO) blinded to the PI. Study investigators, healthcare providers, participants, data collectors, outcome adjudicators and data analysts will be blinded to treatment assignment.
- Follow-up: The follow-up schedule includes:
    - 1 month: 1) remote or in-person safety visit (basic chemistry panel, adverse event assessment, gastrointestinal (GI) symptoms questionnaire, BP, pill compliance); 2) optional outcome measures: FMD_{BA}, plasma AII, aldosterone, ET-1, and serum T_{50}. Of note, aPWV will not be measured at 1 month as it is unlikely to change after only 4 weeks of treatment.
    - 3, 6 and 9 months: safety visit (basic chemistry panel, adverse event assessment, GI symptoms questionnaire, BP, pill compliance).
    - 6 months: in addition to the safety visit, outcome measures: FMD_{BA}, aPWV, plasma AII, aldosterone, ET-1 and serum T_{50} and 24-hour urine collection.

- 12 months: 1) Adverse event assessment, GI symptoms questionnaire, BP, pill compliance; 2) outcome measures: FMD_{BA}, aPWV, LVMI, serum basic chemistry panel, plasma AII, aldosterone, ET-1, serum T50, venous blood gas, and 24-hour urine collection.

4. Study Drug Dosing. The study drugs (bicarbonate and the matched placebo) will be prepared by Belmar Pharmacy and will be identical in size, color, shape and taste. Each sodium bicarbonate capsule contains 7.7 mEq of bicarbonate and 178 mg of sodium. The matching placebo capsule will contain cornstarch. Belmar Pharmacy will bottle the capsules and label the bottles with code numbers to maintain the study's double blind and ship the bottles directly to the investigative site.

a) Sodium bicarbonate: Subjects randomly assigned to sodium bicarbonate therapy will receive 0.5 mEq/kg-lean body weight (LBW)/day for the entire 12 months. Participants will take ½ the daily dose in the morning and the other ½ in the evening. The number of capsules will be rounded to the nearest whole capsule. To reduce pill burden and increase compliance the maximum number of pills per day will be six.

Rationale for sodium bicarbonate dose: A dose of 0.5 mEq/kg-LBW/day has been shown to be safe and effective for treatment of metabolic acidosis in patients with CKD.[75,92] Previous studies using this dose of sodium bicarbonate have not reported significant changes in edema, body weight or BP.[75,76,92] In our **preliminary data**, sodium bicarbonate administration was not associated with an increase in BP or fluid gain in patients with CKD stage 3-4. Additionally, we will dose sodium bicarbonate based on LBW, not actual body weight. The volume of distribution of bicarbonate is approximately that of total body water. Total body water is dependent on lean body mass. We will use the following equations to determine LBW[93]:

$$\text{LBW (kg)}_{male} = \frac{9270 \times Wt}{6680 + (216 \times BMI)} \qquad \text{LBW (kg)}_{female} = \frac{9270 \times Wt}{8780 + (244 \times BMI)}$$

b) Placebo: Subjects randomly assigned to placebo will take the same number of capsules as if they were assigned to receive 0.5 mEq/kg-LBW/day of sodium bicarbonate. Participants will take ½ the daily dose in the morning and the other ½ in the evening. The number of capsules will be rounded to the nearest whole capsule. To reduce pill burden and increase compliance the maximum number of pills per day will be six.

Rationale for placebo: A placebo group is necessary to successfully complete our aims. If a placebo group were not included both the investigators and participants would know that they are receiving sodium bicarbonate, which could influence medical management and perception of side effects in the study. It is not standard of care to give sodium bicarbonate therapy when the serum bicarbonate is >22 mEq/L in CKD. Although unlikely in this 12-month study, serum bicarbonate may fall due to natural progression of CKD, particularly in the placebo group. As discussed in the safety section (see section 7), rescue therapy with open-label sodium bicarbonate will be initiated if the serum bicarbonate falls below 20 mEq/L on two consecutive measurements.

5. Rationale for study duration. The exact duration necessary for improving vascular function and LVMI in patients with CKD is unclear. From the study teams experience with other interventional studies evaluating vascular function, changes in FMD_{BA} can be seen within 4-6 weeks (see preliminary data) and changes in aPWV can be seen after 6 months. We are proposing multiple follow-up measures of FMD_{BA} (1 month (optional), 6 months and 12 months) and aPWV (6 months and 12 months) to determine short-term or long-term effects of bicarbonate. Changes in LVMI require longer periods of treatment and most published data use an intervention period of 6 months to 1 year. Accordingly, we propose to measure LVMI at 12 months.

Study	Population	Measure	Study Duration
Hayoz (2012)[94]	Postmenopausal women (n-125)	aPWV	38 weeks
Frimodt-Moller (2012)[95]	CKD stage 3-4 (n=67)	aPWV	6 months
Kao (2011)[96]	CKD stage 3 (n=67)	LVMI and FMD_{BA}	9 months
Alkaishi (2013)[97]	CKD stage 4 (n=206)	LVMI	48 weeks
Ayus (2005)[98]	CKD stage 4 (n=101)	LVMI	6 months

7. Safety monitoring

- **Expected adverse events:** Sodium bicarbonate therapy may cause bloating, flatulence and abdominal discomfort. Although previous studies using similar doses of sodium bicarbonate have not reported significant changes in edema, body weight or BP,[75,76,92] sodium bicarbonate may cause edema to develop or worsen thereby increasing BP. Previous studies suggest fluid retention is greater when sodium is accompanied by the chloride anion rather than the bicarbonate anion.[99] As shown in our **preliminary data**, sodium bicarbonate did

not result in increased BP. Sodium bicarbonate also has the potential for causing metabolic alkalosis and hypokalemia if it is retained rather than excreted in the urine. These expected adverse events will be monitored during the course of the study and in general, can be medically managed by adjusting antihypertensive and/or diuretic agents, providing potassium replacement and by dietary changes. Of note, none of these adverse events were observed in our short-term pilot study.

- **Dose reduction and discontinuation:**
  - Serum bicarbonate >28 mEq/L: The intervention dose (study drug) will be reduced by 50% by the PI and diuretics will be adjusted as appropriate by the primary nephrologist or PCP. The PI will contact the primary nephrologist or PCP by phone or email and discuss changes in diuretics. The participant will return for a visit one week later to have serum chemistries rechecked. If the bicarbonate remains >28 mEq/L the intervention will be stopped by the PI and the participant will have serum bicarbonate measured weekly until it is <28 mEq/L. Plasma ionized calcium will be checked if bicarbonate is >30 mEq/L at any visit since an increase in pH can decrease the ionized calcium concentration.[100,101] Management of low ionized calcium will be at the discretion of the PI based on symptoms reported by the patient. The PI will contact the primary nephrologist or PCP if she believes calcium supplementation is necessary and the primary physician will order the medication.
  - Systolic blood pressure ≥ 160 or diastolic blood pressure ≥ 80 mmHg: Potential reasons for uncontrolled BP, such as poor compliance or running out of medications will be evaluated and managed appropriately. If an identifiable cause is not found, the PI will contact the primary nephrologist or PCP to increase anti-hypertensive medications and/or diuretics. The participant will return 1 week later for follow-up. If BP remains >160/80 mmHg at the second visit, the PI will again contact the primary nephrologist or PCP for medication changes and the intervention dose (study drug) will be reduced by 50% by the PI. The participant will return 1 week later. If BP remains unchanged at the third visit, the intervention will be stopped by the PI and the PI will contact the primary physician for escalation of medications. Participants will continue to have PRN visits weekly until BP is <160/80 mmHg.
  - Serum potassium < 3.0 mEq/L: The intervention dose will be reduced by 50% by the PI and the PI will contact the primary nephrologist or PCP to discuss adjusting diuretics as appropriate and prescribing potassium replacement. The participant will return 1 week later for repeat labs. If serum potassium remains < 3.0 mEq/L, the intervention will be discontinued by the PI. The participant will return weekly until serum potassium is > 3.0 mEq/L.
  - Severe edema: If in the opinion of the PI severe edema (4+) is present and another etiology cannot be identified the dose of the intervention will be reduced by 50% and the PI will contact the primary nephrologist or PCP to make adjustments to diuretics. If fluid retention remains severe at follow-up visits, the intervention (study drug) will be discontinued.
  - Rescue therapy with oral sodium bicarbonate: Rescue therapy with open-label sodium bicarbonate will be initiated by the PI if serum bicarbonate is <20 mEq/L on 2 consecutive measurements at least 1 week apart since a level <20 mEq/L is associated with adverse outcomes. In this instance, open-labeled sodium bicarbonate will be given to target serum bicarbonate of 20-22 mEq/L. Participants will not discontinue study medications if open-labeled sodium bicarbonate is prescribed.

8. Data Collection

- Demographics, medical history and physical examination will be performed at screening and baseline visit.
- Circulating measures: Subjects will report fasted for standard blood chemistry analysis at baseline, 1, 3, 6, 9 months and end of study. A venous blood gas (for pH and P_{CO2}) will be obtained at baseline and end of study to get a more complete description of the acid-base status of the participant. To assess participant's positive acid balance we will collect 24-hour urines at baseline, 6 months and end of study to measure net acid excretion and net endogenous acid production. A urine pregnancy test will be performed if clinically indicated at the screening visit.
- Outcome measures: FMD_{BA} will be performed at baseline, 1 month (optional), 6 months and end of study. Aortic PWV will be performed at baseline, 6 months and end of study. LVMI will be performed at baseline and end of study. Plasma will be collected for measurement of AII, aldosterone and ET-1 and serum will be collected for measurement of T_{50}, at baseline, 1 month (if patient has in-person visit), 6 months and end of study.

- Other circulating markers of calcification: In addition, will measure serum fetuin-A (inhibitor of VC); serum calcium (promoter of VC); serum phosphate (promoter of VC); inflammatory markers (promoters of VC): serum C-reactive protein and cytokine 12 panel (IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, Interferon gamma, Interleukin 2 Receptor, Interleukin 1 beta, tumor necrosis factor); and markers of bone metabolism (serum parathyroid hormone, bone alkaline phosphatase, beta C-terminal telopeptide). These markers will be measured at baseline, 6 months and end of study.
- Resting BP: Arterial BP will be measured in triplicate while seated at rest using an automated oscillometric machine (Dinamap) at all in person study visits.
- Measures of adherence and safety:
    - Pill counts will be assessed at the quarterly visits and end of study to document subject adherence.
    - A GI symptoms questionnaire will be performed to assess tolerability of the intervention monthly (either via telephone or at an in person visit). Participants will be asked to rate symptoms of nausea, bloating, and diarrhea.
    - Adverse event assessment will be performed monthly either via telephone or at an in person visit. Additional telephone calls and PRN follow-up visits will occur at the discretion of the PI.

Table of Detailed Visits

Screening Visit	**Run-in period**	**Baseline Period**	**Follow-up Period Month 1 (in-person or remotely)**	**Follow-up Period Month 3, 6, 9**	**Follow-up Period Month 12**
Medical history and physical examination	Height, weight, blood pressure, medication review	Height, weight, blood pressure, medication review	Height, weight, blood pressure, medication review	Height, weight, blood pressure, medication review	Height, weight, blood pressure, medication review
Informed consent	Titration of medications if blood pressure not at goal of < 130/80 mmHg	IV catheter, blood draw, 24-hour urine collection	IV catheter, blood draw, urine collection (optional)	Blood draw, urine collection	IV catheter, blood draw, 24-hour urine collection
Blood draw and urine collection		Blood vessel function and nitroglycerin	Blood vessel function and nitroglycerin (optional)	Adverse events assessed	Blood vessel function and nitroglycerin
Pregnancy test if indicated		Aortic pulse wave velocity measurement	Adverse events assessed	Pill count	Aortic pulse wave velocity measurement
		Cardiac MRI	Pill count	*6 month visit only: IV catheter, blood draw Blood vessel function and nitroglycerin Aortic pulse wave velocity measurement and 24-hour urine collection	Cardiac MRI
		Distribution of study drug			Adverse events assessed
					Pill count

9. Outcome Measures.

a) Primary Outcomes

- **FMD_{BA}:** Brachial artery FMD_{BA} will be determined using high-resolution ultrasonography (Toshiba Xario 200) as described originally by Celermajer et al.[102] and more recently by Dr. Jablonski.[103,104] FMD_{BA} will be measured at baseline, 1 month, 6 months and 12 months. 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) will be determined by measuring brachial artery dilation for 10 minutes after administration of sublingual nitroglycerin (0.4 mg).[104-107] A commercially available software package (Vascular Analysis Tools 6.0 Medical Imaging Applications, LLC, Iowa City, IA) will be used to concurrently acquire ECG-gated brachial artery diameters. As recently recommended[108] brachial artery dilation will be determined as the mm and % change from baseline diameter.[104-108] Doppler blood flow velocity will be obtained at baseline and for 2 minutes after cuff release. Shear rate will also be calculated, and if group- or condition- differences exist, FMD_{BA} will be adjusted accordingly.[108-110] Of note, the PI of this proposal has already completed vascular measurements in 128 patients with CKD stages 3B-4 and thus has extensive experience with these measurements (K23 DK087859). The coefficient of variation for baseline and peak brachial diameter in our laboratory is 0.3% and 0.6%, respectively.[104]
- **Large Elastic Artery Stiffness and Compliance:** Aortic PWV, a measure of large elastic artery stiffness, will be determined as described by Seals and colleagues[111,112] at baseline, 6 months and 12 months. Briefly, transcutaneous custom tonometers (Noninvasive hemodynamics Workstation, Cardiovascular Engineering Inc., Norwood, MA) will be positioned at the aorta and femoral artery to measure PWV. Femoral and carotid compliance, also described previously by Seals et al. will be measured non-invasively using simultaneous high-resolution ultrasonography and applanation tonometry of the artery.[111,112]

b) Justification for primary outcomes: As discussed above, EDD and aPWV are key pathophysiological antecedents to LVH in patients with CKD. FMD_{BA} is a the most common, key, non-invasive technique used to assess EDD and aPWV is the gold standard measurement of arterial stiffness. Evaluating these outcomes will allow us to identify the intermediate vascular mechanisms by which bicarbonate administration may reduce the risk of CVD in patients with CKD. The PI and University of Colorado investigative team have expertise in these vascular function measurements.[103]

c) Secondary Outcomes

- **Left Ventricular Mass:** LVMI will be measured using gadolinium-free non-invasive cardiac MRI at baseline and 12 months. Cardiac MRI is the gold standard way to measure LVMI.[113] Cardiac MRI will be performed on 3.0 T imaging system with cardiac sequence package using an electrocardiograph-gated, breath-hold, 2-dimensional, cardiac stead state free precession imaging for assessing ventricular volumes and function. All measurements will take place at the University of Colorado Brain Imaging Center, a research-dedicated facility with a 3 Tesla whole-body MRI scanner and a 3d/cardiac workstation for post-processing and calculation of LVMI. We will assess LVMI, LV end-diastolic and end-systolic volumes, relative wall thickness, left atrial size and ejection fraction. Transmitral flow will be measured to further quantify diastolic dysfunction. LVMI will be calculated by normalizing LVM to height to the 2.7^{th} power.[114] LVM indexed to height has been strongly linked to outcomes in the CKD population.[115] Dr. Nayana Patel (see letter of support), an experienced radiologist, will be reviewing the scans for safety and assessment of LVMI.
- **Plasma angiotensin II, aldosterone and endothelin-1:** Plasma AII, aldosterone and ET-1 will be measured by Dr. Jan Simoni. Plasma AII will be measured with Cayman EIA Kit, Cat No. 589301 (Cayman Chemical, Ann Arbor, MI). Plasma aldosterone will be measured with Cayman EIA Kit-Monoclonal, Cat (Cayman Chemical, Ann Arbor, MI). Plasma ET-1 will be measured with Enzyme Immunoassay Kit (Cayman Chemical, Ann Arbor, MI) after extraction using Bound Elut C_{18} columns (Varian, Harbor City, CA). Dr. Simoni has extensive experience with these plasma tests and has successfully measured them in CKD populations.[4-6,52,58]
- **Serum calcification propensity test:** A serum calcification propensity test (T_{50} assay) will be measured by Dr. Andreas Pasch using a Nephelostart nephelometer (BMG Labtech, Offenburg, Germany), a label-free 96-well plate-based assay that measures the conversion of primary to secondary CPPs by detecting the time-resolved changes of laser light scattering (nephelomtery) associated with it.[67] Dr. Pasch developed this

novel test of calcification and currently has the only laboratory in the world that performs the test. The analytical CV of a pooled serum precipitating at 270 minutes is 8.3%.[67]

d) Justification for secondary outcomes: 1) LVH is very common in patients with CKD and is the strongest predictor of cardiovascular mortality in CKD patients. Cardiac MRI is the gold standard measurement of LVMI. 2) AII, aldosterone and ET-1 increase with acid retention and are likely contributors to arterial dysfunction, LVH and VC. The long-term effect of alkali therapy on plasma AII, aldosterone and ET-1 in CKD has not been evaluated. 3) The effect of alkali therapy on VC is unclear. We will measure T_{50}, a novel test that measures the overall calcification propensity of serum. T_{50} better defines the future calcification risk related to common abnormalities of mineral metabolism factors and improves prediction of calcification beyond single protein measurements. T_{50} predicts aortic stiffness, which is strongly linked to VC.[19-21]

10. Power Calculations

a) Aim 1: Effect sizes were estimated for the primary endpoints of FMD_{BA} and aPWV based on published data of a similar population. We hypothesize that participants randomized to the bicarbonate group will have an improvement (i.e., increase) in FMD_{BA} at 12 months, while those in placebo will have a decrease in FMD_{BA}. We will compare change (from baseline to 12 months) in FMD_{BA} between the two groups. A previous study of paricalcitol vs. placebo in CKD stage 3-4[116] showed a between group difference of 1.8% in changed FMD_{BA} with 95% CI 0.3-3.1% after 12 weeks. Our preliminary data showed a between group difference (bicarbonate vs. control) of 1.6% in changed FMD_{BA} with 95% CI 0.2-2.9% after 6 weeks. Assuming a standard deviation of change of 0.8%, 54 patients in the placebo group and 54 patients in the bicarbonate group achieves 99% power to detect a 1.5% difference among the means (which is a clinically meaningful difference) using the two sample t-test with a two-sided 0.025 significance level (i.e. 5% significance level after Bonferroni correction for 2 outcomes) for intent-to-treat (ITT) analysis. It would also achieve 99% power to detect the same effect size in per-protocol analysis (n=45 per group allowing for 15% attrition). We hypothesize that participants randomized to the bicarbonate group will have no change or a decrease in arterial stiffness measured by aPWV at 12 months, while those in placebo will have an increase in stiffness. We will compare change (from baseline to 12 months) in aPWV between the two groups. In previous studies of CKD patients with comparable kidney function, the mean (SD) increase in aPWV was 1.1 ± 1 m/s over a 12 month period.[117,118] Assuming a common standard deviation within a group of 1.5 m/s,[117,118] 54 patients in each group will provide 88% power to detect a 1.0 m/s difference among the means (which is a clinically significant difference) using the two sample t-test with a two-sided 0.025 significance level (i.e. 5% significance level after Bonferroni correction for 2 outcomes) for ITT analysis. It would also achieve 80% power to detect the same effect size in per-protocol analysis (n=45 per group allowing for 15% attrition).

b) Aim 2: Effect sizes were estimated for the outcome of LVMI (normalized to height to the 2.7^{th} power) based on published data of longitudinal changes in LVMI.[119,120] We hypothesize that participants randomized to the bicarbonate group will have a decrease in LVMI at 12 months whereas those in placebo will have no change or an increase in LVMI. We consider 5-g of between-group difference in absolute LV mass as clinically important based on previous studies [119] where 10-g was considered for 2 years study duration, implying a mean between group-difference of 1.2 in LVMI, given mean height of 1.70 m. We further estimate the SD of the change in LVMI to be 1.8 based on information provided in the same study.[119] Hence, 54 patients in each group will provide 93% power to detect a significant 5-g difference in absolute LV mass or a 1.2 $g/m^{2.7}$ difference in LVMI between the two groups at 12 months using a two sample t-test with a 0.05 significance level.

c) Aim 3: We hypothesize that participants randomized to the bicarbonate group will have a decrease in plasma AII, aldosterone and ET-1 12 months whereas those in placebo will have no change or an increase. Based on data from a small trial of alkali therapy in participants with CKD,[4] we expect the mean ET-1 level to decrease by 0.9 pg/mL with a SD of 0.4 pg/mL and the mean aldosterone level to decrease by 20 pg/mL with a SD of 10.1 pg/mL from baseline to 12 months in the bicarbonate group. The planned sample size of 54 patients in each group will provide >95% power to detect the difference between the two groups. Based on published data in hypertensive patients with normal renal function,[121] we expect the mean AII level to decrease by 30% from baseline to 12 months in the bicarbonate group. The planned sample size of 54 patients in each group will provide >95% power to detect the difference between groups. There is very limited data regarding change in T_{50} levels in CKD patients. We hypothesize that the bicarbonate group will have an increase in T_{50} whereas the placebo will not change or decrease. Based on published data in CKD stage 3-4 patients,[21] we expect the

baseline mean (SD) of T_{50} to be 329 (95) minutes. With a sample size of 54 patients per group, we have 95% power to detect a difference between groups if it is 0.7 SD of changed serum T_{50} from baseline (i.e. the effect size is 0.7) with a two-sided 0.05 significance level.

11. Statistical Analysis

- **General consideration:** The primary analysis will be conducted on ITT basis. Analysis of covariance (ANCOVA) with complete data will be the primary analysis for efficacy. Multiple imputation will apply because of missing data and sensitivity analysis will be conducted in various ways, including linear mixed effects model and pattern-mixture model approach. Compliance of intervention will be summarized using the number of pills taken as the proportion of planned number of pills. The association of compliance with each of the outcomes (FMD_{BA} aPWV and LVMI) will be explored using multiple linear regression. Participants who receive rescue therapy will be analyzed in the ITT analysis but excluded from the per-protocol analysis. We will also analyze the data with and without adjustment for change in eGFR, exploring its potential effect. All analyses will be performed using SAS version 9.4 (SAS Institute, Cary, NC, USA).
- **Descriptive statistics:** Descriptive statistics of baseline characteristics and outcomes variables will be provided for all enrolled participants; mean and standard deviation for continuous variable and proportion for categorical variable. Summaries will be provided for the full cohort and for subgroups defined by age, gender, and baseline kidney function. Highly-skewed variables may be transformed prior to statistical analyses to better approximate normality. Change over time in outcome variables will be presented by using appropriate charts and tables.
- **Analysis Plan for Aim 1:** The goal of this aim is to compare changes from baseline to 12 months in FMD_{BA} and aPWV between the two groups and ANCOVA will be used to test the difference. In all analyses, a $p \leq 0.025$ will be considered significant. Furthermore, we will investigate how FMD_{BA} and aPWV change over time in one year by using longitudinal data analysis with mixed effect models. We will perform analysis of response profile in which we will assess the mean level of FMD_{BA} and aPWV at all time points in each group and examine for differences between groups at each time point. In addition, will assess for an effect of the intervention vs. placebo prior to the end of study. We will also fit parametric curves if exploratory analyses find a curve pattern of change over time in FMD_{BA} and aPWV.
- **Analysis Plan for Aim 2:** The goal of this aim is to compare changes from baseline to 12 months in LVMI between the two groups and ANCOVA will be used to test the difference. In all analyses, a $p \leq 0.05$ will be considered significant.
- **Analysis Plan for Aim 3:** The goal of this aim is to compare changes from baseline to 12 months in plasma levels of AII, aldosterone and ET-1 and serum levels of T_{50} between the two groups and ANCOVA will be used to test the difference. In all analyses, a $p \leq 0.05$ will be considered significant.

12. Potential Problems and Alternative Strategies

- Although subject recruitment and retention are always challenging, we should be able to complete the study in the proposed timeline given our groups excellent track record in recruiting and enrolling participants in clinical trials. The PI completed a randomized clinical trial of 128 patients with CKD stage 3B-4 and all patients were enrolled within 3 years (K23 DK087859).
- We should have few difficulties with the proposed experimental procedures and protocols as they are already establised in the vascular laboratories at the University of Colorado Denver. The PI has extensive experience with the vascular function measurements outlined in this proposal and the investigative team has experience with performing cardiac MRIs in patients with CKD.
- We recognize that alternative approaches exist for examining the effects of alkali therapy on CVD in patients with CKD. For example, one alternative would be to undertake a larger study to examine hard cardiovascular endpoints rather than the investigation proposed in the current application. However, our proposed study is the most cost-effective approach to obtain confirmation of the study hypotheses and to determine the endpoints to use in a future clinical outcomes trial.
- We recognize that different dosages of sodium bicarbonate therapy can be used. However, the proposed dose in the current study has been shown to be safe and effective in treating metabolic acidosis in published reports and in our completed pilot study with CKD stage 3-4 patients.

- We acknowledge that T_{50} has not been validated as a measure of vascular calcification. However, aortic calcification is strongly associated with aPWV[19,20] and reduced T_{50} is independently associated with progressive aortic stiffness in patients with CKD.[21]
- Systolic BP is an important determinant of endothelial function, arterial stiffness and LVH and could be a theoretical confounder when interpreting the mechanisms by which alkali therapy improves EDD, aPWV and LVMI. However, we do not expect this to be an issue as all participants must have their BP controlled prior to entering the study. Patients will undergo a run-in phase to ensure their BP is controlled to <130/80 mmHg and to achieve a stable antihypertensive regimen, such that changes in antihypertensive agents throughout the course of the study are minimal. Additionally, we will adjust for BP if differences in BP control is observed between the groups. In our preliminary pilot study we did not see any differences in BP between the treatment and control conditions (see section 1c).
- We acknowledge that there may be other potential mechanisms by which acid retention results in arterial dsyfunction. Acidosis induces inflammation (risk factor for arterial dysfunction)[122] and net calcium and phosphate efflux from bone (risk factor for calcification).[123] Hence, we will measure markers of inflammation (cytokine 12 panel (IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, Interferon gamma, IL-2 Receptor, IL-1 beta, tumor necrosis factor) and markers of bone metabolism (serum parathyroid hormone, bone specific alkaline phosphatase and beta C-terminal telopeptide).

Optional Cognitive and Motor Function Testing

Participants will be given the option to participate in optional cognitive and motor function testing on the consent form. Cognition refers to the mental processes involved in gaining knowledge and comprehension such as thinking, knowing, remembering, judging and problem-solving. Measurement of cognition is essential to study health and well-being. Motor function is the ability to use and control muscles and movements. Motor performance is indicative of physical health, burden of disease and long term health outcomes. It is also related to a person's daily functioning and quality of life. No additional study visits will be needed. These function measures will be done at the baseline, 6-month and 12-month visits. They will be assessed with the NIH Toolbox tests. These tests will add approximately 38 minutes onto the study visits. There is no additional risk to the patients.

1. Cognition. Cognitive function will be assessed using the NIH Toolbox computerized tests to evaluate 1) attention, 2) episodic memory, 3) working memory, 4) language, 5) executive function, and 6) processing speed. Administering the NIH Toolbox cognitive battery will yield the following summary scores, in addition to individual measure scores: Cognitive Function Composite Score ('g' factor, or general cognitive ability based on performance of each subdomain), Fluid Cognition Composite Score (includes tests of attention, executive function, working memory, and processing speed) and Crystallized Cognition Composite Score (includes Picture Vocabulary and Reading Recognition measures).

Cognitive and Motor Outcomes using NIH Tool Box

	Subdomain	Assessment
Cognitive	Attention	Flanker inhibitory control & Attention Test
	Episodic memory	Picture sequence memory
	Working memory	List sorting working memory
	Language	Picture vocabulary
		Oral reading recognition
	Executive function	Flanker inhibitory control
		Dimensional change card sort
	Processing speed	Pattern comparison
Motor	Dexterity	9-Hole Pegboard Dexterity Test
	Strength	Grip Strength Test

NIH Toolbox Cognitive Domain: computerized tests will be administered at the Kidney Research center during the baseline, 6-month and 12-month visits.

Attention (NIH Toolbox). Attention will be measured by the NIH Toolbox Inhibitory Control and Attention Test. The Flanker task measures both a participant's attention and inhibitory control. The test requires the participant to focus on a given stimulus while inhibiting attention to stimuli (fish for ages 3-7 or arrows for ages 8-85) flanking it. Sometimes the middle stimulus is pointing in the same direction as the "flankers" (congruent) and sometimes in the opposite direction (incongruent). Scoring is based on a combination of accuracy and reaction time.

Episodic Memory (NIH Toolbox). Episodic Memory refers to cognitive processes involved in the acquisition, storage and retrieval of new information and will be measured with the NIH Toolbox Picture Sequence Memory Test. The Picture Sequence Memory Test is a measure developed for the assessment of episodic memory. It involves recalling increasingly lengthy series of illustrated objects and activities that are presented in a particular order on the computer screen. The participants are asked to recall the sequence of pictures that is demonstrated over two learning trials; sequence length varies from 6-18 pictures, depending on age. Participants are given credit for each adjacent pair of pictures (i.e., if pictures in locations 7 and 8 are placed in that order and adjacent to each other anywhere – such as slots 1 and 2 – one point is awarded) they correctly place, up to the maximum value for the sequence, which is one less than the sequence length (if there are 18 pictures in the sequence, the maximum score is 17, because that is the number of adjacent pairs of pictures that exist).

Working Memory (NIH Toolbox). Working Memory refers to a limited-capacity storage buffer that becomes overloaded when the amount of information exceeds capacity and is measured by the NIH Toolbox List Sorting Working Memory Test. This test requires immediate recall and sequencing of different visually and orally presented stimuli. Pictures of different foods and animals are displayed with accompanying audio recording and written text (e.g., "elephant"), and the participant is asked to say the items back in size order from smallest to largest, first within a single dimension (either animals or foods, called 1-List) and then on 2 dimensions (foods, then animals, called 2-List). The score is equal to the number of items recalled and sequenced correctly.

Language (NIH Toolbox). NIH Toolbox focuses on two aspects of language: vocabulary knowledge, which is fundamental to the growth of knowledge and which also has a very high association with overall intelligence, or what has been called the "g factor", and oral reading skill, which reflects level and quality of prior educational experiences, and provides a fairly robust indication of verbal intelligence that is relatively undisturbed by many medical conditions that affect the brain. Vocabulary language will be measured by the NIH Toolbox Picture Vocabulary Test while oral reading will be measured by the NIH Toolbox Oral Reading Recognition Test.
The NIH Toolbox Picture Vocabulary Test measures receptive vocabulary and is administered in a computerized adaptive format. The respondent is presented with an audio recording of a word and four photographic images on the computer screen and is asked to select the picture that most closely matches the meaning of the word.
During NIH Toolbox Oral Reading Recognition Test the participant is asked to read and pronounce letters and words as accurately as possible. The test administrator scores them as right or wrong. The test is given in a computerized adaptive format.

Executive Function (NIH Toolbox). Executive Function is the capacity to plan, organize, and monitor the execution of behaviors that are strategically directed in a goal-oriented manner and is measured by the NIH Toolbox Dimensional Change Card Sort Test (DCCS). DCCS is a measure of cognitive flexibility. Two target pictures are presented that vary along two dimensions (e.g., shape and color). Participants are asked to match a series of bivalent test pictures (e.g., yellow balls and blue trucks) to the target pictures, first according to one dimension (e.g., color) and then, after a number of trials, according to the other dimension (e.g., shape). "Switch" trials are also employed, in which the participant must change the dimension being matched. For

example, after 4 straight trials matching on shape, the participant may be asked to match on color on the next trial and then go back to shape, thus requiring the cognitive flexibility to quickly choose the correct stimulus. Scoring is based on a combination of accuracy and reaction time.

Processing Speed (NIH Toolbox). Processing Speed is either the amount of time it takes to process a set amount of information, or, the amount of information that can be processed within a certain unit of time. It is a measure that reflects mental efficiency, and will be measured with the NIH Toolbox Pattern Comparison Processing Speed Test. This test measures speed of processing by asking participants to discern whether two side-by-side pictures are the same or not. Participants' raw score is the number of items correct in a 90-second period. The items are designed to be simple to most purely measure processing speed.

2. Motor. Motor function will be assessed using the NIH Toolbox tests to evaluate 1) dexterity and 2) strength. ***Dexterity (NIH Toolbox):*** Dexterity is the coordination of small muscle movements, which occur in body parts. Dexterity is measured by the NIH Toolbox 9-Hole Pegboard Dexterity Test. This simple test records the time required for the participant to accurately place and remove 9 plastic pegs into a plastic pegboard. The protocol includes 1 practice and 1 timed trial with each hand. Raw scores are recorded as time in seconds that it takes the participant to complete the task with each hand (a separate score for each).

Strength (NIH Toolbox): Strength refers to a muscle's ability to generate force against physical objects. Measuring hand grip shows how strong ones hand grip is, which in turn provides a good approximation of overall muscle strength. Strength is measured by the NIH Toolbox Grip Strength Test. Participants are seated in a chair with their feet touching the ground. With the elbow bent to 90 degrees and the arm against the trunk, wrist at neutral, participants squeeze the Jamar Plus Digital dynamometer as hard as they can for a count of three. The dynamometer provides a digital reading of force in pounds. A practice trial at less than full force and 1 test trial are completed with each hand.

Optional Additional Study Visit after Treatment is Complete

Participants will be given the option to participate in an additional study visit that occurs after they have completed 12 months on study drug. Participants will return 6 weeks after the 12-month visit. The purpose of this visit is to examine how vascular function, laboratory and urine markers change after being off the study medication for 6 weeks. This visit will last approximately 1.5 hours and the following will occur:

- Vital signs, history and physical examination including medication history.
- IV catheter will be placed and blood will be collected to measure a basic metabolic panel and venous blood gas
- Additional blood will be collected to examine inflammatory markers to see if changes persist after discontinuing treatment
- Participants will return a 24-hour urine collection. This sample will be used to measure 24-hour urine electrolytes, ammonium, pH, creatinine, and urea nitrogen.
- Vascular endothelial function will be measured as described above.

Participants will not be on study medication during this 6-week period. The only risk associated with this visit includes the blood draw and the use of nitroglycerin for measurement of vascular function. Participants will be given the option to consent for this additional study visit. They will receive $25 if they complete this visit.

Protection of Human Subjects

Sources of Research Material: This is a prospective study of newly recruited human subjects with CKD stage 3-4. The data collected will be used exclusively for research purposes. All subject identities and records will remain strictly confidential. We will obtain brachial artery flow mediated dilation, aortic pulse wave velocity, and left ventricular mass measurements, blood samples and demographic and clinical data, including detailed medical and medication histories.

c) Potential Risks: We see no psychological, social, or legal risks beyond those of participation in health related research in general. The potential physical risks of participating in the proposed experiments are reasonably small. The procedures have been used previously by the team of investigators without

complications. Importantly, all of the procedures will be performed in the Clinical Vascular Physiology Laboratory at the University of Colorado Denver and in the Brain Imaging Center MRI lab at University of Colorado Denver, both of which have on-site full-time physician supervision, nursing, and other clinical and research support personnel, safety equipment and established emergency procedures. Moreover, a Data Safety Monitoring Board (DSMB) will be appointed to oversee this ancillary study.

The risks associated with the experimental protocols include:

- ***Venous catheter:*** discomfort associated with insertion of the needle; local bleeding and a small hematoma (~10% of cases); risk of infection of a hematoma or significant external blood loss (<1 in 1000), risk of fainting. Subjects with CKD may have plans in place for future vascular access for hemodialysis and thus we will place the venous catheter in the arm not designated for future vascular access.
- **Endothelium Dependent Dilation (FMD):** 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. Since CKD patients may need future vascular access for hemodialysis, the FMD measurement will be done in the arm not designated for future vascular access for dialysis.
- **Endothelium Independent Dilation (EID):** Sublingual Nitroglycerin is used to determine EID. Nitroglycerin may cause a slight decrease in arterial blood pressure. The subject may experience minor symptoms such as lightheadedness, tingling in the tongue and in the arms and legs, light headaches, fainting and/or increased heart rate. Nitroglycerin will not be given to any subject with a systolic blood pressure less than 100 mm Hg and/or heart rate <60 or >100 on the day the measurements are performed. The dose of Nitroglycerin used is considered safe and is used in patient populations to relieve angina.
- **Magnetic Resonance Imaging:** The risk of performing cardiac MRI is minimal. The magnetic field generated within the MRI is not harmful but can cause metal within the body to heat up or electronics to stop working. All subjects will be questioned regarding the presence of metal or electronic devices inside their body (e.g. pacemakers, etc). All subjects with either metal implants or implanted electronic devices will be excluded from the study. As the MRI is a small round tube, it may make subjects who experience claustrophobia uncomfortable. Thus, all consenting subjects who experience non-severe claustrophobia will be asked to have their private physician prescribe a suitable medication to control their anxiety associated with the procedure. These subjects will be instructed to bring a designated driver with them to the MRI visit. The most common minor side effect of having a MRI exam is flashing lights in the eyes. This is caused by the magnetic waves and is not harmful. Some people also experience warmth and reddening of the skin, which usually goes away after a few minutes. For all female participants of childbearing age, a pregnancy test prior to MRI will be required. All scans will be performed without gadolinium given the risk of nephrogenic systemic fibrosis in patients with CKD.
- **Medications:** With any medication used in testing, there is small risk of an allergic reaction. Reactions reported with the use of the specific medications proposed for use include:
    - **Sodium bicarbonate** is an FDA approved medication for the treatment of metabolic acidosis and as an antacid. It is extremely well tolerated. The main side effect of oral sodium bicarbonate is flatulence and gastric distension. Metabolic alkalosis and weight gain from water retention can also occur but are rare. Serious reactions are uncommon with oral administration of the drug but include edema, CHF exacerbation, hypertension, hypernatremia, hypokalemia and pulmonary edema. Patients will be monitored monthly for side effects (see data and safety monitoring plan below).
    - **Nitroglycerin** may cause a slight decrease in arterial blood pressure, minor symptoms such as lightheadedness, tingling in the tongue and in the arms and legs, headaches, fainting and/or increased heart rate. The dose of nitroglycerin used is considered safe in the proposed population and is also used in patient populations to relieve angina.

There are no alternative methods that would provide the same type and accuracy of information as the state of-the art procedures proposed in this application.

2. Adequacy of Protection Against Risks

a) Recruitment and Informed Consent: We will use the recruitment and adherence strategies and experience previously implemented by the team of investigators as well as the services of the Health Data Compass office. Dr. Kendrick has successfully recruited patients with different etiologies of CKD to clinical trials for several years. Patients for the study will be recruited from nephrology clinics at the University of Colorado Hospital and Denver Health Hospital with access to over 2000 CKD patients.

Written informed consent will be obtained at enrollment using a standardized form approved by the Research Subject Advocate that provides appropriate information about the study and the potential risks and benefits. The subject will then read the consent form and the investigator will answer any questions that the subject may have prior to obtaining the subject's written consent. The consent form will then be signed by both the subject and the investigator as documentation of consent. A copy of the signed consent form will be given to the subject. All of the proposed procedures and protocols will be reviewed and approved by the University of Colorado Institutional Review Board (COMIRB).

b) Protection against Risk: To ensure that participants' confidentiality is maintained, each participant will be assigned a unique study identification number and will be tracked through the study by this number. Only the PI and Study Coordinator will maintain the code linking study identification numbers to specific participants. When data generated from any study is shared, only de-identified information will be exchanged. No protected health information will be sent to any collaborators or be used in any publications of the findings.

The potential general risks of the proposed ancillary study measurements will be minimized by:

- Using only safe, well-established procedures, with only qualified and experienced personnel performing the procedures.
- Ensuring constant personal monitoring of each experimental session by the investigators and clinical staff.
- Providing appropriate clinical supervision and emergency equipment and medications
- All blood pressure, blood draws and FMD_{BA} measurements will be performed in the arm not designated for future vascular access for dialysis.
- Risks associated with sublingual Nitroglycerin will be minimized by not administering Nitroglycerin to subjects with systolic blood pressure <100 mm Hg and/or heart rate <60 or >100. During Nitroglycerin administration, heart rate and blood pressure will be monitored every 2 minutes for signs and symptoms of hypotension. Additionally, an intravenous catheter (dorsal aspect of the hand) will be in place for administration of fluid or drugs in the rare event of symptoms due to hypotension or vasovagal bradycardia.
- Safety monitoring by a data safety monitoring board (DSMB)
- Evaluation for adverse events on a monthly basis either through telephone calls or in person visits.

3. Potential Benefits of the Proposed Research to the Subjects and Others: Because the risks of participating in this study are relatively small, the risk-to-benefit ratio is relatively small. Subjects will receive benefits associated with overall knowledge of their health from the extensive testing performed for screening purposes and the established subject characteristics (i.e. blood pressure, blood chemistries, etc.). The findings we will generate will answer important questions about bicarbonate administration in patients with CKD and help investigators design future clinical trials in this population. Thus, the new knowledge generated from this proposal will yield potential benefit to CKD patients in general and to society as a whole.

4. Importance of the Knowledge to be Gained: The new knowledge generated from this proposal will facilitate planning of future clinical trials in CKD that will aim to reduce the burden of CVD and premature death in this population. In addition, the proposed research should provide important insight into the mechanisms involved in any beneficial effects of bicarbonate administration on vascular function and left ventricular mass in patients with CKD.

5. Data and Safety Monitoring Plan: To ensure the safety of subjects, they will need to meet rigorous

inclusion/exclusion criteria, including comprehensive health screening procedures. A data safety monitoring board (DSMB) including clinicians and a statistician (independent of the study investigators but part of the faculty at the University of Colorado School of Medicine) will be formed to assess potential adverse events. The data will be prepared by the DSMB statistician, ensuring the study statistician remains blinded until the final analysis. The DSMB will meet at least 1 time per year to review the protocol and will follow the guidelines established by the NIH National Center for Research Resources, which include: a) monitoring the progress of the protocol (e.g. reviewing subject recruitment, attrition and minority involvement) and the safety of research participants (e.g. reviewing unblinded data for safety); b) assuring compliance with requirements regarding the reporting of adverse events; c) assuring that any action that results in the temporary or permanent suspension of the protocol is reported to all of the appropriate monitoring bodies (IRB, NIH, etc.) and d) assuring data accuracy and protocol compliance. Any unexpected adverse events will be reported immediately to the Colorado Institutional Review Board, the funding I/C and the NIH Office of Biotechnology Activities. This study is not solidly considered as a confirmatory study and as such no interim analysis has been planned.

- **Expected adverse events:** Sodium bicarbonate therapy may cause bloating, flatulence and abdominal discomfort. Although previous studies using similar doses of sodium bicarbonate have not reported significant changes in edema, body weight or BP,[75,76,92] sodium bicarbonate may cause edema to develop or worsen thereby increasing BP. Previous studies suggest fluid retention is greater when sodium is accompanied by the chloride anion rather than the bicarbonate anion.[99] As shown in our preliminary data, sodium bicarbonate did not result in increased BP. Sodium bicarbonate also has the potential for causing metabolic alkalosis and hypokalemia if it is retained rather than excreted in the urine. These expected adverse events will be monitored during the course of the study and in general, can be medically managed by adjusting antihypertensive and/or diuretic agents, providing potassium replacement and by dietary changes. Of note, none of these adverse events were observed in our short-term pilot study.
- **Dose reduction and discontinuation:**
  - Serum bicarbonate >26 mEq/L: The intervention dose will be reduced by 50% and diuretics will be adjusted as appropriate. The participant will return for a visit one week later to have serum chemistries rechecked. If the bicarbonate remains >26 mEq/L the intervention will be stopped and the participant will have serum bicarbonate measured weekly until it is <26 mEq/L. Plasma ionized calcium will be checked if bicarbonate is >26 mEq/L at any visit since an increase in pH can decrease the ionized calcium concentration.[100,101] Management of low ionized calcium will be at the discretion of the PI based on symptoms reported by the patient.
  - Systolic blood pressure ≥ 160 or diastolic blood pressure ≥ 80 mmHg: Potential reasons for uncontrolled BP, such as poor compliance or running out of medications will be evaluated and managed appropriately. If an identifiable cause is not found, the PI will increase anti-hypertensive medications and/or diuretics. The participant will return 1 week later for follow-up. If BP remains >160/80 mmHg at the second visit, medications will again be increased and the intervention dose will be reduced by 50%. The participant will return 1 week later. If BP remains unchanged at the third visit, the intervention will be stopped and medications will again be escalated. Participants will continue to have PRN visits weekly until BP is <160/80 mmHg.
  - Serum potassium < 3.0 mEq/L: The intervention dose will be reduced by 50%, diuretics will be adjusted as appropriate and potassium replacement will be prescribed. The participant will return 1 week later for repeat labs. If serum potassium remains < 3.0 mEq/L, the intervention will be discontinued. The participant will return weekly until serum potassium is > 3.0 mEq/L.
  - Severe edema: If in the opinion of the PI severe edema (4+) is present and another etiology cannot be identified the dose of the intervention will be reduced by 50% and diuretics will be adjusted. If fluid retention remains severe at follow-up visits, the intervention will be discontinued.
  - Rescue therapy with oral sodium bicarbonate: Rescue therapy with open-label sodium bicarbonate will be initiated if serum bicarbonate is <20 mEq/L on 2 consecutive measurements at least 1 week apart since a level <20 mEq/L is associated with adverse outcomes. In this instance, open-labeled sodium bicarbonate will be given to target serum bicarbonate of 20-22 mEq/L. Participants will not discontinue study medications if open-labeled sodium bicarbonate is prescribed.

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