Official Title: Dietary Acid Load, Kidney Function and Disability in Elderly
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Study Title: Dietary acid load, kidney function and disability in elderly
Principal Investigator Snezana Petrovic**, Co-investigator(s):** Stephen Kritchevsky, Michael Miller, Kristen Beavers, Jeff Williamson
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Background, Rationale and Context

Physical decline and frailty result from age- and disease-related impairments in organs and tissues. Frailty research has focused on the musculoskeletal, neurological and circulatory systems; yet interventions targeting these systems had limited success in preventing and treating functional decline. Given the aging of the US population, additional avenues for intervention development are urgently needed.

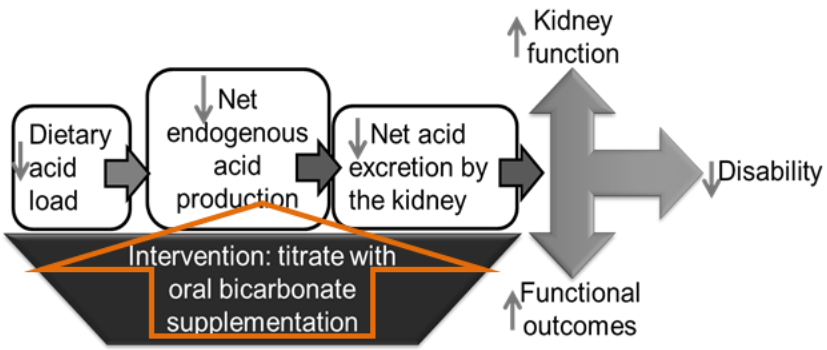
Fragility and disability in people ≥65 strongly correlate with declining kidney function and are evident even in early stages of chronic kidney disease (CKD)(1, 2). Moreover, CKD is highly prevalent in the elderly and associates with sarcopenia, osteopenia, and increased incidence of fractures/falls with hospitalization. Low serum bicarbonate and impaired acid-base homeostasis, also common in CKD, are increasingly appreciated as contributors to functional decline with advancing age(3-6). With aging, the adaptive response of the kidney to low serum bicarbonate and high metabolic acid load becomes maladaptive, facilitating CKD progression. Conversely, in adult patients with CKD, maintenance of serum bicarbonate at 24 meq/L with oral bicarbonate supplementation or increased consumption of base-forming foods slows CKD progression(7).

We proposed the current study and protocol based on the evidence summarized above and our preliminary studies, which suggest that: (1) In the Health Aging and Body Composition cohort (age 70-79) lower dietary acid load associates with stable kidney function over a 7-year follow-up, independent of age, race, gender, BMI, diabetes, hypertension or smoking status(8); (2) metabolomics analysis in participants of the African American Diabetes Heart Study suggested that it is feasible to segregate a urine metabolomics profile in the early stages of CKD (stages 2 and 3), and that lower consumption of base-forming fruits and vegetables and higher rates of acid excretion may be associated with CKD and its progression(9).

We therefore **hypothesized** that decreasing metabolic acid production by titrating dietary acid load may ameliorate the generally expected, age-related decline in kidney function, decrease loss of lean body mass, preserve physical function, and ameliorate disability. This is not a treatment study as we are exploring the effects of bicarbonate on these age-related issues.

Objectives

The objectives of this exploratory R21 project are to establish the feasibility of the proposed approach in the elderly and the project is designed to follow three Specific Aims:
Aim 1. Recruit and randomize up to 83 elderly participants to an oral bicarbonate intervention aimed at titrating net dietary acid load or placebo. The purpose of this aim is to determine the feasibility of achieving ~ 50% reduction in net acid excretion (NAE/Cr meq/g) by the kidney at 6 months in elderly participants following oral bicarbonate supplementation, compared to the placebo group.
Aim 2. Ascertain recruitment yields, adherence to the assessment schedule, compliance with and sustainability of the intervention over 6 months; and collect data on variability and longitudinal correlation structure of the parameters related to potential endpoints of a future full-scale clinical trial (kidney function, lean body mass, and functional outcomes).
Exploratory Aim 3. Explore the feasibility of using metabolomics to detect effects of decreased net acid load on kidney function as a potentially more sensitive method of monitoring kidney function than current clinical markers. Importantly, metabolomics will provide clues about the metabolic pathways activated/deactivated during the intervention, and help determine the mechanism of the beneficial effect of decreased acid load on the kidney.
Impact. This proof-of-principle proposal is a first step towards development of a new intervention to improve prevention and treatment of mobility disability (Fig. 1).



Methods and Measures

Design

This is a randomized, double-blind; placebo-controlled trial designed as exploratory R21 project aimed to establish the feasibility of the oral bicarbonate supplementation in the elderly.

Setting

The study will be conducted at the academic medical center, specifically the Clinical Research Unit (CRU) of the Wake Forest School of Medicine (WFSM) and with the support of the Pepper Center and Translational Science Institute. The participants will be recruited from the general community and evaluated and followed by the CRU.

Subjects selection criteria

Elderly at risk for disability with mild impairment of kidney function will be selected for this study.

Inclusion Criteria	Exclusion Criteria
Age 65+ years of age	Uncontrolled (>160 mg/dl fasting blood glucose), insulin-dependent diabetes and/or uncontrolled hypertension (SBP>160, DBP>100)
SPPB (short physical performance battery) score >3	a current diagnosis of psychotic disorder or taking anti-psychotic medication
eGFR stage 2 (60-89) or 3 (30-59)	take more than 14 alcoholic drinks per week
	plan to relocate out of the study area within the 8 months
Net endogenous acid production (NEAP) ≥40 mEq/d	self-reported inability to walk across a room
Willing to provide informed consent and agrees to randomization	those who reside in nursing homes
Not involved in another intervention study	have difficulty communicating with study personnel due to speech or language or hearing problems
	had cancer requiring treatment in the past 1 year
	lung disease requiring regular use of corticosteroids at a dose of >7.5 mg/d for the last 3 months or use of supplemental oxygen
	cardiovascular disease (Class III or IV congestive heart failure)
	significant valvular disease, uncontrolled angina
	myocardial infarction, major heart surgery (i.e., valve replacement or bypass surgery) in past 6 months
	stroke, deep vein thrombosis, or pulmonary embolus in the past 6 months,
	Parkinson's disease or other progressive neurological disorder
	other medical or behavioral factors that in the judgment of the principal investigator may interfere with study participation or the ability to follow the intervention
	clinical judgment concerning safety or noncompliance
	Individuals with BMI <18.5; or unintentional weight loss >4% in last 6 months
	MoCA score under 24
	End Stage Renal Disease (ESRD) on dialysis or primary kidney disease
	Other illness of such severity that life expectancy is less than 12 months
	Any tobacco or nicotine product use in the past year
	Serum HCO3>30meq/L; serum K+ out of normal range <3.5 or > 5.2

Sample Size

Based on published studies(11-14), we have assumed a 6-month change in NAE/Cr within groups of 16.5 mEq/g. Assuming a conservative 12.5% loss-to-follow-up at 6 months (35 participants/group), we will have 80% power to detect a difference between groups in NAE/Cr as small as 11.4 mEq/g with a two-sided, Type I error rate of 0.05. Considering that expected change in NEA/Cr will be about 20-30 meq/d(11, 12, 14), we should be able to detect clinically important changes in NEA/Cr from the proposed intervention. For Aim#2, we are interested in estimating recruitment yields, compliance and other parameters that will inform the feasibility of a full-scale trial. With up to 83 participants recruited, we will be able to estimate the two-sided, 95% Clopper-Pearson Exact confidence interval with a width of 0.043 if the recruitment yield is 10%, and a width of 0.14 if the recruitment yield is 40%. For correlational analyses, 83 participants will allow us to detect correlation coefficients of 0.31 or larger with at least 80% power assuming a 2-sided 0.05 □.

Interventions and Interactions

Patients will be screened over the phone and in the clinic to see if they meet qualifications for the study. Once screened, they will be randomized into one of two groups- taking an oral bicarbonate supplement or a placebo.

Oral bicarbonate supplementation group. Participants will be advised to continue their usual dietary and exercise pattern, but to take supplementation capsules. They will receive 0.3 meq/kg/day NaHCO3, a dose based on the literature (14) and our Health ABC cohort preliminary study (8). The dose of 0.3 mg/kg/d NaHCO3 was reported to reduce net acid excretion ~50% in individuals with similar daily acid load(14), and was effective in decreasing the rate of yearly eGFR decline in patients with hypertensive nephropathy; the dose has also been reported as safe (14, 15). NaHCO3 will be provided in gelatin capsules containing 10 meq NaHCO3, so that NaHCO3 can be dosed to the nearest amount by total body weight; e.g, a 70kg person will receive 2 capsules daily, and an 85kg person would receive 3 capsules daily (14). Adherence will be assessed by pill count. Compliance will be measured at months 3 and 6 by determining the change from the baseline in net acid excreted (NEA) in 4hr-urine; an advantage of our approach is that compliance can be measured objectively rather than estimated. Oral bicarbonate has been used for decades in patients with CKD or distal renal tubular acidosis to treat metabolic acidosis(7), and is generally considered free from significant side effects. Participants will be withdrawn if any of the following occur: total carbon dioxide >30mmol/L, serum K+ <3.5mEq/L, hospitalization for congestive heart failure, weight gain >2kg due to edema, or increase in blood pressure (>15 mmHg systolic or diastolic) (7, 15).

Placebo group. Participants will be advised to continue their usual dietary and exercise pattern, but to take supplementation capsules (methylcellulose). To ensure participants' blinding, identical capsules will be taken and the total number of capsules kept constant. The placebo group will be followed in the same manner as the bicarbonate group; all assessors will be blinded with respect to group assignment.

Clinical assessments, laboratory analysis and analytical tests.

An 4-hour urine collections were chosen because they can be done during the day in the Clinical Research Unit (CRU). Participants will empty their bladders before beginning the timed collection; then all urine collected over 4hrs will be pooled into a collection container. The specimen will be refrigerated during the collection period and subsequently frozen until analysis. While total urinary nitrogen and Ca^{+2} will be done by LabCorp, a trained technician in our Geriatrics laboratory will determine NEA/Cr (urine ammonium + titratable acidity) using standard methods (16).

For blood gas measurements, a blood sample will be obtained from a cannulated hand vein warmed for 10-15 min at 55˚C before sampling. Samples will be analyzed immediately after phlebotomy(17), using an ABL5 blood gas analyzer to measure blood pH and pCO_2; based on these measurements, the analyzer calculates serum bicarbonate using Henderson-Hasselbalch equation.

Kidney function will be determined using uACR and eGFRcys. (estimated with CKD Epidemiology Collaboration equation (CKD-EPI) (18), based on serum cystatin c levels, which will be measured by a particle-enhanced immunonephelometric assay86). The Nutrition Data System for Research (NDSR) will be used to conduct 24-hour food recalls. NDSR is a computer-based software application which will be used to assess daily protein and K+ intake needed for calculation of NEAP = [54.5x(prot (g/d) / pot (meq/d)] – 10.2, where

prot=g of protein consumed per day and pot=meqs of potassium consumed per day(19). The Short Physical Performance Battery (SPPB) is a well-characterized global measure of lower-extremity function and mobility. It will be assessed during screening (20, 21), at 3 and 6 months. The test consists of gait speed, chair stands, and balance; it is a strong predictor of disability and mortality in initially non-disabled older persons. SPPB scores of 4-9 have already been successfully used as a range associated with risk for developing mobility disability(21). The 400-meter walk is a measure of aerobic endurance where participants are instructed to complete the 400m distance (on a flat indoor surface) at their normal pace and the time to complete the walk is recorded in minutes and seconds. A comprehensive metabolic profile (CMP), including tCO2, electrolytes, blood glucose and creatinine will be assessed at baseline and at 6-month follow-up. In conjunction with data from urine collections and analysis, CMP will allow calculation of fractional excretion of Na+, K+, Cl-, and the anion gap(22).

Bone Health. Because the kidney's capacity to excrete acid declines with age, acid-producing diets lead to a positive balance of protons and mild (often) subclinical acidosis. This acidic milieu facilitates release of Ca+2 from bone and increases bone turnover; it impairs osteoblast function and activates osteoclasts through stimulation of the proton-sensing receptor OGR1 and release of prostaglandin E2. Phosphate and bicarbonate released from bone mineral neutralize acid by a physicochemical reaction, leading to further Ca+2 release from the bone. In addition, renal Ca+2 excretion increases, through suppression of Ca+2 transporters and the calcium-sensing receptor (CaSR), which is exquisitely pH sensitive. CaSR regulates both renal Ca+2 absorption and parathyroid hormone release. Since a dietary acid load does not alter intestinal Ca+2 absorption, negative Ca+2 balance persists as long as the proton balance remains positive (33-73). This pathophysiologic mechanism explains why it is of great interest that we evaluate bone density in our study, which is testing the feasibility of neutralizing diet-dependent acid load in the elderly.

Metabolomics. Metabolomics analysis of the urine samples collected at baseline and the last follow up visit will extend our preliminary findings: 1) to elderly individuals; 2) will test the predictive value of identified metabolites with targeted metabolomics and 3) will repeat the broad spectrum analysis in samples taken before and after the intervention, circumventing the uncertainty of the preliminary data, which relied on statistical associations. All analyses will be overseen by the Wake Forest School of Medicine Proteomics and Metabolomics Core (see the letter from the Director, Cristina Furdui); the metabolomics samples are processed in collaboration with NIH Eastern Metabolomics Core at RTI70-74. We will use targeted metabolomics and broad spectrum 1H NMR spectroscopy.

The copy of **the questionnaire** that will be used during the **initial phone call** to determine initial eligibility (e.g. to exclude individuals with already high consumption of fruits and vegetables (base-forming foods) or those meeting exclusion criteria based on medical history (self-reported insulin-dependent diabetes, advanced heart disease, end stage renal disease) are included in the protocol application. The **questionnaires** that will be used during **follow up telephone call and follow up visits** to evaluate safety and adherence and to collect 24hr-food recall data are also included.

Participants will be required to attend a Screening Visit (SV) after they qualify based on the phone screener. During the screening visit they will be explained the Informed Consent and must sign to agree to participate. They will complete a Short Physical Performance Battery (SPPB) and a MoCA (Montreal Cognitive Assessment) and scores from these tests will determine initial eligibility. Study Coordinators will then ask questions regarding medical history and medications, and a Comprehensive Metabolic Panel (CMP) and urinalysis will be completed. If participants prove to be eligible based on their scores and blood work, they will be asked to come back for their Baseline Visit (BV). Usual dietary intake will also be assessed via a food frequency questionnaire at the BV1, and FV3 visits. We plan to use the VioFFQTM (Viocare, Inc., Princeton, NJ), which is a validated, web-format system for collecting food frequency data. VioFFQ collects data on a subject's dietary behavior and food use patterns, provides estimates of macronutrients, micronutrients, and servings of particular foods of interest, and delivers detailed dietary analysis data and reports.
Dietary intake will also be assessed by 24-hour diet recalls. Data will be collected and analyzed using the NDSR software developed and supported by the Nutrition Coordinating Center (NCC) of the University of Minnesota. NDSR allows use of the multiple pass approach, providing interview prompts to guide and standardize data entry. Subjects will be screened to assure that they are accessible by telephone for the

collection of these data throughout the study. The first recall will be conducted at the SV to review all procedures and tools to be used. A "Food Amounts" booklet containing 2-D visual portions will be provided to the participant for use in standardizing portion sizes. Subsequent recalls will be conducted by telephone with trained interviewers from the CRU placing unscheduled telephone calls to participants at seven additional timepoints. When contacted by the staff, subjects will be asked to recall and report their dietary intake from the previous day's 24-hour period. In addition to the first in-person SV recall, telephone recalls will be done within one week of the SV (1) to further assess eligibility (an average of both protein intake and K+ will be used to calculate the NEAP score), and within a 1-week period of each follow-up visit for a total of eight recalls during the study. Recalls will be randomly scheduled and will reflect both weekday and weekend intakes.

At the baseline visit and remaining in-clinic follow up visits, participants will spend approximately 4hr in the Clinical Research Unit (CRU), have their physical ability tested (FV2 and FV3 only), have blood drawn for blood gas analysis as well as blood for cystatin c and blood for storage. The blood gas draw will be performed in the CRU. Urine will be collected over the 4hr period (this is necessary because urine collections need to monitored and refrigerated). Participants will be encouraged to take 500 ml of water during their stay at the CRU, to stimulate urine production. During these visits (not at FV2) the participants will also provide information about their dietary habits using the VioFFQ™ (Viocare, Inc., Princeton, NJ), which is a validated, web-format system for collecting food frequency data on a subject's dietary behavior and food use patterns. The questionnaire will be self-administered. Participants will also receive a DXA scan of their hip at BV and FV3.

Table showing Study Schedule of events	**SV**	**BV**	**FV1**	**FV2**	**FV3**
Location of Visit	CRU	CRU	Phone Call	CRU	CRU
Study Week Number Activity/Assessment	-1 wk	0	1 mo	3 mo	6 mo
Informed Consent	X				
Cognitive Screen (MoCA)	X				
Fasting blood draw (CMP)	X			X	X
Urinalysis (Spot Urine)	X				X
SPPB	X			X	X
400m walk test		X		X	X
VITALS	X	X		X	X
DXA		X			X
24-hour Diet Recall to calculate NEAP	X		X	X	X
Medical History and Medications	X		X	X	X
Food Frequency		X			X
Blood Gas, Cystatin C, blood for storage		X			X
4hr urine collection to determine (Net acid excretion (NAE); urinary nitrogen and Ca^{+2} excretion, uACR) Urea Nitrogen, Uric Acid, Urine Creatinine,) and obtain urine for storage		X		X	X
Randomization		X			
Urine Metabolomics		X			X
Dispense Medication		X		X	
Medication Adherence				X	X

Outcome Measure(s)

1. Determine the feasibility of achieving **~50% reduction in net acid excretion (NEA/Cr meq/g)** following 6 months of oral bicarbonate supplementation, compared to the placebo group
2. Determine
    a. Recruitment yields
    b. Adherence to the assessment schedule
    c. Compliance with intervention over 6 months

    d. Collect data on variability and longitudinal correlation structure of kidney function, lean body mass and functional outcomes
3. Ascertain feasibility of using metabolomics to detect changes in kidney function following the intervention

Analytical Plan

All data will be entered as collected into web-based forms; analysis datasets will be stored in SAS. All data will be examined monthly by histograms and scatterplots to check for internal inconsistencies, unusual data needing further verification, and outliers. In few case of outliers and unusual data, participant's charts were reviewed to ascertain whether any adverse measurement conditions or circumstances or potential artefacts existed. Only in cases where measurement artefacts were identified or other circumstances were found that adversely affected data collection were the measurements removed. Otherwise all data remained within the data set. Sensitivity analyses will be performed with and without potential outliers included in individual analyses.

For each analysis, regression diagnostics and exploratory analyses will be performed to find appropriate transformations of the variables, if needed. Priority in choosing a transformation will be to satisfy: 1) linearity, 2) homogeneity, and 3) normality assumptions. Initial analyses will follow the "intent-to-treat" principle. Due to having low power to detect potentially important relationships within this pilot, emphasis will be on using confidence intervals to described estimated parameters and relationships. When hypothesis testing is performed, we will set the significance level at $\alpha=0.05$. We have chosen this level for all hypothesis tests within the pilot because risk of missing an association by making a Type II error outweighs the concern for falsely identifying relationships via a Type I error.

Aim 1 Determine the feasibility of achieving ~50% reduction in net acid excretion (NAE/Cr meq/g) by the kidney after 6-months of oral bicarbonate supplementation vs. placebo. (NAE/Cr was measured at each visit.)

We will use mixed effects analysis of covariance (ANCOVA) to estimate change in NAE/Cr within randomized groups, including the baseline value of NEA/Cr and the intervention effect in the model. A contrast will be sued to obtain estimates and 95% confidence intervals after 6-months. This approach utilizes all measurements obtained during follow-up and can provide unbiased estimates, if missing outcomes are related to outcomes obtained at other time points.

Aim 2 Ascertain recruitment yields, adherence to the intervention, adherence to the assessments, and sustainability of the intervention over 6 months; and collect data on variability and longitudinal correlations in kidney function, lean body mass, and functional outcomes to design a future full-scale clinical trial.

For recruitment yields, we will calculate the proportion of screened participants randomized, overall, and within gender and ethnic subgroups.

Adherence to the intervention will be measured as the proportion of the prescribed dose (bicarbonate or placebo) taken, based on pill counts.

Compliance with assessments will be determined by calculating proportion lost to follow-up at each visit and the percent of missing measurements.

Sustainability of the intervention will be assessed via by: a) generalized estimating equations for repeated measures on the proportion of expected pills a participant took.

Means (proportions), variances, and 95% confidence intervals (exact CI where needed) will be calculated within intervention groups and for the difference between groups for all endpoints.

Correlations coefficients (both Pearson and Spearman) will be used to investigate the association between net endogenous acid production (NEAP) and other measures of interest, as well as changes in net acid excretion

(NAE) and measures of interest. (Measures of interest: tCO2, blood bicarbonate (blood gas), eGFR, urineACR, SPPB, 400m walk, bone density).

Power analysis: Based on published studies, we have assumed a 6-month change in NAE/Cr of 16.5 mEq/g within groups. Assuming a conservative 12.5% loss-to-follow-up at 6 months (35 participants/group remaining), we will have 80% power to detect a difference between groups in NAE/Cr as small as 11.4 mEq/g with a two-sided Type I error rate of 0.05. Considering that expected change in NEA/Cr will be about 20-30 meq/d we should be able to detect clinically important changes in NEA/Cr from the proposed intervention. For correlational analyses, 80 participants will allow us to detect correlation coefficients of 0.31 or larger with at least 80% power assuming a 2-sided 0.05 □.

Other Exploratory/Sensitivity Analyses:
Do NEAP and NAE correlate at baseline, when controlled for kidney function and caloric intake?
Do NAE and calcium excretion correlate at baseline and other time points, when controlled for kidney function and calcium intake?
Do NAE and sodium intake correlate? Do NAE and sodium excretion in the urine correlate? (An important one, if so, we will need to treat sodium intake as a confounder. There is physiological feasibility that these two may correlate.)
Does NEAP calculated from food recalls correlate with NEAP calculated from FFQs?

Human Subjects Protection

Human Subjects Involvement and Characteristics
We plan to recruit and randomize up to 83 older men and women who meet the inclusion/exclusion criteria described above. Participants will be randomized to bicarbonate capsules or placebo for 6 months. All assessments will be conducted during following visits and phone calls: screening phone call, 1 screening visit (SV1); 1 baseline visit (BV1); 1-month phone call, 3-months mid-intervention follow-up visit (FV1); and 1 post-intervention follow-up visit at 6-month (FV2).

The nature, purpose, and risks of all procedures and protocols will be explained to each participant prior to obtaining the written consent. All examiners are trained in the standardized conduct of all assessments before data collection. Participants will be instructed to wear appropriate and comfortable clothing and standardized written instructions will be provided prior to each study visit. The primary outcome of interest (change in net acid excretion (NAE) by the kidney) was selected as an indicator of feasibility of implementing this intervention in elderly individuals. Oral bicarbonate will titrate dietary acid load, decrease net endogenous acid production (NEAP) and this will result in decreased acid excretion by the kidney. This dose (0.3mg/kg) was selected according to published studies, which indicated that it is generally safe and can decrease NEA ~ 50%(14). We have extensive experience measuring all of these outcomes in our research studies and the fact that there is such an easy-to-assess and inexpensive (simple chemical titration of titratable acid and ammonium) outcome to measure is an important advantage of our approach. Importantly, decreased NEA will also serve as quantitative measure of compliance.

Sources of Research Material
Participants will self-report personal information on paper survey forms including personal demographic data, race, health information, and psychological and social data. Results will also be recorded in a computer database. Data from the paper forms will be entered into an electronic database on a computer workstation. All material and data will be obtained solely for research purposes.

Potential Risks
The potential risks to study participants receiving bicarbonate supplementation have recently been reviewed by Dr. Wesson who is a consultant on this study (7). In summary, the potential risks of this study include:

1) Blood Draw. Participants may experience temporary pain, bruising, bleeding and a small risk of infection or fainting or dizziness during the blood sample collection process. Only trained staff will be responsible for the collection of blood samples.

2) Metabolic alkalosis (increased serum bicarbonate). Theoretically, administration of bicarbonate can cause metabolic alkalosis, but that is unlikely because the kidney excretes excess of bicarbonate efficiently. We will be enrolling participants with CKD 2 or 3; at this level of kidney function, the participants should not experience problems with elimination of any excess of bicarbonate. Moreover, it has been demonstrated that oral bicarbonate supplementation for 1 year at 1 meq/kg/bw/day (70 mEq/day for 70 kg =5.9g/d) even in patients with substantially decreased glomerular filtration rate (stage 4 CKD) did not increase serum bicarbonate above normal levels (24). An exception would be a participant who developed e.g. vomiting and hypokalemia because of some unrelated e.g. viral illness. In that case, the participant would be advised to discontinue bicarbonate supplementation until recovery, since these individuals might be susceptible to develop metabolic alkalosis.

3) Volume overload. Studies that used bicarbonate supplementation for up to 5 years did not report increases in blood pressure or required increased dose of anti-hypertensive agents (7, 24). This is not surprising given that NaCl supplementation, but not sodium bicarbonate supplementation has been shown to affect blood pressure. Regarding other electrolyte and volume abnormalities, we will initially select participants who do not have edema. Nevertheless, the participants will be carefully monitored for edema development. It is important to note that a recent pilot trial, which tested safety and dose of sodium bicarbonate in CKD patients did not find development of edema as a side effect (15). Of interest for our application is that the dose we proposed to use is in the range which according to Abramowitz et al.(15) did not show any difference in adverse effects compared to placebo:

Table 5. Side effect profile before and during treatment

Adverse Effect	Baseline	Placebo	Oral Sodium Bicarbonate Dose (mEq/kg per day)			*P* Value
			0.3	0.6	1.0	
Gastrointestinal symptoms						
Bloating	3 (15)	4 (20)	2 (10)	2 (10)	4 (22)	0.96
Flatulence	10 (50)	15 (75)	11 (55)	12 (60)	10 (55)	0.39
Stomach upset	3 (15)	4 (20)	3 (15)	3 (15)	3 (11)	0.84
Nausea	3 (15)	2 (10)	0 (0)	3 (15)	4 (22)	0.28
Total	12 (60)	16 (80)	11 (55)	12 (60)	11 (61)	0.24
Edema						0.61
None	11 (55)	12 (60)	13 (65)	11 (55)	10 (55)	
1+	7 (35)	5 (25)	4 (20)	6 (30)	7 (39)	
2+	2 (10)	3 (15)	3 (15)	3 (15)	1 (6)	
Shortness of breath	4 (20)	1 (5)	1 (5)	3 (15)	2 (11)	0.97
Hospitalization	0	0	0	0	0	—

Data are expressed as number (%). Data are presented for all 20 participants at each time point except for the 1.0-mEq/kg per day dose, when 18 participants remained in the study.

4) Increased risk of cardiovascular disease. Lower all-cause mortality has been reported for individuals with serum bicarbonate >23 mmol/L vs. <23 mmol/L. A recent study however reported slightly higher risk of heart failure in kidney patients with serum bicarbonate >24 mmol/L. Serum bicarbonate >24 mmol/L did not however associate with any increase in mortality or ischemic events in the same group of patients (25). As an additional precaution, we will calculate bicarbonate deficit (if any) for each individual (=serum bicarbonate x 50% body weight (kg)) to help adjust dosing regimen and will monitor serum bicarbonate after the initiation of the intervention to adjust the amount of bicarbonate administered, if needed.

5) Predisposition to vascular calcifications as a result of reduced solubility of calcium. There is a theoretical concern from *in vitro* cell culture studies that raising extracellular pH may reduce solubility of calcium and predispose participants to vascular calcifications(26). There are currently no studies suggesting that this occurs *in vivo* with bicarbonate therapy, but this issue has not yet been addressed specifically in clinical trials. This may be more relevant to patients with advanced kidney disease and patients with Ca x Pi ratio >50, receiving phosphate binders, who are in fact prone to vascular calcification, than to population that we plan to recruit (CKD2 and 3a)(27).

6) Simultaneous ingestion of bicarbonate and non-absorbable antacids (aluminum and magnesium carbonate in combination with cation-exchange resins). This causes formation of easily absorbable bicarbonate in the small intestine and in this case metabolic alkalosis can occur, if bicarbonate excretion by the kidney is limited, again unlikely in participants with CKD 2 and 3a. Nevertheless, participants will be advised to avoid concomitant use of non-absorbable antacids and bicarbonate.
7) DXA scans. Exposure to radiation from the hip DXA (20 mRem total) for baseline and follow-up scans.

Adequacy of protection against risks

Recruitment and Informed Consent

We will use a number of recruitment strategies, including the use of mass mailing, newspaper ads, letters to potential participants, community advertising, and volunteers among participants from previous studies. We will also advertise in the VITAL newsletter (BG99-559) and participate in community outreach events. Participants who are eligible via a telephone screening interview, and who agree to go through the screening process, are invited to a screening visit. The informed consent process will follow the procedures of the WFSM Institutional Review Board. The study interviewers explain the purpose, methods and extent of the study to prospective participants. The potential participant is asked to read the informed consent form and ask questions. The form is written in simple easy to understand language. We require study staff to review all of the key aspects of the study verbally with the potential participants. Staff are provided with a structured checklist for this purpose. Staff are then required to question potential participants to ascertain whether they understood the information. Potential participants who are illiterate or have impaired vision must have the consent read to them, followed by review of the checklist, opportunity for questions, and discussion. A copy of the signed and dated consent form will be given to participants, and the original document will be placed in participants' individual study files, which will be stored in a secure location.

Safety measures during the bicarbonate intervention period

There is plethora of published data on use of bicarbonate supplementation and that has simplified determination of a safe dose for this study. An average dose per 85 kg patient will be ~ 25 meq/d as gel capsules containing 10 meq $NaHCO_3$ with sucrose in 3 daily doses; placebo capsules will contain sucrose only. Per published studies(14), each subject will be dosed to the nearest "one-half capsule" by lean body weight in kg (e.g., a 70 kg subject will receive two doses daily; capsules with half a dose can easily be prepared by our pharmacy as needed)). Participants will be monitored for evaluation of medication tolerability. Adherence to study medication will be assessed by pill count and deducted from net acid excretion measurements. Participants will be withdrawn from the study at their FV2 visit if their total carbon dioxide >30 mmol/L, their eGFR falls below 15 ml/min, they experience weight gain of more than 2 kg due to edema, develop serum potassium<3.5mmol/L, or have an increase in blood pressure (systolic or diastolic) of more than 15 mmHg (7, 15). These parameters will be assessed at each visit.

Safety measures during the assessments

All study assessments will be conducted by trained and certified staff. Safety precautions will be taken during all testing (e.g. gait speed) by applying standardized stopping criteria. If the participant reports pain, tightness or pressure in the chest, feeling faint, lightheaded or dizzy, or significant other medical problems, the test will be stopped. The PI and Co-I Dr. Williamson, will review lab data for medical concerns. When there are medically relevant findings, the participant will be told the cause for concern, and may be advised to consult his or her physician. If given permission by the participant, a letter will be sent to her/his primary care physician stating the concern.

Adverse events include any event that occurs during the course of the study that results in a participant suffering physical or mental injury, pain or suffering. Adverse events can be major, such as a subject who suffers cardiac arrest during neuromuscular function testing, or minor such as a subject pulling a muscle during neuromuscular function testing. This includes any events occurring while a subject is enrolled in the study, even if the event did not occur while s/he was actively participating in the activities called for in the research protocol. Deviations from the study's protocol are also considered an adverse, unexpected, or notable event and will be reported to the PI.

Both major and minor events will be reported using the study's Adverse, Unexpected, or Notable Event Reporting Form. Any major event, i.e., any serious injury or life-threatening event, will be reported immediately right after completing any and all actions that are necessary to protect the subject's health and safety. Minor events will be reported within seven days. A description of the event, and the date and location of the event will be recorded on this form, which will be kept in the subject's research file.

Confidentiality

Data will be used only in aggregate and no identifying characteristics of individuals will be published or presented. Results of testing will be sent to participants' private physicians if participants agree to this. Confidentiality of data is maintained by using research identification numbers, which uniquely identify each individual. The information collected from participants in this study has a low potential for abuse because the data does not address sensitive issues. Nevertheless, appropriate measures are taken to prevent unauthorized use of study information. Data other than demographic information do not use names as an identifier. The research ID number is used. The research records are kept in locked cabinets in the Geriatric Research Clinic. The files matching the participants' names and demographic information with the research ID numbers are kept in a separate room and locked file that uses a different key from that of all other files. Files may not be obtained from the research unit by persons other than research personnel, who are asked to sign a document agreeing to maintain the confidentiality of the information. After the study is completed, the local data will be stored with other completed research studies in a secured storage area.

Potential benefits of the proposed research to the participants and others

Individuals may experience clinical benefit from knowing some of their physiological parameters, such as their muscle strength and physical performance. Participants randomized to bicarbonate intervention may experience improved physical function. Individuals will be informed of their results at the end of the study and provided a copy to give to their health care providers. Those who do not qualify will be told the reasons for disqualification and referred for appropriate care. Therefore, the risk/benefit ratio is acceptable since potential risks of these research procedures are minimal and/or infrequent, and possible complication will be carefully guarded against. Potential risks are reasonable in relation to anticipated benefits to health from the intervention, which may include improved kidney function, positive nitrogen balance, improved muscle strength, and increased bone density.

Importance of the knowledge to be gained

These studies focus on using base (bicarbonate) to titrate high dietary acid load, which is emerging as a new risk factor for disability mediated through the progression of the aging associated-decline of kidney function. We plan to determine the feasibility (this application) and ultimately effectiveness (the subsequent R01) of a comparably simple and generally safe intervention to decrease dietary acid load in elderly, which, in spite of strong evidence in support of its potential effectiveness, has not been tested in the age group who is also most likely to benefit from this intervention and at risk for disability. This intervention may ultimately help protect kidney function and reduce the associated disability in elderly, with a hope that it may work synergistically with exercise, the only other intervention demonstrated in a randomized, controlled clinical trial to ameliorate disability, albeit modestly (the LIFE trial). The importance of the high dietary acid load in face of declining kidney function is a public health issue pointed out ~ 20 years ago, at which time the term "presbynephria" was coined to alert the medical community to consequences of the diminishing capacity of the aging kidney to excrete acid, including osteopenia, sarcopenia, adverse functional outcomes and disability(30-32).

Data quality and management

The study investigators and staff have experience and expertise in quality control procedures and successful participant tracking in randomized controlled trials. We will use a computerized tracking system to document participant attendance, generate quality control reports for identifying protocol deviations and to assess protocol adherence over time. All data will be entered into web-based forms on a continuous basis as collected, and analysis datasets will be stored in SAS. All data will be examined monthly by histograms and scatterplots to check for internal inconsistencies, unusual data needing further verification, and outliers. Telephone screening and in-person assessment interviews will be conducted by trained study staff who will use computer-assisted technology, entering the data directly into desktop computers.

Data entry is facilitated by automated skip patterns and alerts for out of range values. We will develop standardized reports that will be used for quality control. Careful development of data collection forms is crucial (a) to allow for systematic and uniform recording of participants' data, (b) help maintain complete data collection, and (c) to minimize the possibilities for data entry errors. Data entry screens are created to mimic the forms. Forms are designed so to make data entry easy and straightforward. In addition the above precautions, procedures to monitor screening, data collection, follow-up, clinical measures, forms and data entry will also be used.

Data and Safety Monitoring Plan
We will use the WFU Pepper Center's appointed Data Safety Monitoring Board. This board meets twice a year and reviews all studies that are supported by the WFU Pepper Center. A copy of the report will be reported to the IRB after each meeting.

Informed Consent
Informed consent will be obtained by a trained study coordinator in a clinic room with closed doors. The study interviewers explain the purpose, methods and extent of the study to prospective participants. The potential participant is asked to read the informed consent form and ask questions. We require study staff to review all of the key aspects of the study verbally with the potential participants. Staff is then required to question potential participants to ascertain whether he/she has understood the information. The consent form will only be signed once the participant has read the form and is satisfied with all information given. The person administering consent will also sign and a copy of the signed and dated consent form will be given to the CRU, and to participants, and the original document will be placed in subjects' individual study files, which will be stored in a secure location.

Storage and disposal of biological material
Blood and urine samples will be stored at Wake Forest Baptist Medical Center for twenty years after the end of the trial at which time the samples will be destroyed. Biological specimens will be stored in locked –70oC alarmed Revco freezers located in a locked room. The Wake Forest Pepper Center Integrative Biology Core lab coordinator (Karin Murphy) and the Core Leader (Dr. Nicklas) have access to the keys of the freezers. All the specimens will have numerical study IDs with no personal identifiers of the participants. These are stored under the Pepper Center Tissue Repository (IRB#1219).

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