PROTOCOL

Jean Mayer USDA Human Nutrition Research Center on Aging (HNRCA) at Tufts University

TITLE: Impact of protein and alkali supplementation on skeletal muscle in older adults

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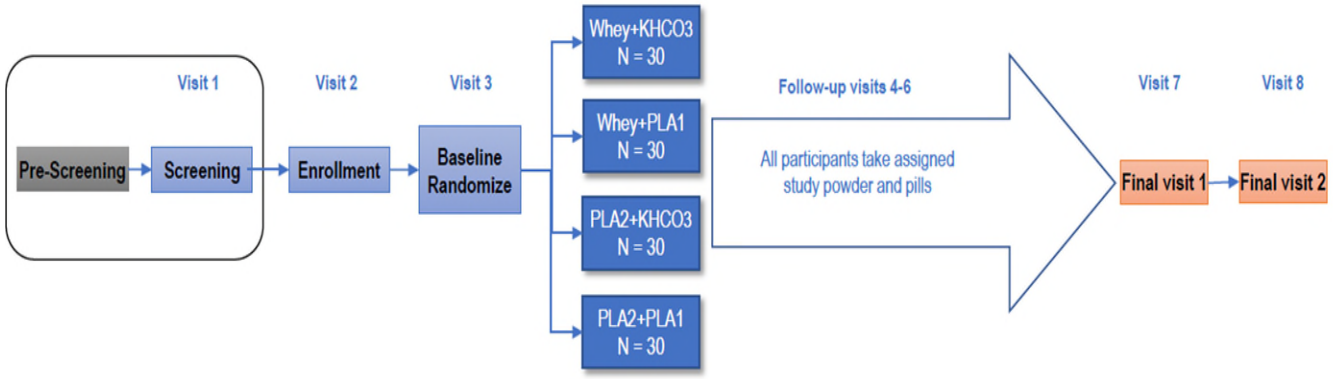
PROTOCOL SUMMARY

Synopsis

Skeletal muscle loss and impairment – prominent features of the aging process – lead to physical disability. There is plausible evidence that altering components of the diet, mainly *protein intake* and *acid/base balance*, may improve indices of muscle health in older adults and thus translate to a reduction in physical disability. A growing number of studies suggest that increasing protein intake using a whey protein supplement may benefit muscle health in older adults. However, a main concern is that high protein results in a large dietary acid load from the breakdown of protein to acidogenic byproducts, which could in turn promote muscle degradation particularly in older adults with age-related declines in renal excretion of acid. Thus, our hypothesis is that the balance between the amount of protein in the diet (anabolic component) and the net acid load of the diet (catabolic component) in part determines whether the diet as a whole has a net anabolic or catabolic effect on muscle. Preliminary data from our group have suggested that a daily potassium bicarbonate ($KHCO_3$) supplement improved lower extremity muscle power in postmenopausal women. Based on our preliminary data, we plan to conduct a randomized, double-blind, placebo-controlled, 2x2 factorial study in which underactive men and women age 65 and older on baseline lower protein diets will be enrolled. Participants will be assigned to one of four groups: either a whey protein supplement (to raise protein intake to 1.5 g/kg/d) with or without $KHCO_3$ 81 mmol/d or an isocaloric placebo supplement with or without $KHCO_3$ 81 mmol/d for 24 wks. The primary outcome is lower extremity muscle power at 24 weeks. Secondary outcomes are lower extremity muscle power at 12 weeks, knee extension strength/torque at 12 and 24 weeks, physical performance at 24 weeks, lean mass at 24 weeks, and 24-hr urinary nitrogen excretion adjusted for nitrogen intake at 24 weeks. Exploratory outcomes will include a measure of muscle mass – D_3-creatine dilution – and how it correlates with the older method – lean mass. Our central hypothesis is that higher protein intake and a neutralizing alkaline salt will improve muscle performance and mass, compared to their respective placebos, in older men and postmenopausal women. If successful, this research could result in a paradigm shift in dietary advice to older adults to reduce physical disability. As 1 in 5 US adults will be over age 65 by year 2040, a safe dietary intervention that reduces age-related musculoskeletal decline will be an important preventative health step for our aging society.

Study Schema



INTRODUCTION

Study Rationale

Epidemiological studies[1-5] report that older adults with protein intakes ≥1.2 g/kg/d have significantly greater lean mass and muscle performance than those consuming 0.8 g/kg/d (Recommended Daily Allowance, RDA) or less. A recent pilot weight loss study in older adults indicated that increasing dietary protein from 0.8 to >1.2 g/kg/d improved physical function as measured by a short physical performance battery (SPPB) over 24 wks.[6] Other intervention studies in exercised older adults show muscle benefits of added protein supplements particularly whey protein.[7-9] Results, however, remain mixed.[10] A main concern demonstrated in many metabolic studies[11-14] including our pilot study[15] is that high protein intake increases the dietary acid load from the breakdown of protein to sulfur-containing amino acid byproducts. Acidosis is an established stimulus for muscle catabolism.[16] Older adults have age-related decline in renal function causing decreased renal excretion of acid; thus, high dietary protein potentiates a low-grade metabolic acidosis[17] that could limit the anabolic effect of protein. *It is currently unknown whether reducing the acid load of higher protein intakes allows protein to have an enhanced effect on skeletal muscle health.*

Alkalinogenic foods (e.g., fruits and vegetables) that lower dietary acid load, are linked to better muscle health.[18-23] A surrogate for alkalinogenic foods is an alkaline salt supplement (i.e. potassium bicarbonate $KHCO_3$). Our group found a significant improvement in lower extremity muscle power and endurance following 12 wks of a HCO_3 supplement in younger postmenopausal women.[24] HCO_3 also reduced 24h urinary nitrogen excretion (UNi; a marker of muscle breakdown) in postmenopausal women on self-selected diets.[24] Changes were directionally similar but not statistically significant in older men. *We then asked the question of what is the impact of HCO_3 supplementation combined with high protein intake on muscle health in older adults?*

Our 6-wk randomized, protein cross-over, controlled feeding pilot[15] found that increasing dietary protein up to 1.5 g/kg/d and $KHCO_3$ at 90 mmol/d reduced by almost 50% the rise in UNi that accompanied a higher protein intake in both men and postmenopausal women suggesting a decrease in muscle breakdown. Our most recent trial[25] found better tolerability with an 81 mmol/d dose of $KHCO_3$ without compromising efficacy, thus making the 81 mmol/d dose more desirable in a longer-term study. *Based on our pilot data conducted under controlled feeding conditions, a plausible hypothesis that we aim to test in this larger study is that a high protein intake and $KHCO_3$ will enhance muscle power, endurance, physical performance and mass in an older sedentary population on lower protein intake.*

In this proposal, we plan to conduct a 24-wk, double-blind, randomized, placebo-controlled 2x2 factorial study in underactive women and men aged ≥65 yrs and on a ≤0.8 g/kg/d protein diet, to achieve the following specific aims:

Primary Aim: To test the effects of supplemental whey protein (to achieve 1.5 g/kg/d) and/or $KHCO_3$ 81 mmol/d on lower extremity muscle power by double leg press at 24 wks;

Secondary Aims: To test the effects of supplemental whey protein (to achieve 1.5 g/kg/d) and/or $KHCO_3$ 81 mmol/d on changes in:
2a) lower extremity muscle power at 12 wks;
2b) knee extension peak torque at 12 and 24 wks;
2c) handgrip strength at 24 wks;
2d) muscle mass as measured by appendicular non-fat non-bone lean mass by dual energy x-ray absorptiometry (DXA) at 24 wks;
2d) modified SPPB at 24 wks;
2e) 24h UNi excretion;

Exploratory Aims:
To test the individual and additive effects of supplemental whey protein (to achieve 1.5 g/kg/d) and/or $KHCO_3$ 81 mmol/d on 24-wk changes in muscle mass as measured by D_3-creatine dilution;

Background
Age-related muscle loss. After age 50, muscle mass declines at a rate of 1-2% per year[26,27] in association with a decrease in muscle strength of about 3% per year.[28-30] By age 65 years and over, more than 30% of adults experience falls, loss of independence, or physical disability.[31,32] These factors need to be considered in the context of a rapidly expanding older population, which is projected to increase from 12% in 2000 to 20% in 2030 in the US.[33] Given that a large proportion of these older individuals will experience declines in muscle mass and some limitation in physical function, the costs for support services and lost productivity associated with disability will place an enormous burden on society as a whole. Nutrition is an important modulator of healthy aging and enhanced physical function in older adults. Malnutrition is a contributor to age-related muscle loss and impairment. As life expectancy continues to rise, it is important to consider optimal nutritional recommendations that will improve physical independence in older adults. Dietary protein has received a great deal of attention as a promising nutritional intervention in the last decade.

Protein and muscle. Protein is a main building block of skeletal muscle. According to the Institute of Medicine in 2005, the RDA for all adults young and old is for 0.8 g/kg/d.[34] Yet, a series of recent epidemiologic studies and expert panels report that older adults may need as much as twice this daily protein intake for better skeletal muscle health outcomes.[1-5,35-37] Older US adults with protein intakes ≥1.2 g/kg/d had 43% less loss of lean mass by DXA over a 3-year period compared to those with lower intakes closer to the current RDA of 0.8 g/kg/d.[5] A separate Finnish observational study found that older women with protein intakes ≥1.2 g/kg/d had greater muscle strength (as measured by knee extension strength, one-leg stance, chair rise, walking speed for 10m) and higher SPPB scores compared with those with moderate and lower intakes of ≤0.8 g/kg/d. This discrepancy in dietary protein needs in older adults compared to a younger adult population is, at least in part, rooted in the concept of aging-related anabolic resistance,[38] which is defined as an impaired muscle protein anabolic response to a meal resulting in inadequate muscle protein synthesis. Augmenting dietary protein provides the needed amino acid substrate to stimulate muscle protein synthesis[39] and it stimulates the production of muscle growth factors, such as IGF-1. In older patients with recent hip fractures, 20 g/d of protein supplement increased serum IGF-1 levels.[40] Our group also found that increasing protein intake from 0.8 to 1.5 g/kg/d significantly increased serum IGF-1 in 32 healthy older men and women.[41]

Several interventional studies have shown favorable results of adding protein intake up to 1.5 g/kg/d to an exercise training intervention on skeletal muscle mass, strength and performance.[7,9,42] However, trials testing the muscle effects of high protein intake without an added exercise intervention in older adults have been scarce. A recent randomized controlled trial found that older obese nonexercised adults on a high protein (>1.2 g/kg/d) hypocaloric diet versus a lower ≤0.8 g/kg/d protein diet, had better physical function as measured by the SPPB after 24 wks.[6] Conversely, a different study showed no benefit in lean mass or muscle performance with a single 30 g bolus of whey protein after 2 yrs, but notably all participants were allowed to be on higher protein uptakes <1.5 g/kg/d in this trial which may have washed out the ability to see a difference between the low and high protein groups.[10] *In summary, there is a clear research gap in evaluating whether a high protein diet impacts muscle performance and mass without an added exercise intervention.*

Whey protein has been the favored form of protein supplementation due to being rich in certain key amino acids, such as leucine,[43] and leading to higher rates of muscle protein synthesis in metabolic studies in older adults.[43] Many experts suggest that protein supplements need to be distributed throughout the day with a meal[44-49] rather than in a single bolus.

There are concerns that high protein intake induce a mild chronic metabolic acidosis in an older population with age-related declines in renal function.[17] Due to the breakdown of dietary protein to acidogenic sulfur-containing amino acids, high protein diets result in a large dietary acid load.[13] An aging population whose declines in renal

function result in impaired renal acid excretion are prone to a low-grade chronic metabolic acidosis. Dietary protein's acidogenic effect was shown in our pilot trial using 1.5 g/kg of protein from meat sources with a 3-fold increase in 24h urinary net acid excretion (NAE), an established measure of endogenous acid load.[15] Another key determinant of dietary acid load is the potential renal acid load (PRAL).[13,14] In our pilot, the PRAL of the 1.5 g/kg/d diet was +16 based on a 60 kg individual.[15] The quantity of whey isolate used in our proposal has a similar PRAL estimate of +15 to the meat-based intervention in our pilot; thus, we expect our whey intervention to have a similar acidogenic impact. An acidic environment is an established stimulus for muscle catabolism.[50] Muscle catabolism appears to be an adaptive response to acidosis.[50,51] The efflux of amino acids from muscle increases with acidosis.[16,52] The released glutamine is used by the kidney to synthesize ammonia.[53] With the availability of glutamine, the kidney can increase its production of ammonia, which accepts protons and is excreted as ammonium ions, thereby mitigating the acidosis. The impact of acidosis on muscle mass in humans has been studied in chronic kidney disease patients and manifests itself as an increased excretion of nitrogen in the urine.[54] *The question that we have raised is: Does targeting the dietary acid load of high protein diets with alkalinogenic foods or supplements enhance the anabolic benefits of the high protein in an otherwise healthy older adult population?*

Alkali and muscle. Alkalinogenic foods (e.g., fruits and vegetables) that lower dietary acid load, are linked to better muscle health.[18-23] In a very recent observational study in Iranian older adults, those in the highest tertile of consumption of fruits and vegetables, had a lower odds ratio for sarcopenia (measured by DXA) than those in the lowest tertile.[55] Another recent study from the UK found positive associations between women on the Mediterranean diet (rich in fruits and vegetables) and leg power and percent lean mass by DXA.[56] Other observational studies in older adults found similar positive associations between Mediterranean-style diet and SPPB score.[57]

A surrogate for alkalinogenic foods is an alkaline salt supplement (i.e. $KHCO_3$). Currently, there are few data describing the impact of dietary alkali on muscle health in older adults. The earliest study was conducted in postmenopausal women on standardized high protein diets treated with an alkaline $KHCO_3$ supplement of 60-120 mmol/d for 18-days followed by a 12-day recovery.[58] With reductions in NAE from $KHCO_3$ there were concomitant reductions in UNi and vice versa during the $KHCO_3$ withdrawal period.[58] In our trial, we also found that postmenopausal women on self-selected diets treated with HCO_3 had significant reductions in NAE and UNi.[24] A similar pattern was noted in the men but it did not reach statistical significance. Further, we found that women on HCO_3 significantly improved lower extremity muscle power (as measured by double leg press) and isokinetic muscle endurance of knee extensors (as measured by knee extension) following 12 wks vs placebo.[24]

Based on the data on protein and alkali for muscle health, we conducted a pilot to explore whether neutralizing the dietary acid load of a high protein diet (1.5 g/kg/d) with an alkaline salt $KHCO_3$ (90 mmol/d) favored the enhancement of intermediate indices of muscle metabolism.[15] Although the sample was small (19 adults age 50 yrs and over), this 6-wk protein-crossover randomized-controlled study showed that $KHCO_3$ decreased the protein-induced rise in UNi by 50% compared to placebo. Furthermore, both the increase in protein intake and the alkali showed increases in serum IGF-1 levels over the 6 wks. In our most recent $KHCO_3$ trial,[25] we found excellent tolerability of a slightly lower, 81 mmol/d, dose of $KHCO_3$ compared to the 90 mmol/d in the pilot, thus, making the 81 mmol/d more feasible in a longer-term study without compromising efficacy. *The impact of these two dietary factors on muscle performance $and\ mass$ has not been studied to date.*

RISK BENEFIT ASSESSMENT

Known Potential Risks to Participants
Potential of $KHCO_3$ to cause hyperkalemia
Administration of 81 mmol/d could cause significant hyperkalemia in older patients with advanced chronic kidney disease; however, in our study population with mild age-related renal declines, it is much less likely.

In our recent trial in 233 men and women age 60 years and older,[25] participants were randomized to treatment with placebo, 81 mmol/d or 122 mmol/d of $KHCO_3$ and followed for 12 wks. Each participant had a safety serum potassium (K) level measured on study days 10, 13, 16, 19, 22, 50, and on the final visit.

The results of these measurements are summarized in the table below.

	Placebo	81 mmol dose	122 mmol dose
N	79	79	75
Serum K measures	553	553	525
Initially elevated	9	7	13
Confirmed elevated	1	1	5

As shown in the Table above, confirmed hyperkalemia occurred in one person in the placebo group (serum K 5.8 mEq/L, reference range 3.5-5.3 mEq/L), one person in the 81 mmol/d dose group (serum K 5.8 mEq/L), and 5 persons in the 122 mmol/d dose group (5.4, 5.5, 5.5, 5.7, and 5.8 mEq/L).

Given the very large number of measurements, the occurrences in the placebo and 81 mmol/d dose groups likely occurred by chance. Mean serum K levels did not change significantly during the study in any group. Baseline and final mean (±SEM) serum potassium levels were 4.4±0.03 (SEM) and 4.3±0.03 meq/L in the placebo group, 4.4±0.04 and 4.5±0.03 meq/L in the 81 mmol/d group, and 4.4±0.04 and 4.4±0.04 meq/L in the 122 mmol/d group. This is consistent with the experience of others. Sebastian et al. reported that 90 mmol/d of $KHCO_3$ increased serum K levels only modestly and within the reference range (from 3.9 ± 0.15 to 4.03 ± 0.20 mEq/L; reference range 3.5-5.3 mEq/L) in postmenopausal women age 50 to 77 yrs.[59] Sellmeyer et al. administered 90 mmol/d of Kcitrate to 60 postmenopausal women.[60] She did not measure (or at least did not report) serum K values, but exclusion criteria for her study included a 2-hr creatinine clearance of <40 ml/min.[60] Moseley administered 90 mmol of Kcitrate daily for 6 months to 52 postmenopausal women and reported one episode of hyperkalemia during dose escalation when the dose was 40 mmol/d.[61] *From our previous work and the experience of others, we conclude that there is minimal risk of hyperkalemia from $KHCO_3$ supplements in the dose we plan to give in the proposed trial, 81 mmol/d.*

Potential of $KHCO_3$ to cause alkalosis

The dose of bicarbonate to be administered is not expected to produce alkalosis in participants with normal baseline serum HCO_3^- levels and GFR ≥ 50 ml/min at entry, as in this study. In our recent trial, treatment with 81 mmol/d for 84 days increased serum HCO_3^- levels from 25.3±0.27 to 25.9±0.27 mmol/L and treatment with 122 mmol/d increased mean HCO_3^- levels from 25.0±0.30 to 25.9±0.22 mmol/L (our reference range is 21-33 mmol/L). No episodes of elevated bicarbonate were found with either dose. This is consistent with a metabolic study in which Sebastian administered 60 to 120 mmol/d of $KHCO_3$ and reported that plasma $HCO3^-$ increased from 23.7±1.3 to 25.6±1.3 mmol/L, a value that was well within their normal range of 22 to 29 mmol/L [59].

Tolerability of $KHCO_3$

Of the 233 participants in our recent trial, 5 participants stopped taking their pills because of gastrointestinal complaints - 2 in the placebo group, 1 in the 81 mmol/d group, and 2 in the 122 mmol/d group.[25] Adherence with the study capsules averaged 92.2% in the placebo group, 91.3% in the 81 mmol/d $KHCO_3$ group, and 87.4% in the 122 mmol/d $KHCO_3$ group. Based on these findings, we expect that the 81 mmol/d dose of $KHCO_3$ we plan to give in the proposed study will be well-tolerated.

Tolerability of whey protein and placebo isocaloric maltodextrin powder

Whey protein powder and its isocaloric placebo powder will be prepared by our Metabolic Kitchen staff in packets. The powder will be flavorless and sample recipes on how to incorporate it into a beverage or meal will be provided to the participants to maximize tolerability.

Blood draws

Samples to be obtained include blood. Expected risks associated with blood draws may include slight discomfort on puncturing of the skin and possible bruising at the site. Uncommon or rare risks may include phlebitis and/or scarring. The amount of blood to be drawn, 15 ml for the screening tests and will not exceed 90 ml during the study, is well within the limit for research participants (maximum allowable <500 ml over 8 weeks according to American Red Cross).

DXA scans

Radiation exposure to the region of interest is less than 1 mrem/0.01 mSv from the single total body DXA scan. Participants are to have two DXA scans during the 24 week study period which will total 1 mrem/0.01 mSv. The exposure is less than the amount of natural background radiation received over 5 days at sea level.

Muscle Strength and Physical Performance Testing

Risk of injury from testing of physical capacity is very low. The only risk expected to be associated with these tests is a risk of losing balance during the gait speed. The examiner will remain close to help volunteers who are unsteady.

D_3-Creatine Dilution

This is an oral dose of deuterium-labelled creatine (stable isotope) to determine total skeletal muscle mass via estimation of total body creatine pool size. Giving 60 mg of D_3-creatine has no known side effects.

Known Potential Benefits to Participants

This study is not designed to benefit individual participants. Screening lab results will be made available to the participants. If any adverse medical conditions are discovered during the study, participants will be notified in person or by telephone by the study physician/PI, Dr. Ceglia. If the adverse condition is mild, the participant will be referred to their doctor for an clinic evaluation. If the adverse condition is severe, the participant will be referred to urgent care or the emergency room.

Assessment of Potential Risks and Benefits

The risks incurred are minimal relative to the potential benefit of gaining information about one's health.

OBJECTIVES AND ENDPOINTS

We propose to conduct a 2x2 factorial design single center double-blind randomized controlled clinical trial. We plan to enroll 300 participants to obtain 120 completers. Each treatment arm will aim to include 30 participants.

Treatment Arms:

- Arm 1: Whey protein packet + $KHCO_3$ supplement
- Arm 2: Whey protein packet + placebo supplement
- Arm 3: Isocaloric placebo packet + $KHCO_3$ supplement
- Arm 4: Isocaloric placebo packet + placebo supplement

The primary outcome is the lower extremity muscle power at 24 weeks as measured by the double leg press. Secondary outcomes are lower extremity power at 12 weeks, knee extension torque at 12 and 24 weeks, and handgrip strength, physical performance as measured by the modified SPPB, appendicular non-bone non-fat lean tissue mass as measured by whole body DXA scan, and 24-h urinary nitrogen excretion adjusted for nitrogen intake at 24 weeks. Our exploratory outcome is muscle mass as measured by D_3-creatine dilution at 24 weeks.

ENROLLMENT AND WITHDRAWAL

Inclusion criteria:

This study will enroll ambulatory community-dwelling men and women age ≥65 years. Participants must have a habitual dietary intake of protein of ≤0.8 g/kg/d and not be vegetarian. Participants must not change their habitual diet, habitual exercise regimen, or not enter into a weight loss program during the 24-wk study. Participants will be underactive based on a screening questionnaire, the validated Rapid Assessment of Physical Activity (RAPA).[62] Participants must have an estimated GFR ≥ 50 ml/min/1.73 m^2. Participants who are taking a daily calcium supplement (as carbonate, acetate, citrate) can participate in the trial as long as they are willing to switch to a calcium triphosphate supplement provided to them by the study.

Exclusion criteria:
Daily protein intake >0.8 g/kg/day and highly fit older adults (based on RAPA) will be excluded from the study. Drug and medical exclusions are imposed to maximize participant safety. Apart from safety considerations, there are medications known to have a large effect on our muscle outcomes (e.g., glucocorticoids, hormones) and that are likely to dilute the effect of whey protein and $KHCO_3$ supplementation on our outcomes. However, we expect to have only small numbers of users of any specific medication so it is unlikely that we will be able to explore each of these. Therefore, we plan to exclude users of these medications.

Medications and supplements
1. oral glucocorticoid use for > 10 days in the last 3 months
2. anabolic and gonadal hormones in the last 6 months
3. Tamoxifen/raloxifene in the last 6 months
4. regular use of alkali-producing antacids (> 3 times per week)
5. potassium containing supplements or products (i.e., KCl or salt substitutes)
6. NSAIDS >3 times per week
7. antacids containing calcium carbonate, aluminum hydroxide, magnesium hydroxide, or calcium acetate
8. insulin
9. sulfonylureas
10. SGLT2 inhibitors

Conditions/Diseases
1. a lower extremity fracture in the last year
2. kidney stones in the past 5 years
3. creatinine clearance < 50 ml/min (assessed from serum creatinine with use of the Modification of Diet in Renal Disease (MDRD) Study equation).[63]
4. hyperkalemia (serum potassium >5.3 mEq/L; normal range 3.5-5.3 mEq/L)
5. elevated serum bicarbonate (>33 mmol/L; normal range 21-33 mmol/L)
6. serum calcium outside the range of 8.3-10.2 mg/dl
7. uncontrolled diabetes mellitus defined as having fasting blood >150 or hemoglobin A1c >8%
8. untreated thyroid or parathyroid disease
9. significant immune disorder
10. current unstable heart disease
11. Crohn's disease
12. active malignancy or cancer therapy in the last year
13. alcohol use exceeding 2 drinks/day
14. current peptic ulcers or esophageal stricture
15. milk protein allergy
16. other condition or abnormality in screening labs, at discretion of the study physician (Dr. Ceglia)

Withdrawal of subjects:

Participants are free to stop participation at any time for any reason. Should a participant decide to withdraw from the study, the study measurements made prior to withdrawal may be included in the scientific analysis and publication of the study results. Upon completion of the study and data analysis, which may take up to

four years, any remaining blood samples will be discarded. To withdraw from the study, a participant can contact the study PI (Dr. Ceglia).

RECRUITMENT AND RETENTION

This team has successfully recruited for $KHCO_3$ intervention trials. In our dose-finding $KHCO_3$ trial, we recruited and enrolled 244 older adults over a 31-month period,[25] completing enrollment 7 months ahead of schedule. Recruitment will be done in ways that have been successful for us in the past, including:

Local recruitment methods:

1) We will identify appropriate potential candidates (i.e., those who meet our age criteria and whose participation in prior studies would not make them ineligible) in the HNRCA Recruitment database, and send them a recruitment letter and an email. We will also send a modified recruitment letter and email to potential candidates who received a recruitment letter for this study >1 year ago, to reassess their interest. Interested individuals may call or reply via email for more information about the study and their potential eligibility (opt in). A follow up call or email will be made to those people who do not reply. A voicemail will be left for those who do not answer the telephone.
2) We will also recruit subjects with use of mailing lists purchased from Act One Lists, Marblehead, MA (available by age, sex, and zip code). This involves contacting men and women in the Boston area by mail with a brief description of the study and the inclusion/exclusion criteria. Interested candidates will call. An advertisement will be run in local newspapers, giving a call-in number to the Bone Metabolism Lab for more information, as above. Flyers with a brief study description and contact number will be posted throughout Boston and neighboring cities in supermarkets, churches, subways, buses, and senior centers. Written permission will be obtained from supermarkets, churches, senior centers, and Tufts Medical Center before flyers will be placed there. These permissions will be collected and retained by the Bone Metabolism Laboratory. For subway and bus ads, we will purchase the advertising space/time and submit our IRB-approved ads to the MBTA. Posting of IRB-approved, study-specific flyers on bulletin boards at HNRCA is the responsibility of the Recruitment Department, and their posting of such flyers constitutes HNRCA permission.
3) We will also send recruitment letters to individuals whose electronic medical record at Tufts Medical Center (TMC) indicates that they are 65 years and over with a GFR >60. We will query the Tufts Medical Center's Research Data Warehouse (TRDW) (https://www.tuftsctsi.org/research-services/informatics/tufts-medical-center-research-data-warehouse/) to identify eligible patient population. The TRDW is a centralized clinical data warehouse that aggregates clinical information from various Tufts Medical Center's systems. We will query the system using the ICD10 (CPT, [other]) codes for concepts required to conduct a study. If feasibility is confirmed, we will request patient names, contact information, demographics, and diagnostic codes to facilitate recruitment. Once potential participants are identified we will use IRB approved recruitment materials for the process.

Telephone Prescreening:
Interested candidates will be invited to call our dedicated line at the HNRCA. They will have a prescreening telephone interview about their medical history, height and weight. A short physical activity questionnaire (RAPA) and a short diet questionnaire will be administered. Interested and potentially eligible candidates will be invited to HNRCA for a screening visit. Screening will consist of: a comprehensive medical history and supplement use questionnaire, physical measures, an EKG, fasting blood chemistries including serum Cr, K, HCO^-, and a dietary consultation. Participants who pass the screening evaluation will be enrolled.

Screening visit:
Screening and enrollment procedures as well as documents related to MRU standard policies and procedures, approved under **IRB #6701**, will be used for this protocol. Interested candidates will undergo a brief medical evaluation, a fasting lab draw, and electrocardiogram to assess eligibility. If a candidate screened for HNRCA

study 3035 (IRB #13390) in the prior 4 weeks but deemed ineligible, screening labs and electrocardiogram can be applied to this study's screening evaluation in order to reduce burden of repeat testing.

Payment plan:
Each participant who completes the screening process will be paid $25. Each participant who completes the study will be paid an additional $1000 for transportation and miscellaneous expenses. Of this, $200 will be given after the completion of the enrollment and baseline visits, $100 will be given after the 6 week visit, $250 will be given after the 12 week visit, $100 will be given after 18 week visit, and $350 will be given after the completion of final visits 1 and 2. If a subject is taking an angiotensin-converting-enzyme inhibitor (ACEI), angiotensin II receptor blocker (ARB) or a potassium-sparing diuretic, they will receive an added $25 for an added safety serum K level at 1 week. If a subject needs to remain in the study for up to 10 extra days for any reason (such as snow days, unrelated minor illness, unanticipated schedule change), he/she will continue on the study, however the stipend will not be increased. If a subject drops out of the study or is obliged to skip any of the visits, his/her payment will be pro-rated to the portion of the protocol that he/she completed according to the payment schedule described above. The HNRCA will mail a check to participants about two to three weeks after each visit.

Retention:
Steps to encourage retention include: a welcoming and cordial environment on each study visit and recipes that contain information about how to incorporate the powder packets into each meal throughout the study. ***All participants*** will be counseled by our dietary team at the start of the study and throughout the study (weeks 6, 12, 18) to promote adherence to their usual protein intake and to the powder packets. For study pills, participants will record compliance in diaries and return these diaries at each visit. Nursing staff will also perform pill counts. Diaries will also be kept for the powder packets. Participants will contact our research coordinator if they have concerns about the powder packets or other concerns (scheduling, etc). More objective adherence measures for the whey protein intervention will be recorded in 24h UNi measurements, and for the $KHCO_3$ intervention will be recorded in 24h net acid excretion (NAE) measurements.

STUDY DESIGN

This study is a 2x2 factorial design single center double-blind randomized controlled clinical trial. Study duration is 24 weeks. See study timeline chart and study procedures chart below.

Study Timelines

	Year 1		Year 2		Year 3		Year 4		Year 5	
Preparation of study powder packets and pills										
Preparation of recruitment materials										
Subject recruitment/screening/randomization										
Enrollment/intra-study visits										
Muscle strength and performance testing										
Bioassays										
Quality control complete/Data files locked										
Data analyses and interpretation										
Manuscript preparation and submission										

Study Procedures

	Pre-screen	Screen	Enroll Day -3	Baseline Day 0	1 wk Day 7	3 wk Day 21	6 wk Day 42	9 wk Day 63	12 wk Day 84	15 wk Day 105	18 wk Day 126	21 wk Day 147	Final 1 Day 164	Final 2 Day 168
Study Visit		**1**	**2**	**3**	**3a**		**4**		**5**		**6**		**7**	**8**
Consent		X	X											
Medical history		X	X											X
Height		X												
Weight		X		X			X		X		X			X
Blood pressure		X		X					X					X
EKG		X												
Protein intake assessment[a]	X													
Physical activity assessment[b]	X			X										X
Whole body DXA scan				X										X
D_3-Creatine Dilution[c]			X	X	X								X	X
Double leg press				X					X					X
Knee extension				X					X					X
Handgrip				X										X
Modified SPPB				X										X
Diet assess (recall)[d]				X			X		X		X			X
Diet counseling[e]		X		X			X		X		X			
Phone call (Adherence/ Tolerance)						X		X		X		X		
Screening blood[f]		X												
Study blood[g]				X	X		X							X
Return 24h urine[h]				X										X
Study capsules[i]				X			X		X		X		X	
Study powder packet[j]				X			X		X		X		X	
Adherence[k]							X		X		X			X

[a] Two-item protein intake questionnaire on telephone prescreening.
[b] To recruit participants who are underactive at baseline, we will use the RAPA, a validated 9-item questionnaire at prescreening and recruit those with scores <5. Intrastudy, changes in physical activity could modify the anticipated responses to treatment. Therefore, we will assess leisure, household, and occupational activity in the past 40 days with use of the Physical Activity Scale for the Elderly (PASE) questionnaire with 10 items and a score ranging from 0 to 400 with higher scores reflecting greater levels of activity at Baseline and Final Day 2.[64]
[c] A stable deuterium (D_3)-labelled creatine capsule (60 mg) will be administered to participants at the Enrollment and Final Day 1 visits. A second void of fasting morning urine will be collected on the Baseline and Final Day 1 and 2 visits for analysis of creatine to creatinine. In a subset of participants coming in for a Week 1 safety lab, we will also request a second void of fasting morning urine for analysis of creatine to creatinine on the supplements early in the course of the study. This is to measure the creatine component coming from the diet load.
[d] Three consecutive 24-hr dietary recalls will be done for 3 days at the beginning of the study (between the Enrollment and Baseline visits). The third recall day will coincide with the 24-hr urine collected. We will also collect 24-hr recalls on weeks 6, 12, 18, and Final Day 2 to track their diet during the study.
[e] Our research dietitian will counsel participants on how to mix the powder packets in water at Baseline and provide a variety of flavors. On weeks 6, 12, and 18, the dietitian will be providing counseling to maintain protein intake in self-selected diets intra-study.
[f] Screening blood for routine fasting blood chemistries including serum creatinine (Cr), potassium (K), HCO_3^-.
[g] Fasting study blood:

- Baseline: serum 25-hydroxyvitamin D (25OHD), IGF-1, HCO_3^-
- If the participant is on an ARB, ACEI or potassium-sparing diuretic, a 1 week safety blood for serum K will be drawn. A 6 week safety blood draw to check serum K and Cr (for GFR estimation) will be drawn in all participants.
- At Final Day 2: IGF-1, HCO_3^-
- Ten 0.5 ml aliquots of serum will be archived on the Baseline visit and Final 2-wk visit.

[h] Return 24-hr urine

- Baseline visit and Final 2 visits: Ni, NAE, Cr
- Five 5-ml aliquots will be archived at the Baseline and Final 2 visits.

[i] Capsules of $KHCO_3$/placebo and Ca/D supplements dispensed. Participants will take 1 capsule after each meal for 14 days (to adapt to the $KHCO_3$) and then increase to 2 after each meal (6/d total) thereafter.

[j] Protein packets will be dispensed by the dietary team. Subjects will take the whey protein supplement with each meal except on the days 164 and 168 (Final Days 1 and 2) when they will skip the packet with their evening meal. The goal of the packets is to bring total intake of protein to 1.5 g/kg/d (or 15-25 g of additional protein with each meal depending on body weight).

[k] Adherence to study pills will be assessed by capsule counts and by capsule diaries. Adherence to the protein packets will be assessed by packet counts and diaries. Objective measures of adherence are NAE and UNi.

Medical history questionnaire

The medical history includes medication and supplement use, general medical history, and past and current cigarette smoking and alcohol use. The questionnaire will be conducted in person or by telephone if preferred by the study participant.

Study interventions

<u>*Whey protein powder*</u>. Protein packets will contain 15-25g of whey protein each. We will advance all participants to approximately 1.5 g/kg/d based on their body weight and typical baseline protein intake. The whey protein and its isocaloric placebo (maltodextrin - an easily digestible flavorless starch) powder will be flavorless and purchased in bulk at http://www.bulkfoods.com. The dietary team will make the packets and distribute to participants at baseline and weeks 6, 12, and 18. The packets will be mixed with water and the dietary team also will provide a menu for how to incorporate the powder into recipes. The whey and placebo powder packets will be isocaloric.

Weight category	**A (< 70 kg)**	**B (70 – 90 kg)**	**C (91+ kg)**
Dosing group	15g per meal	20g per meal	25g per meal

<u>*$KHCO_3$ supplement*</u>. The 81 mmol of $KHCO_3$ is available in six 13.5 mmol capsules daily; an equal volume of microcrystalline cellulose (placebo) will be placed in 6 identical gelatin capsules. All capsules will be taken with 8-oz of water or the supplement drink immediately after meals. On days 1-14, participants will take 1 capsule after each meal (adaptation period to minimize risk of GI intolerance); thereafter, they will take 2 capsules after each meal (6/d). $KHCO_3$ capsules and matching placebo capsules will be purchased from Life Enhancement Products, Inc. (Reno, NV). Independent analysis by Eurofins (Madison, WI) will be carried out to verify contents.

Dietary intake assessment

<u>*Dietary telephone prescreening questionnaire*</u> – Usual protein intake in grams per day will be assessed with a two-item questionnaire developed in the NEPS laboratory to target individuals on no more than 0.8 g/kg/day of dietary protein. Each of the two questions has multiple parts. For example, participant responses to the following question: “How many servings of per day do you have of a) meat, poultry, and fish? b) eggs? c) dairy such as milk, yogurt and cheese?” For part a), think of a serving as being about the size of a deck of cards. For part c), think of a serving as a cup of milk or yogurt or 2 slices of cheese. The second question asks about vegetable and other non-animal protein foods. From servings, the questionnaire calculates g/d of dietary protein which we can then arrive at the g/kg/d with a body weight. This daily intake will be reviewed and confirmed at screening with an in-person dietary assessment.

3-day dietary records-assisted recall – Three consecutive 24hr dietary recalls will be done for 3 days at the beginning of the study. The third recall day will coincide with the 24-hr urines collected for NAE, Ni, and Cr. Both the recalls and urine collections will be done on "normal days" so as to be most representative of the participant's normal diet pattern. Having 24-hr recalls on the day of the 24-hr urine collections will enable us to link specific foods to daily dietary protein intake and NAE. This diet information will also enable us to adjust our 24-hr UNi measurements for nitrogen intake, and thereby refine the assessment of UNi as a surrogate for protein intake and muscle catabolism at end of study following unblinding. Recalls will also be conducted on weeks 6, 12, 18 and Final Day 2 to track protein intake and NAE throughout the study. 24-hr recalls were used in NHANES, and this will allow us to compare intakes of our participants to national averages. Subjects will be given detailed instructions and measuring aids (a measuring cup, spoons, a ruler and a Food Amounts Booklet) and asked to record everything they eat and drink. They will report what they have consumed to a trained diet technician each day. The 1st recall will highlight what level of food intake detail will be needed to report for the remaining recall days. The dietary interview training is adapted from the 24-hr recall protocol used by the Nutrition Coordinating Center, Minn, MN, http://www.ncc.umn.edu/index.html. It is recognized that having to write food items down may alter what participants choose to eat; however, this approach with frequent contact and coaching is likely to give more accurate intake information in our study population than the feasible alternatives, including diet records which do not involve daily staff interaction. The food lists will be coded and analyzed with use of the Minnesota database (NDSR software version 2018, developed by the Nutrition Coordinating Center, U. of Minn., Minn, MN). From this we will attain specific information on food group and nutrient intake, as well as protein nitrogen intake.

Dual energy x-ray absorptiometry (DXA) scans for lean tissue mass
Lean tissue mass will be measured on total body DXA scans with a precision in our laboratory of 0.67%.[65] It will be measured on a Hologic Horizon-A (Bedford, MA). The scanner utilizes IRIS Enterprise-APEX for systems on APEX 4.xx which includes DICOM Storage, DICOM Modality Worklist, Remote Physician's Viewer Physician's Report, Writer HL7 Enterprise Data Management Installation. Participants will be scanned wearing light clothing and no shoes. All participants will be scanned at the same time of day at baseline and final day 2 under similar conditions to minimize the impact of fluid effects. DXA operators will be ISCD-certified.

D_3-creatine Dilution Method for Muscle Mass
This technique is a novel method to measure skeletal muscle mass based on creatine and creatinine biology.[66,67] Participants will ingest a single 60 mg dose of stable D_3-creatine on Day -3 enrollment and again on final day 1. The D_3-creatine is procured from Cambridge Isotope Laboratories (catalog# DLM-1302-MPT). D_3-Creatine is encapsulated by Greenpark Pharmaceuticals (Houston, TX) as a 60 mg dose in each capsule. To measure total body creatine pool size and muscle mass, the second void of fasting morning urine will be collected 3 days following the dose (baseline and final day 2 visits). A urine will also be collected on final day 1 to subtract any remaining D_3-creatinine from the baseline dose. The urine will be measured for D_3-creatinine enrichment in the urine, which will be determined by LC-MS/MS at the University of California at Berkeley. To minimize variability in this muscle mass measurement, participants will be asked not to eat foods containing creatine or creatinine (animal products) after their mid-day meal on the day prior to these urine collections on day 0 (Baseline), day 164 (Final Day 1) and day 168 (Final Day 2). In a subset of participants coming in for a Week 1 safety lab, we will also request a second void of fasting morning urine. The purpose of performing this additional urine sample in our ongoing study is to evaluate if it is necessary to account for the dietary creatine load from the whey. By adding a spot urine 1 week on supplementation, we will calculate the 1-week difference in the D_3-creatine tracer pool size from Baseline. Should we detect a difference, then this difference would be an adjustment factor in our analysis. A handout will be provided to guide participants in what they should and should not eat on these three occasions.

Muscle Measures and Physical Activity
Isokinetic Muscle Strength: Muscle strength will be assessed by one repetition maximum (1-RM) testing using the Keiser double leg press. 1-RM is defined as the maximum load that can be lifted one time and one time only

using proper lifting technique. This testing method has been used extensively to monitor change in muscle strength, and has been shown to be safe in a wide range of populations.[68] For each machine, participants will have proper form and lifting technique explained and demonstrated for them. Participants will be familiarized with the equipment and learning proper exercise and breathing technique. The participant will then perform five to eight repetitions at a resistance equal to 50% of their estimated 1-RM as an exercise-specific warm-up. Each participant will then perform and additional 5-6 sets of one repetition at increasingly heavier loads until they are no longer able to lift the weight using proper form and technique. Participants will rest 1-2 min between each set during the 1-RM testing. Testing will be conducted at baseline, week 12, and final day 2 visits under supervision of Dr. Fielding (Co-I).

Leg power - Peak power will be assessed by the Keiser double leg press. Following baseline 1-RM measurement, 1-RM at 40% and 70% is established. The participant performs 5 lifts at each established % as fast as possible through their full range of motion. The highest power output achieved at each % is determined to be the peak power. Reliability of this measurement in older subjects in our lab is excellent (ICC = 0.901).[69] Testing will be conducted at baseline, week 12, and final day 2 visits under supervision of Dr. Fielding (Co-I).

Isokinetic Muscle Endurance of Knee Extensor -Muscle endurance of knee extensors will be assessed using a standard protocol on a Biodex isokinetic dynamometer which consists of a lever arm that can be attached to a part of the body and moved through a range of motion. After a period of warm-up and familiarization, subjects are encouraged to exert maximal force for 5 repetitions with the speed of rotation set at 60°/sec. After a 2-min rest, subjects will perform 60 maximal contractions at 240°/sec. The peak torque and its corresponding angle will be recorded as the maximal effort. The right and left side will initially be measured separately and in a random order, and the highest result taken as their actual peak torque. We have previously shown that two tests are necessary to achieve maximal results due to learning effect.[70] Pearson's correlation coefficients ($P < 0.01$) between peak torque of the knee and muscle mass ranged from 0.68 to 0.77 and from 0.58 to 0.74, in men and women, respectively.[70] Isokinetic muscle endurance of knee extensors will be assessed at baseline, week 12, and final day 2 visits under the supervision of Dr. Roger Fielding (Co-I).

Handgrip strength - Handgrip strength is a convenient, safe, and reliable measure of overall muscle strength. Handgrip strength of both hands will be determined separately using a hand held dynamometer. The highest of three consecutive readings will be recorded as the maximum force produced with each hand. Up to 20 seconds will be allowed between measurements. This will be captured at baseline and final day 2 visits.

Modified SPPB (HABC-PPB) - The short physical performance battery (SPPB) is a performance-based measure of lower-extremity function developed by the NIA.[71] It is highly predictive of subsequent disability; however, in well-functioning older adults, the SPPB may not adequately discriminate physical performance.[72] To broaden the applicability, Health ABC investigators expanded the SPPB by increasing the duration of balance stands (to 30 seconds), adding a single foot stand, and adding a 6-meter narrow walk test of balance. They also adapted the scoring method to reduce the ceiling effects that are common in well-functioning groups.[72] The Health ABC Physical Performance Battery (HABC-PPB) has been used to assess lower extremity function in Health ABC, a population-based study designed to identify determinants of functional decline in well-functioning older adults because it discriminates over a broad range of lower extremity function.[72] In Health ABC, the 3-year decline in lower-extremity performance assessed using the HABC-PPB was consistent with clinically meaningful declines in usual gait speed and walking endurance.[73-75] This will be captured at baseline and final day 2 visits.

If the participant cannot perform one of the above muscle and physical performance tests on the scheduled day or there is an equipment problem during testing that prevented acquisition of these data, the participant will have the option of returning on a separate day to repeat measurements. Participants will be compensated for travel with an extra $25 for the additional visit.

Biochemical measurements

Bloods will be drawn after a 12-hr overnight fast and between 7 and 9:30 am. All samples from individual participants will be batched for analyses. Serum K, HCO_3^-, serum chemistries, Cr and urinary Cr will be measured on an automated clinical chemistry analyzer (Olympus AU400, Olympus America Inc, Melville, NY). Estimated GFR will be assessed from serum Cr with use of the Modification of Diet in Renal Disease (MDRD) Study equation (see *Exclusion Criteria* in the *Protection of Human Subjects*). Serum 25OHD levels will be measured by LC/MS/MS on a Waters Acquity UPLC with TQD triple quadrupole mass spectrometer with a coefficient of variation (CV) of 6%. Separation is on a C18 UPLC column. NIST 25OHD standards are run to calibrate the assay. Serum IGF-1 will be measured by chemiluminescent immunoradiometric assays on an automated immunoassay system (IMMULITE® 1000, Diagnostic Product Corporation, Los Angeles, CA). The CV ranges from 3.0 to 9.0%. Urinary nitrogen will be measured with a model FP-2000 nitrogen/protein determinator (LECO, St. Joseph, MI) with intra- and inter-assay CVs of 6.5% and 8.6%. Urinary NAE (=titratable acid + NH_4^+ - HCO_3) will be measured by a modification of the Jorgensen titration method [76], as described by Chan,[77] with precision in our laboratory of 10.1%.[78]

ETHICS AND PROTECTION OF HUMAN SUBJECTS

Human Subjects Involvement, Characteristics and Design

This proposal involves the participation of human volunteers in a 2x2 factorial design, double-blind, randomized, placebo-controlled study. Participants take an added whey protein packet to achieve a 1.5 g/kg/d protein intake or isocaloric placebo daily with meals and either a $KHCO_3$ 81 mmol/d supplement or a matching placebo daily over a 24-wk period to assess changes in lower extremity muscle power, endurance, handgrip, physical performance, lean and muscle mass, and 24-h UNi excretion. Participants agree to attend up to 9 intrastudy visits (Screening, Enrollment, Baseline, week 1 if on certain medications ONLY, week 6, week 12, week 18, Final Day 1 and 2). These visits will involve (1) a blood draw (Baseline, week 1, week 6, and Final Day 2), (2) return of a 24h urine collection (Baseline and Final Day 2), (3) second fasting urine sample (Baseline and Final Day 1 and 2), (4) double leg press power testing, knee extensor strength testing (Baseline week 12, and Final Day 2), (5) diet recall interviews in person (Baseline, week 6, week 12, week 18, and Final Day 2), (6) hand grip, modified SPPB test, and whole body DXA scan for body composition analysis (Baseline and Final Day 2), and (7) physical activity and medical history questionnaires (Baseline and Final Day 2). Up to 300 men and women age 65 years and over will be screened to attain 120 completers. Participants will be generally healthy and will continue their usual diet and activity level. We will include participants on ≤0.8 g/kg/d of dietary protein and are underactive. The study will be conducted at the Jean Mayer USDA HNRCA at Tufts University.

Sources of Materials

At the screening visit, records will be made of the response to screening questionnaires, medical history and physical examination, electrocardiogram, and laboratory results. Daily dietary protein at baseline will be confirmed. Daily physical activity will be assessed. During the course of the study, additional data in the form of records from visits, responses to questionnaires, and testing procedures will be obtained.

In addition to the testing performed during the study, blood and urine specimens will be archived for future measurements. All blood and tissue samples will be used for the purpose of this study only and will be discarded once all study-related analyses are complete. All laboratory tests that will be performed in the Nutrition Evaluation Laboratory at the HNRCA are in compliance with Clinical Laboratory Improvement Amendments (CLIA) of 1988. Participants will be issued an individually identifiable number linking records, specimens and data. The participants' private information will be kept confidential, and access to this information will be restricted to the investigators only.

Informed Consent

- Informed consent for both the screening and study will be obtained from each participant by the PI or registered nurses on the Metabolic Research Unit. It will be obtained at the beginning of the screening and enrollment visits. The consenting process will take place in a private area. The PI or one of the

registered nurses on the research team will go over the study information and will obtain the participants' consent. The full nature of the study (purpose, procedures, risk) will be provided to participants. Participants will be encouraged to ask questions and will be told that they can cease participating in the study at any time for any reason. Participants' consent will be documented in written form, signed by the participant and by the PI or her designee. The study protocol and consent form will have been approved by the Tufts Institutional Review Board (IRB). All personnel coming in contact with the participant will be trained and certified by the Tufts IRB in the ethical conduct of research. Subjects must be able to read and, in the view of study staff, indicate understanding of the study purpose and procedures to be eligible.

- Reasons for not enrolling non-English and illiterate participants are:
    - This study involves a large number of assessment tools, surveys, questionnaires and adherence calendars that are only available in English and in written form and there are no oral/video alternatives.
    - Translators are not readily available at the HNRCA.
    - This is an early phase clinical study without a prospect for direct benefit and it will provide preliminary results for a larger longer-term clinical study.

Protections Against Risk

To optimize intra-study safety and tolerability, we will take the following steps:

- We will strictly adhere to protocol inclusion and exclusion criteria.
- Blood sampling will be done by experienced nurses on the Metabolic Unit. They will use aseptic techniques.
- DXA scans will be performed by DXA technicians who are experienced and certified by International Society for Clinical Densitometry and the State of MA.
- We will exclude subjects with eGFR < 50 ml/min and serum potassium levels above the normal range.
- The muscle performance measurements will be administered by an experienced research coordinator who will stay in close proximity to the subject during testing.
- We will instruct participants to notify Dr. Ceglia of any changes in health status or of any hospitalizations or emergency room visits.
- We will have participants who are unable to tolerate all the study pills and powder to remain in the study and take the maximal amount that they are able to tolerate.

The study will be approved by the Tufts Institutional Review Board (IRB) before anyone will be recruited for the study. All study personnel have been certified by the Tufts University IRB to work with clinical research participants. All research activity will take place at the Jean Mayer USDA HNRCA with the exception of the statistical analyses which will in part be conducted across the street at the Tufts CTSI Statistical Department. Written informed consent will be obtained from all participants. To ensure confidentiality of participant information, personal information will not be given to anyone unless the law requires it. The Tufts Medical Center IRB or the NIH appointed Safety Monitor, Dr. Elena Volpe, may check records that identify specific participants. This might include medical records from this study and the informed consent forms. The records of this study might also be reviewed by the National Institutes of Health (NIH) to make sure all rules and guidelines were followed. Participant information will be kept in a locked medical records room at the HNRCA. The computer files contain identification numbers but not personal identifiers of participants.

Protection of study blinding

Discontinuation of study medications will be managed without unblinding. Participants will be encouraged to remain in the study and complete measurements per protocol to be included in the intention-to-treat analysis. One exception to this is if a participant develops a "serious" Adverse Event in which case he/she will not be asked to remain in the study. If there is an Adverse Event which is thought by the staff to be "probably related" or "related" to the coded medication, the PI, when necessary for the safety of the participant, will contact Greg Matuszek the unblinded team member, and ask him to provide the treatment assignment to the participant's physician. A detailed written report will be submitted to the IRB.

To optimize intra-study safety and tolerability, we will take the following steps:

- upon enrollment, give each participant a wallet card containing name, dates in study, study treatments (whey protein or isocaloric placebo powder per day; 81 mmol $KHCO_3$ or matching placebo per day) and contact information where the study physician (Dr. Ceglia, PI) can be reached during the day and evening/weekend hours;

- instruct participants to notify Dr. Ceglia *before* starting *any* new drugs during this study (because some medications impair potassium excretion and increase risk of hyperkalemia);

- instruct participants to notify Dr. Ceglia of any changes in health status or of any hospitalizations or emergency room visits;

- monitor serum potassium (K) at baseline, 6 wks on full 81 mmol/d dose and at the end of study at 24 wks. In addition, we will measure serum K at 1 week if the participant is on an ACEI, ARB or potassium sparing diuretic. We will measure serum K at any other time, should a participant experience an AE which might be related to the trial. In the unlikely event that it is elevated, the measurement will be repeated immediately. If the repeat measure is elevated, all study pills will be stopped. Serum K will be re-measured after one day off of the study pills to ensure that it has returned to normal. If it remains elevated, the participant will be referred immediately to his/her doctor. Discontinuation of study medications will be managed without unblinding. Participants will be encouraged to remain in the study and complete measurements per protocol to be included in the intention to treat analysis. One exception to this is if a participant develops a "serious" AE in which case he/she will not be asked to remain in the study. If there is an AE which is thought by the staff to be "probably related" or "related" to the coded medication, the PI, when necessary for the safety of the participant, will contact Greg Matuszek, the unblinded team member, and ask her to provide the treatment assignment to the participant's physician. A detailed written report will be submitted to the IRB.

- monitor GFR after 6 wks in the study and at the final 24-wk visit. GFR will be monitored because the higher protein intake and progressive renal failure increases risk of hyperkalemia. The study inclusion criteria include a GFR ≥ 50 ml/min/1.73m^2. If GFR falls below 45 ml/min/1.73 m^2, participants will be asked to return for a repeat measurement the following day. Should the participant be unable or unwilling to return for the repeat study blood, study interventions (powder packets and pills) will be discontinued. Participants who fail to show up for repeat monitoring will be contacted and advised to obtain care as soon as possible. If the repeat measure is normal, the participant continues per protocol. If the repeat GFR is below 45 ml/min/1.73 m^2, the study powder and pills will be stopped and the participant referred to his/her physician for follow-up care. If the repeat GFR is in the range of 45-50 ml/min/1.73m^2, the participant will remain on study capsules and be asked to return for a follow-up GFR measurement in the next week. If the GFR is again less than 45 ml/min/1.73, the participant will stop study capsules and be referred to his care giver for follow-up. If it is above 45 ml/min/1.73, the participant will continue taking study pills. Participants discontinuing study capsules will remain in the study for the intent-to-treat analysis. The threshold of 45 ml/min/1.73 m^2 was chosen to allow for some day to day fluctuation in creatinine and hydration status. Any decline below 45 ml/min/1.73 m^2 will be recorded as an Adverse Event.

- have all pill supplements taken in split doses after meals with a full (8-oz) glass of water to increase tolerability;

- have all powder packet supplements incorporated in each meal in split doses to increase tolerability;

- phase-in treatment for study pills (taking half dose for the first 14 days) to increase tolerability

- have participants who are unable to tolerate an escalation of the study pills or packets remain in the study and take the maximal number of capsules or powder packets that they are able to tolerate.

Data Safety and Monitoring Board (DSMB)
To help ensure safety of participants and the validity and integrity of the data, a DSMB was established to monitor this trial. This was done in accordance with NIH policy. This board have reviewed and approved the plans and safety and overall operations and have approved the Manual of Operating Procedures before the trial begins. The members of the DSMB were chosen by the NIA to have the relevant scientific and medical knowledge to monitor this trial. This board includes two experts in the field of dietary protein and acid-base and a statistician. Names of candidates have been provided by the NIA. In addition, the Program Officer, Giovanna Zappala PhD, from the NIA who is responsible for this trial is included. DSMB members have no conflicts of interest related to the trial. Administrative and safety summaries will be sent to the DSMB at the requested intervals during the trial. These reports will describe the numbers of participants screened, enrolled, and completed, and summaries of the baseline characteristics of the enrollees and of adverse events.

Adverse event (AE) reporting
- All AEs ("expected or "unexpected") and/or laboratory abnormalities and/or problems will be recorded on AE forms and include a brief narrative description including onset, duration, whether the AE has resolved, whether it was expected or unexpected, and whether any treatment was administered for the AE.
- Severity of AEs will be described ("serious" or "non-serious"). Serious AEs, whether or not related to the trial, will be reported to IRB and DSMB immediately (within 24 hours) by phone and in writing. Other AEs will be reported within 5 days to the IRB and at the requested interval to the DSMB.

Other IRB reports
- All serious AEs and any AE that results in the participant's withdrawal from the study or deviation from the study protocol.
- New information that may adversely affect safety of participants or conduct of trial.
- If applicable, recent literature relevant to the hypothesis related to the trial to determine whether continuation of the trial is ethical in the setting of new information.
- All proposed protocol modifications or additions.
- Annual continuing review, listing study progress and all AEs.

Unanticipated problem and adverse event (AE) reporting
This will be done in accordance with the Tufts IRB Reportable New Information policy.

Confidentiality

Dr. Ceglia will ensure that study documents are stored in a manner that protects the privacy of subjects and the confidentiality of study data. The participants' private information will be kept confidential, and access to this information will be restricted to the investigators and staff who are directly involved with the participants. Authorized regulatory personnel have the right to inspect and copy all records pertinent to this study. All data used in the analysis and reporting of this evaluation will have no identifiable reference to participants.

Protection and management of data: All information and materials will be obtained for research purposes only and the data will be kept in strict confidence for use in this research only. Confidentiality will be assured by the use of subject ID numbers rather than personal identifiers on questionnaires, blood collection and storage tubes. All subject paper records will be kept in locked file cabinets in the Medical Records Room at HNRCA and will be accessible only to the investigative team. The master list linking subject IDs to identifying information will be secured in a computer database. All data will be accessible only with a login and protected password. After the study is completed, all data will be kept in a locked file.

Record retention
Subjects' records will be retained for a minimum of 10 years after the study is closed with the IRB.

Research related Injury
Emergency medical treatment will be given to participants if s/he is hurt or gets sick as a direct result of being in this research study. The participant or their insurance carrier will be asked to pay for any such medical care. There is no money to pay for the treatment if the participant gets hurt or sick as part of this study. The HNRCA does not have any money available to pay for a research-related injury or illness. If a participant experiences a research-related injury, they will be instructed to contact the Principal Investigator, Dr. Ceglia, at 617-556-3085 or after hours or on weekends at 617-230-7545.

Economic Burden to Subjects
There will be no cost to the participant for participating in this study.

Vulnerable Populations
This study does not include vulnerable populations.

STATISTICAL CONSIDERATIONS

See Statistical Analysis Plan

Importance of Knowledge to be Gained

Importance of the knowledge: The goal of this study is to identify a dietary intervention that is acceptable over the long term to healthy adults and that will lower NAE and potentially improve body composition, lower body weight, and improve bone and muscle mass and function in adults. If effective, the proposed dried fruit regimen would have significant clinical benefit in lowering the chronic disease burden of adults.

Risk/Importance Assessment: There is minimal risk to participants and no direct benefit from the proposed research study. The potential for serious adverse events is small and the potential benefit to society is high enough to justify conducting this study.

Study sponsorship
This study is funded by the NIH/NIA.

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