Impact of protein and alkali supplementation on skeletal muscle in older adults - Statistical Analysis Plan

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1. SAP purposes

This document provides a detailed description of the analysis plan for the Protein & Alkali trial. This document is meant to be used in conjunction with the study protocol. This document does not subsume the protocol, but several elements of the protocol, such as the sample size justification are reproduced here. This document:
1. Provides a written agreement between the investigators, trial statisticians, and data analysts regarding the analyses to be performed.
2. Provides a record of the analysis plan specified prior to examining any outcome data by group.
3. Provides clear specifications for the data analysts performing variable derivations, statistical analyses, and generating reports.
This document follows the guidance published by Gamble et al. in JAMA 2017.

2. SAP contributors and signatures

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3. Introduction

a. Background and rationale

With aging, skeletal muscle mass and performance decline leading to an increased risk of falls and physical disability. There is ongoing research on whether increasing dietary protein intake in older adults improves indices of muscle health and thus translates to a reduction in physical disability. 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. The scientific premise of this project 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 have suggested that a daily alkaline salt supplement (potassium bicarbonate, $KHCO_3$) lowered the dietary acid load and improved lower extremity muscle power in postmenopausal women.

The investigator's 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. To test the hypothesis, the investigators conducted a randomized, double-blind, placebo-controlled, 2x2 factorial study in underactive men and women age 65 and older on baseline lower protein diets. Participants were assigned to one of four groups: either a whey protein (WP) supplement (to raise protein intake to 1.5 g/kg/d) with or without potassium bicarbonate ($KHCO_3$) 81 mmol/d or an isocaloric placebo supplement with or without $KHCO_3$ 81 mmol/d for 24 wks.

b. Objectives

Primary aim: Evaluate the effects of whey protein (WP) and of $KHCO_3$ on lower extremity muscle power at 24 weeks. This factorial (“at-the-margins”) analysis will evaluate the effects of WP vs. placebo-WP and of $KHCO_3$ vs. placebo-$KHCO_3$ by using an ANCOVA approach comparing the primary outcome between groups with adjustment for baseline lower extremity muscle power, sex, age and factorial design.

Secondary aims: Evaluate the effects of WP and of $KHCO_3$ in terms of lower extremity muscle power at 12 weeks, knee extension torque at 12 and 24 weeks, physical performance at 24 weeks, lean mass at 24 weeks, handgrip strength at 24 weeks, physical performance battery at 24 weeks, and 24-hr urinary nitrogen excretion (UNi).

4. Study Methods

a. Trial design

This is a single-center, individually randomized, 2x2 factorial, placebo-controlled trial. Participants were randomized in a 1:1:1:1 fashion to:

- whey protein isolate + $KHCO_3$
- whey protein isolate + microcrystalline cellulose
- maltodextrin powder + $KHCO_3$
- maltodextrin powder + microcrystalline cellulose

b. Randomization

Randomization was stratified by sex, by using block randomization (randomly permuted block sizes of 4 or 8). The random allocation sequence was computer-generated by a biostatistician. The randomization sequence was incorporated into the online Electronic Data Capture system. Central randomization via the online EDC system maintained allocation concealment.

c. Sample size

Sample size calculations focused on the change from baseline to 24 weeks in muscle power and were conducted for a factorial analysis (at-the-margins analysis), i.e. a) the comparison between all participants allocated to $KHCO_3$ vs. all those not allocated to $KHCO_3$ and b) the comparison between all participants allocated to whey protein vs. all those not allocated to whey protein. We will test each of these two primary hypotheses with a two-sided test and significance level 0.05. There will be no adjustment for multiplicity because, under the assumption of no interaction (the effect of whey protein is the same whether the patient is allocated to $KHCO_3$ or not, and similarly for $KHCO_3$), that is inherent to a factorial analysis, our trial answers two distinct research questions, and statistical simulation support that no adjustment is needed in this scenario. All secondary and exploratory analyses are considered as supporting the primary analysis, with two-sided tests and significance levels of 0.05.

Table 1 shows the hypothesized mean changes in muscle power in each of the 4 randomization groups. For the comparison of $KHCO_3$ vs. no $KHCO_3$, the hypothesized mean change is 10% (with) and 4% (without). We assumed a common standard deviation (SD) of change in power to be approximately 12%, based on the subset of men and women over age 65 in a previous study of the effect of HCO_3^-(1). Sample sizes of 60 and 60 in $KHCO_3$ vs. no $KHCO_3$ yield 80% power to detect a between-group difference of at least 6%. This effect size was observed in the subset of older men and women in the previously referenced study (1). For the comparison of whey protein vs. no whey protein, the hypothesized mean change in muscle power from baseline to follow-up is 11% (with) and 3% (without). We assumed a common SD of change in power to be 15%. Sample sizes of 60 and 60 in whey protein vs. no whey protein yield 80% power to detect a between-group difference of at least 8%. This effect size was observed when subjects in the previous study (1) were divided into high and low protein groups based on their self-selected diets.

Table 1: Hypothesized change in leg power by intervention

		$KHCO_3$		
		yes	**No**	**Overall**
Whey protein	**Yes**	14% N=30	8% N=30	N=60
	No	6% N=30	0% N=30	N=60
	Overall	N=60	N=60	

d. Framework

For all objectives, we use a superiority framework and a factorial analysis (at-the-margins analysis), i.e., testing the superiority of whey protein against no whey protein and of $KHCO_3$ against no $KHCO_3$.

e. Statistical interim analyses and stopping guidance

No formal statistical interim analysis was planned. The Data and Safety Monitoring Board (DSMB) periodically evaluated enrollment data, AE, and SAE by randomization group.

f. Timing of final analysis

Final analysis will take place after all data for the primary and secondary outcomes have been collected, data cleaning is complete, and after this SAP is finalized and posted on clinicaltrials.gov.

g. Timing of outcome assessments

Outcomes are measured at baseline, 12 weeks, and 24 weeks after randomization.

5. Statistical Principles

a. Confidence intervals and P values

All statistical tests will be 2-sided. We will use $p \leq 0.05$ to indicate statistical significance for the primary outcome for each of the two primary comparisons of interest in a factorial analysis (at-the-margins analysis): a) the comparison between all participants allocated to $KHCO_3$ vs. all those not allocated to $KHCO_3$ and b) the comparison between all participants allocated to whey protein vs. all those not allocated to whey protein. Under the assumption of no interaction that is inherent to a factorial analysis, the primary analyses test two independent hypotheses, and no multiplicity adjustment is needed in this scenario. All secondary and exploratory analyses are considered as supporting the primary analyses, with two-sided tests and significance levels of 0.05.

b. Adherence and protocol deviations

Adherence to study pills was assessed by capsule counts and by capsule diaries. Adherence to the protein packets was assessed by packet counts and diaries. Objective measures of adherence are urinary net acid excretion (NAE) and UNi.

Major protocol deviations for the trial include:

1. Participant found ineligible after randomization
2. No informed consent signed
3. Participant didn't receive the randomly allocated intervention
4. Withdrawal of consent for future data collection, and/or ongoing use of previously collected study data

c. Analysis populations

The full analysis set will include all randomized subjects. Following the intention-to-treat (ITT) principle, subjects will be analyzed according to the group they were assigned to at randomization regardless of treatments received.

In a modified ITT analysis set, we will exclude from the ITT sample those participants who –took less than 10% of whey protein isolate or $KHCO_3$ and those with any major protocol deviation.

In a per protocol analysis set, we will further restrict the mITT sample to participants who took whey protein isolate and $KHCO_3$ throughout the 24 weeks with an average compliance of at least 75%.

6. Trial Population

a. Eligibility

The inclusion criteria are:

- ambulatory community-dwelling men and women age ≥65 years
- habitual dietary intake of protein of ≤0.8 g/kg/d and not be vegetarian

- willing not to change their habitual diet, habitual exercise regimen, or enter into a weight loss program during the 24-wk study
- underactive based on the validated Rapid Assessment of Physical Activity (RAPA)
- estimated GFR ≥ 50 ml/min/1.73 m2.

Participants who are taking a daily calcium supplement (as carbonate, acetate, citrate) can participate in the trial if they are willing to switch to a calcium triphosphate supplement provided to them by the study.

The exclusion criteria are

- users of the following medications or 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
- individuals with the following 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 (MDRD equation)
  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 (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

b. Recruitment

We will use a CONSORT flow diagram to summarize the number of participants who:
• were assessed for eligibility at screening
• were eligible at screening
• were invited to participate
• provided consent
• were randomized
• withdrew prior to receiving treatment

• included in the primary analyses
The flow diagram will also show the numbers who were eligible but not randomized, who did not receive the randomized allocation, who discontinued the intervention, and we will describe the reasons.

c. Withdrawal/follow-up

The level of withdrawal will be tabulated and classified as “consent to continue follow-up and data collection”, “consent to continue data collection only”, “complete – no further follow-up or data collection”. The numbers (with reasons) of losses to follow-up (drop-outs and withdrawals) over the course of the trial will be summarized by randomization group.

d. Baseline patient characteristics

Participants will be described at the time of randomization with respect to age, sex, race and ethnicity, weight, BMI, blood pressure, physical activity assessment, DXA lean tissue mass, D_3-creatine dilution, double leg press power testing, knee extensor strength, handgrip strength, modified SPPB, total energy intake, dietary protein intake, and fruit and vegetable intake. All characteristics will be first tabulated overall and separately for whey protein vs. no whey protein and $KHCO_3$ vs. no $KHCO_3$. We will then tabulate the characteristics in each of the four cells of the 2x2 design. Categorical data will be summarized by numbers and percentages, and continuous data by mean, SD (or median, Q1-Q3 if data are skewed). No tests of statistical significance will be performed; any imbalance of clinical importance will be noted.

7. Analysis

a. Outcome definitions

Primary outcome: Keiser double leg press power at 70% at 24 weeks. Following one-repetition maximum measurement (1RM), the participant performs 5 presses at 70% of their 1RM as fast as possible through their full range of motion. The highest power output is determined to be the peak power.

Secondary outcomes:

- Double leg press peak power at 70% of 1RM at 12 weeks
- Double leg press peak power at 40% of 1RM at 12 and at 24 weeks
- Knee extension peak torque at 60°/sec at 12 and 24 weeks
- Maximum handgrip strength at 24 weeks
- Appendicular lean mass/ht^2 by DXA at 24 weeks
- Health ABC-PPB score at 24 weeks
- 24hr urinary nitrogen excretion

Exploratory:

- muscle mass as measured by D_3-creatine dilution at 24 weeks

b. Analysis methods

Summary statistics. For each outcome, we first will report summary statistics at each timepoint for whey protein vs. no whey protein and for $KHCO_3$ vs. no $KHCO_3$. We will then report summary statistics in each of the four cells of the 2x2 design. We also will create box plots that display the distribution of the data at each time point for each comparison.

Primary Outcome. Primary analyses of the primary outcome will be performed on the ITT sample based on an ANCOVA comparing 24-week leg power with fixed effects for intervention groups and further adjustment for leg power at baseline, age and sex. Moreover, the effect of each intervention will be adjusted for the other intervention. Type III sum-of-squares will be used to test significance of each intervention. Least squares mean difference between intervention groups, and 95% confidence intervals, based on the fitted linear models will be reported.

Table 2: Presentation of the results of the primary analyses

	Whey protein	No whey protein	$KHCO_3$	no $KHCO_3$
Leg power, mean (SD)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
LS mean difference (95% CI)*	-xx.xx (-xx.x to -xx.x)		-xx.xx (-xx.x to -xx.x)	
p value	xxx		Xxx	

**adjusted for sex, age, leg power at baseline, and factorial design*

Secondary analyses and secondary outcomes.

- We also will conduct factorial analyses of all secondary outcomes by using similar analytic approach to the primary outcome.

- In addition of ANCOVA analyses, we will fit longitudinal model based on all repeated measurements of the outcome, i.e., a linear model with 12-week and 24-week measurements as outcome observations and fixed effects for the intervention group, time treated as categorical, intervention group by time interaction, baseline measure, baseline measure by time interaction, and sex. The corresponding treatment effect measure is the between-group difference in the slopes of the mean responses. Restricted maximum likelihood estimation will be used with the Newton–Raphson algorithm. An unstructured covariance matrix will be used to model the variance-covariance matrix for random intercept and random slope. In the case of nonconvergence with UN, a toeplitz covariance matrix will be used. In the case of nonconvergence with TOEP, an autoregressive covariance matrix [AR (1)] will be used.

Difference in estimated least square mean slopes, the corresponding 95% CI, and p-value will be reported. We will use the p value from the type III F-test of the fixed effect parameter for the intervention group by time interaction. Denominator degrees of freedom will be estimated using the Kenward-Roger approximation with an unstructured covariance matrix and the between-within method otherwise.

- We also will present results for outcome measures at follow-up within each of the four factorial groups in the 2 × 2 design. We will test for interaction by extending the multivariable linear models described above by simply adding the appropriate interaction terms. However, we anticipate insufficient precision on the interaction estimates to rule out interaction.

- We will repeat analyses in the mITT and per protocol samples.

c. Subgroup Analyses

We will perform subgroup analyses by sex, NAE (<15 mmol/day vs. ≥15 mmol/day), protein intake based on average 3-day food recall (≤0.8 gm/kg/day vs.> 0.8 gm/kg/day), no sarcopenia vs. sarcopenia (defined as maximum grip strength <35.5 kg (men) and <20 kg (women) in either hand and/or gait speed <0.8 m/sec.

d. Missing data

Descriptive tables of baseline characteristics stratified by arm will include the frequency and percentage of missing values. In attrition analyses, we will then compare these variables between subjects who drop out and subjects with complete outcome data. The number of missing primary outcome data points at each time point, by intervention arm, will be reported in the CONSORT participant flow diagram.

For the primary analyses, we will analyze all available follow-up outcome data using restricted maximum likelihood estimation, under the assumption that data are missing at random.

As sensitivity analyses, we will use multiple imputation. Multiply imputed datasets will be generated by using imputation by chained equations. We will use predictive mean matching and logistic regression to impute continuous and binary outcomes, respectively. We will consider fully conditional specification. We will treat repeated measurements as distinct variables, so longitudinal data will be in wide format, with one row per participant, so that the within-subject correlation is maintained. We will impute at least 10 imputed datasets or a larger number corresponding to the fraction of missing outcome data. Randomization group, stratification factors, baseline variables, and outcome data will be considered for inclusion in the imputation model as auxiliary variables. We will compare the distribution of observed and imputed values to assess the adequacy of the imputation model. We will fit the linear models on each imputed dataset and combine results according to Rubin's rules. Depending on the amount and pattern of missing data, we will consider alternative strategies to handling missing data.

e. Harms

We will summarize the number and percentage of participants experiencing adverse events, both for the overall safety population and in each randomization group. We will not perform statistical testing. We will assess the clinical significance of the differences.

f. Statistical software

Analyses will be carried out using R and SAS.

References:

1. Dawson-Hughes B, Castaneda-Sceppa C, Harris SS, Palermo NJ, Cloutier G, Ceglia L, Dallal GE. Impact of supplementation with bicarbonate on lower-extremity muscle performance in older men and women. Osteoporos Int. 2010;21(7):1171-9. Epub 2009/09/04. doi: 10.1007/s00198-009-1049-0. PubMed PMID: 19727904; PMCID: 2888724.