Published in final edited form as:

Osteoporos Int. 2017 December ; 28(12): 3355–3359. doi:10.1007/s00198-017-4196-8.

Increasing alkali supplementation decreases urinary nitrogen excretion when adjusted for same day nitrogen intake

Lisa Ceglia^{1,2} and Bess Dawson-Hughes^{1,2}

¹Nutrition, Exercise Physiology and Sarcopenia Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA

²Division of Endocrinology, Diabetes and Metabolism, Tufts Medical Center, Boston, MA

Abstract

Purpose—Neutralization of dietary acid load with alkali supplementation (i.e. potassium bicarbonate [KHCO₃]) has been hypothesized to have muscle protein-sparing effects. In controlled feeding studies with fixed nitrogen (N) intake/day, 24-hour urinary N excretion is a good marker of muscle breakdown. However, in studies with self-selected diets, changes in 24-hour urinary N excretion can be influenced by shifts in N intake.

Methods—We evaluated changes in 24-hour total urinary N excretion as a ratio of N excretion to concurrent N intake in 233 older men and women who participated in an 84-day KHCO₃ supplementation randomized placebo-controlled trial.

Results—After adjustment for relevant cofactors, escalating doses of KHCO₃ (1 mmol/kg/day [low] or 1.5 mmol/kg/day [high]) resulted in a progressive decline in urinary N excretion/N intake compared to placebo (overall P for trend = 0.042). The 84-day change in urinary N excretion/N intake in the high dose KHCO₃ group was statistically significantly lower compared to placebo (P=0.012) but not compared to the low dose KHCO₃ group (P=0.276). The 84-day change in urinary N excretion/N intake in the low dose KHCO₃ group did not differ significantly from placebo (P=0.145).

Conclusions—Urinary N excretion expressed as ratio to same day N intake declined steadily with increasing doses of KHCO₃ supplementation from low 1 mmol/kg/day to high 1.5 mmol/kg/day, suggesting a nitrogen-sparing effect. Compared to urinary N excretion alone, this ratio could be a more reasonable measure of muscle protein metabolism in large-scale long-term human studies.

Mini-Abstract

We examined whether escalating doses of potassium bicarbonate [KHCO₃] supplements alter urinary nitrogen excretion expressed as a ratio to same day nitrogen intake (measure of muscle-

Corresponding Author: Lisa Ceglia, MD MS, 711 Washington Street, Boston, MA 02111, 617-556-3085 (voice), 617-556-3305 (fax), lisa.cegla@tufts.edu.

Disclosures

Lisa Ceglia and Bess Dawson-Hughes declare that they have no conflicts of interest.

Clinical Trial: NCT00986596

protein breakdown). The ratio declined significantly from placebo to low to high dose of KHCO_3 supplementation in older adults over 3 months, suggesting muscle-sparing.

Keywords

alkali; potassium bicarbonate; skeletal muscle; nitrogen excretion

Introduction

Chronic metabolic acidosis increases muscle catabolism and causes increased excretion of nitrogen (N) in the urine [1–3]. This muscle response is purported to mitigate acidosis by releasing amino acids like glutamine which provide ammonia which will facilitate renal acid excretion as ammonium ions [4]. Healthy aging is also associated with a progressive low-grade metabolic acidosis due to the ingestion of acidogenic Western diets and to decreases in the ability of the kidney to excrete acid with age [5]. The modern Western diet is rich in protein and cereal grains, which are metabolized to acidic residues, and low in fruit and vegetables, which are metabolized to alkaline residues.

Reducing metabolic acidosis with alkali in patients with chronic kidney disease, reduces urinary N excretion and normalizes N balance [6]. Alkalinogenic foods that lower dietary acid load are linked to better muscle health in older adults [7–12]. A surrogate for alkalinogenic foods is an alkaline salt supplement such as potassium bicarbonate (KHCO_3). Two previous intervention studies in older adults and one in rats that targeted the neutralization of dietary acid load with alkali supplementation, such as KHCO_3 , have reported significant decreases in urinary N excretion in healthy older adults [13–15]. These studies involved controlled feeding conditions using a fixed N intake daily. N intake in the diet can influence 24-hour urinary N excretion as shown previously in numerous studies [16–18]. Thus, in such controlled conditions, 24-hour urinary N excretion can be a good indicator of muscle protein breakdown.

Studies using self-selected diets may present more challenges in translating alterations in urinary N excretion as muscle protein effects. Measuring and adjusting for baseline N intake, as was done in two previous 84-day (~3-month) alkali supplementation trials [19,20], is a reasonable approach to account for this influential factor in a relatively short-term trial. Yet, in one study [19], alkali supplementation significantly reduced urinary N excretion in older postmenopausal women but not older men. In the other KHCO_3 dose escalation trial in older men and postmenopausal women [20], there was no alkali effect on urinary N excretion in the group as a whole or separately in men or women ($p>0.242$).

Given the impact of N intake on urinary N excretion on the same day of the urine collection, this study sought to evaluate whether adjusting the urinary N excretion for same day N intake expressed as a ratio at each timepoint may alter results in our recent KHCO_3 dose escalation trial [20].

Methods

Participants and study design

The design, execution, and outcomes of the KHCO_3 dose escalation trial have been described in detail elsewhere [20]. Briefly, this was an 84-day randomized, double-blind, placebo-controlled trial to determine the optimal dose of KHCO_3 (1 mmol/kg/day [low] or 1.5 mmol/kg/day [high]) vs. placebo to maximally suppress bone resorption (as measured by 24-hour urinary N-telopeptide [NTX]) and muscle catabolism (as measured by 24-hour total urinary N excretion) in healthy ambulatory men and women aged 60 years and older. Other inclusion criteria were an estimated glomerular filtration rate (GFR) of at least $50 \text{ mL} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^2$. Participants were encouraged to maintain stable diets and physical activity during the trial. This protocol was approved by the Tufts Medical Center-Tufts University Institutional Review Board, and written informed consent was obtained from each participant. All participant visits took place at the U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University. The study is registered at [Clinicaltrials.gov](http://clinicaltrials.gov) (<http://clinicaltrials.gov/show/NCT1475214>). The current observational study was conducted in all participants randomized to placebo, KHCO_3 1 mmol/kg/day, or KHCO_3 1.5 mmol/kg/day in the main trial [20].

Supplements

Each capsule contained 13.5 mmol of KHCO_3 each. The matching placebo contained microcrystalline cellulose. Briefly, participants took either 6, 9, or 12 capsules depending on baseline body weight. The proportion of KHCO_3 and placebo capsules within the daily total capsules varied across the three treatment arms. KHCO_3 and placebo capsules were purchased from Life Enhancement Products, Inc. (Petaluma, CA, USA), with independent analysis (Covance; Princeton, NJ, USA) confirming that the treatment capsule contained 13.6 mmol of KHCO_3 . A calcium and vitamin D supplement was supplied to all participants. Adherence was measured by pill counts and 24-hour urinary net acid excretion (NAE), which is a validated measure of endogenous acid load. Further details are described elsewhere [20].

Diet assessment

Record-assisted 24-hour dietary recalls were collected at the beginning and end of the study under the supervision of a research dietitian, on days coinciding with the 24-hour urine collections. Subjects were instructed to record everything they ate and drank and the dietary supplements taken and report these to a diet technician on the baseline and final visits. The dietary interview training was adapted from the 24-hour recall protocol described at <http://www.ncc.umn.edu/index.html>. Dietary intake data were coded and analyzed using Nutrition Data System for Research software version 2011, Nutrition Coordinating Center, University of Minnesota. This version defines food group servings according to the Dietary Guidelines for Americans, 2005. Additional details described elsewhere [20].

Anthropometrics and Body Composition

Baseline height was measured using a wall mounted stadiometer and baseline weight using a calibrated digital scale. A baseline dual-energy x-ray absorptiometry (DXA; Hologic, Bedford, MA) was used to assess body composition (fat mass and fat-free mass).

Biochemical measurements

All 24-hour urine samples were batched for analyses. Serum and urinary creatinine (UCr) was measured on an automated clinical chemistry analyzer (Olympus AU400; Olympus America Inc., Melville, NY, USA) with CVs of 3.0% to 6.0%. Total urinary N was measured with a model FP-2000 nitrogen/protein determinator (LECO, St. Joseph, MI, USA) with intra-assay and inter-assay CVs of 6.5% and 8.6% [19]. Urinary pH was determined on the Accumet Excel pH meter (Fisher Scientific, Pittsburgh, PA, USA). Urinary NAE (NAE = titratable acid + NH_4^+ - HCO_3^-) was measured in urine by a modification of the Jorgensen titration method, [21] as described by Chan [22] with precision in our laboratory of 10.1% [23].

Statistical analysis

In accordance with intention-to-treat principles, analyses were conducted on all 233 subjects who completed the final visit, whether or not they were compliant with treatment. There were several missing laboratory values. For analyses in which one or more value was missing, the resulting sample size is indicated in parentheses in the relevant table or figure. Data were examined graphically to rule out the presence of outliers and to evaluate the linearity of bivariate associations. One urinary N excretion change of 2071 mmol was set aside because it was implausibly high (the next highest value of change among all subjects was 951).

As expected, urinary N excretion was strongly associated with same day N intake (baseline $r=0.526$, $p<0.001$; final $r=0.592$, $p<0.001$), thus we analyzed urinary N excretion as a ratio to same day nitrogen intake (baseline urinary N excretion/N intake and final urinary N excretion/N intake).

Participant characteristics and other means were compared across groups by analysis of variance for unadjusted values (overall differences and/or linear trends) and by analysis of covariance (ANCOVA) with least-squares means for adjusted values. The effect of KHCO_3 on 84-day changes in urinary N excretion/N intake was examined by ANCOVA. These analyses included the computation of means by treatment group adjusted for relevant factors and covariates. IBM SPSS Statistics for Windows Version 22.0 (IBM Corp. Armonk, NY) was used for all statistical analyses, and P values <0.05 were considered to indicate statistical significance. We did not stratify by gender because we found no significant interactions of gender with KHCO_3 on this endpoint. Adjustment for gender, GFR, or creatinine index (24-hour urine creatinine/body weight) did not influence the results.

Results

Baseline characteristics of the participants are shown by group in Table 1. Baseline clinical, dietary, and biochemical characteristics were not significantly different in the three groups. Furthermore, baseline urinary N excretion/N intake did not differ significantly in the three groups (Table 2). Adherence to the study capsules was reported to be >87% in all three groups and was also evidenced by the significant differences in final urinary NAE values of 16.5 ± 29.1 in the placebo, -0.7 ± 11.1 in the low dose, and -7.6 ± 23.9 mmol/d (P for between group differences <0.001).

After adjustment for relevant factors (i.e., baseline urinary N excretion/N intake, age, baseline weight, baseline lean body mass, baseline physical activity score, and baseline urinary NAE), we found a significant and progressive reduction in urinary N excretion/N intake ratio with escalating doses of KHCO_3 compared to placebo (overall P for trend = 0.042; Table 2; Figure 1). Furthermore, the 84-day change in urinary N excretion/N intake in the high dose KHCO_3 group was statistically significantly lower by $-9.57 \pm \text{SE } 3.79$ compared to placebo (P=0.012) but not compared to the low dose KHCO_3 group (P=0.276; Table 2; Figure 1). The 84-day change in urinary N excretion/N intake ratio in the low dose KHCO_3 group did not differ significantly from placebo (P=0.145).

Discussion

In this experimental intervention of generally healthy older men and postmenopausal women, we found that escalating doses of KHCO_3 supplementation to lower the dietary acid load led to a decline in urinary N excretion when expressed as a ratio to concomitant N intake. The progressive decline was statistically significant below the 0.05 level after adjusting for several influential factors including baseline urinary N excretion/N intake ratio, age, weight, lean body mass, physical activity, and endogenous acid load. This treatment effect was not noted in our initial analysis [20] which accounted for N intake at baseline but not at the end of the study. The N intake was collected via a 24-hour recall by a dietary technician at both baseline and final timepoints in the clinical trial. Thus, by creating a ratio of N excretion to N intake at baseline and final visits, we accounted for small shifts in N intake. These results support prior clinical data demonstrating N sparing in older adults on alkali supplementation [13,14,19]. Two of these prior studies involved controlled feeding conditions where participants were fed a fixed N intake daily while evaluating N excretion [13,14]. Conducting controlled feeding studies is costly; thus, such studies are often less than 8 weeks. The current study suggests that in large-scale longer-term studies involving participants on self-selected diets, using a 24-hour recall on the day of urine collection and creating a ratio with urinary excretion could be a more reasonable measure of change in N status compared to urinary N excretion alone. Furthermore, this ratio is a simpler method than N balance studies where N excretion would need to be measured in other bodily secretions such as stool. However, it would be an important next step to compare this ratio to a traditional N balance study to better evaluate its accuracy.

In this study, we observed that the N sparing effect of KHCO_3 supplementation was more pronounced in the higher dose group than the lower dose group. This finding differs from

the KHCO_3 effect on suppression of bone turnover which was more notable at the lower KHCO_3 dose without any additional suppressive benefit at the higher dose [20]. This discrepancy in dose effect raises interesting questions about whether there should be different thresholds of alkali supplementation depending on the clinical goals. Additional longer-term studies including evaluation of muscle and bone mass are needed to better determine these dose effects.

This study had some important strengths, including the fact that our participants' adherence to the intervention was high (range 87.4 – 92.2% in the 3 groups) and their dropout rate (2.5 – 6.0%) was low, as described previously [20]. We chose a parallel-arm, blinded, placebo-controlled design to study the KHCO_3 effects. The present study also had limitations. We do not have verification (by using para-aminobenzoic acid or other means) that the 24-h urine collections are complete; however, the mean ($\pm$ SD) creatinine index (24-hour urinary creatinine/body weight) for our sample was in 16.8 ± 5.1 at baseline and 16.5 ± 5.2 at the final collections – values that were similar and in the normal range. We were also not able to compare our results using the ratio of urinary N excretion/N intake to a gold standard nitrogen balance method for accuracy; thus, a future study comparing these measures would be important to validate this study. Lastly, the findings relate to the specific starting point and/or achieved level of urinary NAE. It remains to be determined what is the optimal urinary NAE level for muscle preservation.

In conclusion, urinary N excretion expressed as ratio to same day N intake declined steadily with increasing doses of KHCO_3 supplementation from low 1 mmol/kg/day to high 1.5 mmol/kg/day, suggesting a nitrogen-sparing effect. Compared to urinary N excretion alone, this ratio could be a more reasonable measure of muscle protein metabolism in large-scale long-term human studies.

Acknowledgments

This study was funded by NIH/NIAMS grant number 1R01AR060261. This material is based upon work supported by the U.S. Department of Agriculture, Agricultural Research Service, under agreement No. 58-1950-7-707. Any opinions, findings, conclusion, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Dept of Agriculture.

References

1. Garibotto G, Russo R, Sofia A, Sala MR, Sabatino C, Moscatelli P, Deferrari G, Tizianello A (1996) Muscle protein turnover in chronic renal failure patients with metabolic acidosis or normal acid-base balance. *Mineral and electrolyte metabolism* 22 (1–3):58–61 [PubMed: 8676826]
2. Williams B, Layward E, Walls J (1991) Skeletal muscle degradation and nitrogen wasting in rats with chronic metabolic acidosis. *Clin Sci (Lond)* 80 (5):457–462 [PubMed: 1851685]
3. Ballmer PE, McNurlan MA, Hulter HN, Anderson SE, Garlick PJ, Krapf R (1995) Chronic metabolic acidosis decreases albumin synthesis and induces negative nitrogen balance in humans. *The Journal of clinical investigation* 95 (1):39–45. doi:10.1172/JCI117668 [PubMed: 7814640]
4. Cersosimo E, Williams PE, Radosevich PM, Hoxworth BT, Lacy WW, Abumrad NN (1986) Role of glutamine in adaptations in nitrogen metabolism during fasting. *The American journal of physiology* 250 (6 Pt 1):E622–628 [PubMed: 3521309]
5. Frassetto L, Sebastian A (1996) Age and systemic acid-base equilibrium: analysis of published data. *The journals of gerontology* 51 (1):B91–99 [PubMed: 8548506]

6. Papadoyannakis NJ, Stefanidis CJ, McGeown M (1984) The effect of the correction of metabolic acidosis on nitrogen and potassium balance of patients with chronic renal failure. *The American journal of clinical nutrition* 40 (3):623–627 [PubMed: 6089541]
7. Cesari M, Pahor M, Bartali B, Cherubini A, Penninx BW, Williams GR, Atkinson H, Martin A, Guralnik JM, Ferrucci L (2004) Antioxidants and physical performance in elderly persons: the Invecchiare in Chianti (InCHIANTI) study. *The American journal of clinical nutrition* 79 (2):289–294 [PubMed: 14749236]
8. Houston DK, Stevens J, Cai J, Haines PS (2005) Dairy, fruit, and vegetable intakes and functional limitations and disability in a biracial cohort: the Atherosclerosis Risk in Communities Study. *The American journal of clinical nutrition* 81 (2):515–522 [PubMed: 15699243]
9. Myint PK, Welch AA, Bingham SA, Surtees PG, Wainwright NW, Luben RN, Wareham NJ, Smith RD, Harvey IM, Day NE, Khaw KT (2007) Fruit and vegetable consumption and self-reported functional health in men and women in the European Prospective Investigation into Cancer-Norfolk (EPIC-Norfolk): a population-based cross-sectional study. *Public health nutrition* 10 (1):34–41. doi: 10.1017/S1368980007222608 [PubMed: 17212840]
10. Neville CE, Young IS, Gilchrist SE, McKinley MC, Gibson A, Edgar JD, Woodside JV (2013) Effect of increased fruit and vegetable consumption on physical function and muscle strength in older adults. *Age* 35 (6):2409–2422. doi:10.1007/s11357-013-9530-2 [PubMed: 23543264]
11. Kim J, Lee Y, Kye S, Chung YS, Kim KM (2015) Association of vegetables and fruits consumption with sarcopenia in older adults: the Fourth Korea National Health and Nutrition Examination Survey. *Age and ageing* 44 (1):96–102. doi:10.1093/ageing/afu028 [PubMed: 24646604]
12. Dawson-Hughes B, Harris SS, Ceglia L (2008) Alkaline diets favor lean tissue mass in older adults. *The American journal of clinical nutrition* 87 (3):662–665 [PubMed: 18326605]
13. Frassetto L, Morris RC Jr, Sebastian A (1997) Potassium bicarbonate reduces urinary nitrogen excretion in postmenopausal women. *The Journal of clinical endocrinology and metabolism* 82 (1):254–259. doi:10.1210/jcem.82.1.3663 [PubMed: 8989270]
14. Ceglia L, Harris SS, Abrams SA, Rasmussen HM, Dallal GE, Dawson-Hughes B (2009) Potassium bicarbonate attenuates the urinary nitrogen excretion that accompanies an increase in dietary protein and may promote calcium absorption. *The Journal of clinical endocrinology and metabolism* 94 (2):645–653. doi:10.1210/jc.2008-1796 [PubMed: 19050051]
15. Ceglia L, Rivas DA, Pojednic RM, Price LL, Harris SS, Smith D, Fielding RA, Dawson-Hughes B (2013) Effects of alkali supplementation and vitamin D insufficiency on rat skeletal muscle. *Endocrine* 44 (2):454–464. doi:10.1007/s12020-013-9976-0 [PubMed: 23666769]
16. Chan H (1968) Adaptation of urinary nitrogen excretion in infants to changes in protein intake. *The British journal of nutrition* 22 (2):315–323 [PubMed: 5673552]
17. Huse DM, Nelson RA, Briones ER, Hodgson PA (1974) Letter: Urinary nitrogen excretion as objective measure of dietary intake. *The American journal of clinical nutrition* 27 (8):771–773 [PubMed: 4846507]
18. Bingham SA, Cummings JH (1985) Urine nitrogen as an independent validity measure of dietary intake: a study of nitrogen balance in individuals consuming their normal diet. *The American journal of clinical nutrition* 42 (6):1276–1289 [PubMed: 4072961]
19. Dawson-Hughes B, Castaneda-Sceppa C, Harris SS, Palermo NJ, Cloutier G, Ceglia L, Dallal GE (2010) Impact of supplementation with bicarbonate on lower-extremity muscle performance in older men and women. *Osteoporos Int* 21 (7):1171–1179. doi:10.1007/s00198-009-1049-0 [PubMed: 19727904]
20. Dawson-Hughes B, Harris SS, Palermo NJ, Gilhooly CH, Shea MK, Fielding RA, Ceglia L (2015) Potassium Bicarbonate Supplementation Lowers Bone Turnover and Calcium Excretion in Older Men and Women: A Randomized Dose-Finding Trial. *J Bone Miner Res* 30 (11):2103–2111. doi: 10.1002/jbmr.2554 [PubMed: 25990255]
21. Jorgensen K (1957) Titrimetric determination of the net excretion of acid/base in urine. *Scandinavian journal of clinical and laboratory investigation* 9 (3):287–291 [PubMed: 13495348]
22. Chan JC (1972) The rapid determination of urinary titratable acid and ammonium and evaluation of freezing as a method of preservation. *Clinical biochemistry* 5 (2):94–98 [PubMed: 5039597]

23. Dawson-Hughes B, Harris SS, Palermo NJ, Castaneda-Sceppa C, Rasmussen HM, Dallal GE (2009) Treatment with potassium bicarbonate lowers calcium excretion and bone resorption in older men and women. *J Clin Endocrinol Metab* 94 (1):96–102. doi:10.1210/jc.2008-1662 [PubMed: 18940881]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1.

Adjusted means $\pm$ SE by treatment group of 84-day change in urinary N excretion/N intake by group. Adjustment made for baseline urinary N excretion/N intake, age, baseline weight, baseline lean body mass, baseline physical activity score, and baseline urinary NAE.

*p=0.012 compared to placebo.

Table 1.

Mean baseline ($\pm$ SD) clinical characteristics and biochemical measurements of the 232 study participants by group.

	Placebo	Low dose KHCO_3	High dose KHCO_3
N	79	79	74
Age (yr)	67.0 $\pm$ 6.2	67.2 $\pm$ 5.3	66.4 $\pm$ 5.0
Sex (% female)	46.8	49.4	50%
Weight (kg)	72.7 $\pm$ 13.6	74.2 $\pm$ 13.9	74.0 $\pm$ 13.3
BMI (kg/m^2)	25.5 $\pm$ 3.7	25.6 $\pm$ 4.3	26.1 $\pm$ 3.8
Activity score ⁺	149.1 $\pm$ 69.4	143.4 $\pm$ 66.0	139.7 $\pm$ 75.0
Lean tissue mass (%)	66.6 $\pm$ 9.3	64.5 $\pm$ 9.6	64.2 $\pm$ 10.6
Caloric intake (kcal)	2045 $\pm$ 675	1989 $\pm$ 594	2056 $\pm$ 769
Nitrogen intake (g)	14.2 $\pm$ 5.8	13.6 $\pm$ 6.0	14.3 $\pm$ 5.8
Urinary N excretion (g/d)	12.1 $\pm$ 4.2	10.8 $\pm$ 4.0	11.7 $\pm$ 4.8
Urinary NAE (mmol/d)	16.7 $\pm$ 22.4	19.8 $\pm$ 27.6	20.0 $\pm$ 32
Urinary creatinine excretion (mmol/d)	11.2 $\pm$ 3.9	10.7 $\pm$ 3.5	10.9 $\pm$ 4.3

Table 2.

Baseline, unadjusted and adjusted 84-day changes in ratio of urinary N excretion/N intake by treatment group expressed as adjusted means ($\pm$ SE). Adjusted for baseline ratio of urinary N excretion/N intake, age, baseline weight, baseline lean body mass, baseline physical activity score, and baseline NAE. Overall P=0.042.

*Compared to placebo P=0.012

Group	Ratio Urinary N excretion/N intake		
	Baseline Mean $\pm$ SE	Unadjusted Change Mean $\pm$ SE	Adjusted Change Mean $\pm$ SE
Placebo	67.0 $\pm$ 27.7	0.023 $\pm$ 0.049	0.071 $\pm$ 0.037
Low dose KHCO ₃	60.9 $\pm$ 20.3	0.020 $\pm$ 0.043	-0.006 $\pm$ 0.037
High dose KHCO ₃	63.3 $\pm$ 24.0	-0.033 $\pm$ 0.040	-0.063 $\pm$ 2.68*