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SHORT COMMUNICATION

Preliminary evidence of effects of potassium chloride on a metabolomic path to diabetes and cardiovascular disease

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Abstract

Introduction Low potassium intake can affect cardiovascular disease (CVD) risk and cardiometabolic risk factors.

Objective We hypothesize that potassium chloride (KCl) supplementation can improve cardiovascular risk metabolomic profile.

Methods In this secondary analysis of a pilot randomized clinical trial (RCT) of 26 participants with prediabetes randomized to KCl or placebo, we performed targeted mass-spectrometry-based metabolomic profiling on baseline and 12-week (end-of-study) plasma samples. Principal component analysis (PCA) was used to reduce the many correlated metabolites into fewer, independent factors that retain most of the information in the original data.

Results Those taking KCl had significant reductions (corresponding to lower cardiovascular risk) in the branched-chain amino acids (BCAA) factor ($P=0.004$) and in valine levels ($P=0.02$); and non-significant reductions in short-chain acyl-carnitines (SCA) factor ($P=0.11$).

Conclusions KCl supplementation may improve circulating BCAA levels, which may reflect improvements in overall cardiometabolic risk profile.

Clinical Trials Registry Clinicaltrials.gov identifier: NCT02236598; https://clinicaltrials.gov/ct2/show/NCT02236598.

Keywords Potassium chloride · Potassium supplements · Prediabetes · Metabolites · Branched-chain amino acids · Cardiovascular disease risk

Abbreviations

A1c	Hemoglobin A1c
BCAA	Branched-chain amino acids
CVD	Cardiovascular disease
IQR	Interquartile range
KCl	Potassium chloride
LCA	Long-chain acylcarnitines
OGTT	Oral glucose tolerance test
PCA	Principal component analysis
SCA	Short-chain acylcarnitines

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1 Introduction

Studies have found associations between low potassium levels and elevated blood pressure, increased stroke and cardiovascular (CVD) risk, as well as impaired glucose metabolism and increased diabetes risk (Aburto et al. 2013; Filippini et al. 2017; O'Donnell et al. 2014; Mente et al. 2014; Chatterjee et al. 2010; Peng et al. 2017). Interventions of potassium supplementation have been shown to lower blood pressure. Interventions of potassium supplementation may reduce longer-term risk of stroke, diabetes, and CVD, but clinical trials to demonstrate this have not been conducted.

Novel biomarkers, such as ones identified through metabolomic profiling, including amino acids and fatty acids and

their cognate acylcarnitines, have been found to have strong positive associations with presence of CVD, risk of CVD, insulin resistance, and risk of diabetes (Newgard et al. 2009; Shah et al. 2009, 2010a; Guasch-Ferre et al. 2016a; Ruiz-Canela et al. 2017). These biomarkers have been shown to change with interventions, such as pharmaceuticals and different dietary patterns, within a short time period (Wang et al. 2017; Rebholz et al. 2018; Ruiz-Canela et al. 2018). Thus, these metabolomic biomarkers may serve as early biomarkers of efficacy in clinical trials of interventions to prevent diabetes and CVD.

We recently conducted a pilot randomized clinical trial among African-American participants with prediabetes testing the effect of potassium supplementation with potassium chloride (KCl) 40 mEq/day compared with placebo for three months. The primary outcome of this pilot trial was changes in glucose (Chatterjee et al. 2017). This pilot trial found that, compared to placebo, those taking KCl had significant improvements in fasting glucose and trends in improvement in insulin sensitivity and glucose disposition (Chatterjee et al. 2017). In this secondary analysis, we used metabolomic profiling in samples collected within this pilot trial of KCl supplementation to identify metabolomic pathways and changes in biomarkers associated with KCl supplementation which could potentially indicate effect on longer-term risk of diabetes and CVD.

2 Methods

2.1 Study population

The Effects of Potassium on Glucose Metabolism in African Americans study was a randomized, controlled, double-blinded single-center pilot clinical trial of 29 participants with prediabetes at baseline (defined by A1c levels only) to determine the impact of KCl supplementation at a dose of 40 mEq/day compared to placebo on measures of potassium and glucose metabolism (Chatterjee et al. 2017).

Eligibility criteria for the pilot trial included African-American race by self-report, age ≥ 30 years, A1c of 5.7–6.5%, serum potassium 3.3–4.0 mEq/L, and estimated glomerular filtration rate ≥ 60 mL/min. The trial and this analysis were approved by Duke University's Institutional Review Board and conducted at the Duke Center for Living (Durham, North Carolina; clinicaltrials.gov identifier: NCT02236598). All participants provided written informed consent. All participants agreed to not alter their diet or physical activity patterns for the duration of the trial. At baseline and at 12 weeks, each participant underwent phlebotomy in a fasting state. Plasma samples were immediately centrifuged and aliquoted on site, frozen in a – 20 °C freezer, and then transferred to a – 80 °C freezer for storage.

Of the original 29 participants enrolled in the study, two did not have follow-up data and one did not have a stored baseline sample for metabolite measures; therefore, these three participants were excluded from the current analyses. Thus, 12 participants in the placebo arm and 14 participants in the KCl-supplement arm were included in these primary analyses (Supplement Fig. 1).

2.2 Metabolomic profiling

Tandem flow injection mass spectrometry with inclusion of stable-isotope-labeled internal standards was used for targeted metabolomic profiling of 60 metabolites (45 acylcarnitines, 15 amino acids) (Acquity TOD Triple Quadrupole; Waters) (Shah et al. 2009). Quantification was facilitated by addition of mixtures of known quantities of stable-isotope internal standards from Isotec (St. Louis, MO), Cambridge Isotope Laboratories (Andover, MA), and CDN Isotopes (Pointe-Claire, Quebec, CN) to samples.

2.3 Statistical analyses

Quality control was performed on metabolomics data, including visual inspections of histograms, boxplots, and density plots to assess distributional form and outliers; and run order plots with LOESS lines to inspect any batch effects or trends in timing of lab measurements (none were present). We identified two metabolites (C6 and C7-DC) with ≥ 25% of values below the lower limit of quantification and excluded them from further analyses.

Principal components analysis (PCA) was used given the large number of metabolites and known collinearity in the metabolite data reflecting shared biological pathways (Shah et al. 2010a). Given the small sample size of the pilot trial, PCA-factor scores were calculated using factor weights from the CATHGEN study, an established cohort of 9334 participants. Details of the CATHGEN repository and use of its data for creation of factor weights for metabolites have been described previously (Shah et al. 2010b; Kraus et al. 2015). In brief, PCA was performed on the remaining 58 metabolites of interest in the CATHGEN study to create fewer uncorrelated components that were weighted sums of the 58 metabolites. Factors with an eigenvalue ≥ 1 were retained, and varimax rotation was used to aid in interpretation of the components. Metabolites with a factor load ≥|0.4| were considered to comprise a given component. In this study, weights from the PCA factors created in CATHGEN were applied to each metabolite for each participant and summed to create PCA factor scores. Factors were sign-transformed as needed so that higher factor scores corresponded to higher levels of the metabolites that loaded heavily on the factor. A change score for each participant was created by subtracting the baseline factor value from the follow-up value.

Bivariate analyses were performed using two-sample t-tests to compare the mean changes in metabolite factors from baseline to follow-up between the two study arms. We also compared the mean change in factors and individual metabolites using nonparametric Mann Whitney tests to determine if results were affected by normality assumptions. Multivariable analyses were conducted using linear regression models, in which follow-up factor score was regressed on study arm indicator, baseline factor score, age, and sex. For factors found significant in these analyses (nominal unadjusted $P < 0.05$), individual metabolites within each factor were then compared between study arms. In bivariate analyses, we conducted two-sample t-tests to compare mean changes in individual metabolites between the two study arms. We additionally fit multivariable linear regression models in which changes in individual metabolites were regressed on baseline metabolite level, age, and sex.

Due to the small sample size and exploratory, hypothesis-generating goal of these analyses, adjustments were not made for multiple testing. Hypothesis tests were two-sided at the 0.05 significance level, and analyses were performed using SAS 9.4 (SAS Institute, Cary, NC) and R 3.4.4 (R Core Team, Vienna, Austria).

3 Results

Baseline characteristics of the 26 participants are shown in Table 1. Median [IQR] age was 53.1 [47.6–63.7], nearly 70% were female, and median [IQR] screening serum K was 3.8 [3.7–3.9] mEq/L.

Thirteen PCA factors had been derived in CATHGEN; of these, mean levels of a factor consisting of long-chain acylcarnitines (LCA) increased significantly in the KCl supplementation arm compared with the placebo arm in bivariate analyses ($P = 0.02$) (Table 2). Compared to placebo, both factor 13, composed of branched-chain amino acids (BCAA), as well as factor 10, composed of the short-chain acylcarnitines (SCA), which are byproducts of BCAA catabolism, decreased in the KCl arm (P-values 0.07 for both comparisons) (Table 2). Sensitivity analyses using Mann Whitney tests yielded similar results (results not shown).

In multivariable linear regression models, factor 13 (BCAA) was significantly reduced in the KCl arm ($P < 0.01$), but changes in factor 10 (SCAs) and factor 5 (LCAs) were no longer significantly different between arms (Table 2). Results of multivariable analyses of individual metabolites within factor 13 (BCAA) demonstrated a significant reduction in valine (P-value = 0.02) (Supplement Table 1).

4 Discussion

In this secondary analysis of a pilot randomized clinical trial testing the effects of KCl supplements on measures of potassium and glucose metabolism, we found that circulating BCAA levels were reduced with KCl supplementation. These results not only identify potential biomarkers reflecting efficacy of this intervention, but also suggest an underlying biological pathway through which the salutatory effects of KCl supplementation on glucose metabolism and risk of diabetes and CVD may be mediated.

There is a rich body of literature around the role of BCAA metabolism and BCAA biomarkers in obesity, insulin resistance, diabetes and CVD. These studies have found that BCAAs (which include valine, leucine, and isoleucine), the aromatic amino acids (tyrosine and phenylalanine, which share a large neutral amino acid transporter with BCAA), and the mitochondrial catabolic byproducts of BCAA metabolism (C3 and C5 short-chain acylcarnitines

Table 1 Baseline characteristics of study participants

	Total (n = 26)	KCl arm (n = 14)	Placebo (n = 12)
Age, years	53.1 [47.6, 63.7]	56.2 [48, 66.8]	51.1 [47, 59.2]
Female, N (%)	18 (69.2)	10 (71.4)	8 (66.7)
BMI, kg/m^2	33.6 [30.7, 37.2]	33.6 [30.8, 36.5]	33.5 [31.1, 37.9]
Baseline systolic blood pressure, mmHg	126.8 [118.8, 132.9]	125.5 [115, 136.9]	129.0 [121.5, 132.6]
Screen serum potassium, mEq/L	3.8 [3.7, 3.9]	3.9 [3.7, 4]	3.8 [3.7, 3.9]
Screen sodium, mEq/L	141 [141, 142]	142 [141, 142]	141 [140.8, 142.2]
Screen eGFR	99 [84.2, 112.8]	103 [86.5, 115]	90.5 [83.2, 112.2]
Screen A1c, %	6.0 [5.9, 6.2]	6.2 [6, 6.2]	6 [5.8, 6.1]
Fasting glucose, mg/dL	98.0 [92.0, 101.8]	98.5 [94.0, 110.2]	98.0 [90.0, 100.2]
Glucose 1-h, mg/dL	149 [130.2, 161.8]	154 [132.5, 183.2]	145 [126.8, 152.8]
Glucose 2-h, mg/dL	124 [101.2, 141]	119 [100.5, 140.2]	124.5 [105.8, 143.2]
Matsuda insulin sensitivity index	3.1 [2.2, 5]	3 [2, 4.2]	3.7 [2.3, 5.2]
Utz disposition index	0.1 [0.1, 0.2]	0.1 [0.1, 0.2]	0.1 [0.1, 0.2]

Values are median [IQR] unless otherwise indicated

Table 2 Comparison of mean factor change scores by study arm

Factor	Description	Factor change, KCl arm, mean (SD)	Factor change, placebo arm, mean (SD)	*P*-value (t-test)	*P*-value* (multivariable)
1	Medium- and long-chain acylcarnitines (C8, C10:1, C10, C12:1, C12, C14:2, C14:1, C14, C14:1-OH/C12:1-DC, C16:2, C16:1)	− 0.29 (0.61)	− 0.40 (1.00)	0.74	0.92
2	Medium-chain dicarboxyl-and hydroxyl-acylcarnitines (Cit, Ci4-DC/C4-DC, C5-DC, C6-DC, C10-OH/C8-DC, C12-OH/C10-DC, C6:1-DC/C8:1-OH, C8:1-DC)	− 0.28 (0.49)	− 0.34 (0.88)	0.83	0.86
3	Aromatic amino acids (Ala, Phe, Tyr)	0.30 (0.87)	− 0.03 (0.80)	0.34	0.22
4	Long-chain dicarboxyl- and hydroxyl-acylcarnitines (C12-OH/C10-DC, C14-OH/C12-DC, C16-OH/C14-DC, C18:1-OH/C16:1-DC, C18-OH/C16-DC, C20, C18:1-DC, C20-OH/C18-DC)	− 0.23 (0.52)	− 0.12 (0.79)	0.68	0.86
5	Long-chain acylcarnitines (LCA) (C16, C18:2, C18:1, C18, C16:1-OH/C14:1-DC, C20:4)	0.21 (0.49)	− 0.37 (0.66)	**0.02**	0.05
6	Amino acids (Gly, Ser, Pro, Met, Orn, Cit, Arg)	0.37 (0.81)	0.26 (0.94)	0.74	0.81
7	Miscellaneous acylcarnitines (C2, C4-OH, C14:2, C14:1-OH/C12:1-DC)	− 0.32 (0.93)	− 0.15 (0.81)	0.63	0.90
8	Miscellaneous (Gly, C5:1)	0.14 (0.55)	− 0.10 (0.82)	0.40	0.74
9	Medium-chain acylcarnitines (C8:1, C10:3, C10:2, C10:1)	0.24 (0.93)	0.45 (1.05)	0.58	0.76
10	Short-chain acylcarnitines (SCA) (C3, C4/Ci4, C5′s)	− 0.04 (0.94)	0.67 (0.98)	0.07	0.11
11	Amino acid and short-chain acylcarnitines (Asx, C5-OH/C3-DC)	− 0.41 (0.95)	− 0.06 (0.62)	0.28	0.58
12	Amino acids (His, Glx)	0.28 (0.72)	− 0.27 (0.78)	0.08	0.30
13	Branched-chain amino acids (BCAA) (Val, Leu/Ile, Glx)	− 0.42 (0.88)	0.20 (0.75)	0.07	**< 0.01**

Bold values indicate hypothesis tests were two-sided at the 0.05 significance level

*Adjusted for baseline factor score, age, sex

[SCAs]) are associated with increased type 2 diabetes risk and insulin resistance (Newgard et al. 2009; Guasch-Ferre et al. 2016a). Additionally, studies have found associations between BCAAs and CVD prevalence and risk (Shah et al. 2010a; Guasch-Ferre et al. 2016b; Tobias et al. 2018). The Prevención con Dieta Mediterránea (PREDIMED) trial found significant associations between baseline BCAAs and subsequent risk of CVD and risk of stroke.(Guasch-Ferre et al. 2016b) The Women's Health Study cohort found significant associations between BCAAs and CVD with the strongest associations in women who also developed type 2 diabetes (Tobias et al. 2018).

There are physiologic mechanisms that can explain the associations between these BCAAs and risk of diabetes and CVD, but the directionality of the associations is not clear and is likely to be bidirectional (White and Newgard 2019). With regards to BCAAs, people with obesity have higher levels of circulating BCAAs and BCAA metabolites, in part due to greater intake of protein, but also due to decreased oxidation of BCAAs in adipose tissue, suppression of enzymes that are responsible for BCAA catabolism, as well as impairments in uptake of BCAAs from blood, particularly by the liver. Obesity and certain types of diets could also lead to altered BCAA metabolism through effects on the gut microbiome (White and Newgard 2019). Additionally, higher levels of BCAAs can lead to increased insulin secretion in response to glucose loads but can also impair glucose uptake and insulin sensitivity by skeletal muscle (Newgard et al. 2009). Higher levels of circulating BCAAs have been found to precede the detection of insulin resistance by more traditional methods, such as the Homeostatic model assessment of insulin resistance (HOMA-IR); and levels of these metabolites have been found to precede and predict improvement in traditional measures of insulin resistance with interventions such as weight loss (Shah et al. 2012). Finally, increased insulin resistance in tissues can also lead to decreased protein synthesis, increased protein breakdown, and higher levels of free amino acids (Kalhan 2009; Abdulla et al. 2016).

While certain metabolomic biomarkers have been well-studied as predictors of chronic disease risk, it is less clear if these biomarkers can help to predict the long-term efficacy/effectiveness of interventions being tested to reduce long-term risk of chronic diseases. Trials such as the Diabetes Prevention Program (DPP) and the PREvención con DIeta MEDiterránea (PREDIMED) trial, a clinical trial testing

the effects of a Mediterranean diet on risk of cardiovascular disease, found improvements in high-risk metabolomic biomarkers with interventions (Walford et al. 2016; Guasch-Ferre et al. 2017).

In the context of this study, after 3 months of taking the intervention, in adjusted analyses, compared to placebo, participants in the KCl supplement arm had an overall reduction in circulating BCAA levels as reflected by improvements in the BCAA factor. The mechanism by which KCl could lead to reduced BCAA levels is unclear. It is possible that increases in potassium levels could lead to improvements in the oxidation of BCAAs, improvements in the function of enzymes that break down BCAAs, or improvements in the uptake of circulating BCAAs. Or, increases in potassium levels could lead to improvements in insulin sensitivity by other mechanisms, which allowed for improvements in BCAA catabolism. Although not observed in this small study, it is possible that KCl supplementation could lead to altered dietary intake or weight loss, which could lead to changes in BCAA catabolism; however, participants were advised not to alter their diet or physical activity habits. Or it is possible that KCl supplementation could alter the gut microbiome, which could also affect BCAA levels. Further study is needed to determine the mechanism of effect of KCl supplementation on circulating BCAA levels.

There are limitations of this study that should be mentioned. The main limitation is the small sample size, as well as analyses which are conducted without adjustment for multiple comparisons; and thus, these results should be viewed as hypothesis-generating, and not meant to draw inference. In addition, while factor scores for this study were created using weights from a larger validated cohort, these results cannot be generalized without confirmatory testing in a larger population. Finally, these results may only be specific to African Americans with prediabetes, and generalizability to other races and with earlier or later stages of insulin resistance cannot be made.

In conclusion, exploratory analyses of changes in metabolites from a targeted metabolomics assay from our pilot trial suggest that an intervention as simple as KCl is associated with salutatory changes in circulating BCAA levels, suggesting that alterations in BCAA levels could underlie the reason that KCl supplementation resulted in improvements in insulin sensitivity in our pilot trial. Further studies with larger sample sizes and longer-term follow-up are necessary to determine if KCl supplementation may be an effective intervention for diabetes and CVD prevention.

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Author contributions RC, LPS, DD, PHL, DE, SHS designed research (project conception, development of overall research plan, and study oversight); RC, CAD, CS, JJ, OI, LK conducted research (hands-on conduct of the experiments and data collection); OI, LK provided essential reagents or provided essential materials (applies to authors who contributed by providing animals, constructs, databases, etc., necessary for the research); CAD, LK analyzed data or performed statistical analysis; RC, CAD, LK, PHL, CS, JJ, OI, LPS, DD, DE, SHS wrote paper (only authors who made a major contribution); RC, SHS had primary responsibility for final content.

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Data availability De-identified data described in the manuscript, code book, and analytic code will be made available upon review of requests.

Compliance with ethical standards

Conflict of interest No authors have any conflicts of interest.

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