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Metabolic Changes with Base-Loading in CKD

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In small, randomized studies, treatment with sodium bicarbonate slowed kidney function decline in patients with CKD, possibly by lowering urine ammonium or inhibiting the renin-angiotensin-aldosterone or endothelin-1 pathways (1). Understanding the metabolic effects of alkali supplementation may reveal new candidate mechanisms. With this goal in mind, we profiled changes in systemic metabolites after treatment with sodium bicarbonate within a previously performed crossover trial of oral sodium bicarbonate (2).

The trial enrolled 20 adults with moderate CKD (mean±SD eGFR: 33±9 ml/min per 1.73 m^2) and serum bicarbonate concentration of 20–24 mEq/L (2). Mean age was 63±11 years, 85% had diabetes mellitus, and the mean±SD body mass index was 31.2±6.4 kg/m^2. Each participant was treated sequentially for 2 weeks with placebo, followed by sodium bicarbonate at escalating doses (0.3, 0.6, and 1.0 mEq/kg per day) under a protocol approved by the Institutional Review Board of the Albert Einstein College of Medicine (protocol #2008-376). We extracted a volume containing 2 μmol of creatinine into ethyl acetate from stored 24-hour urine samples collected after the placebo and 1.0 mEq/kg per day sodium bicarbonate period in all participants. From these, we generated nontargeted urine metabolite profiles using gas chromatography/electron-ionization mass spectrometry (GC/EI-MS). Blood samples were not fasting, therefore we performed plasma metabolomics among 11 out of 20 individuals, each of whom had similar fasting duration at their paired timepoints. Fasting duration ranged from 90 to 330 minutes, with a maximum difference in fasting time between paired samples of ±150 minutes. Plasma metabolomics included nontargeted GC/EI-MS from samples after methanol extraction, targeted panels using the Absolute*IDQ* p180 kit (Biocrates, Innsbruck, Austria), and conventional plasma metabolites (*i.e.*, lactate, ketones, and pyruvate) measured on a Beckman Unicel DxC 600 autoanalyzer. Full details of GC/EI-MS protocols at the Duke Molecular Physiology Institute (DMPI) have been previously reported (3).

Nontargeted data were deconvoluted and annotated using the DMPI's custom spectral libraries with entries from external libraries (Fiehn laboratory and Golm Metabolome Database), public libraries such as the National Institute of Standards and Technology database, and the DMPI's own additions. Integrated peak areas were $\log_2$ transformed. Metabolites missing in >50% of samples were not analyzed further, and other missing values were imputed using k-nearest neighbor (k=6) for nontargeted platforms or set to the limit of detection for targeted results. We used mixed linear models to evaluate pre-post metabolite changes and express the exponentiated β coefficients (2^{β}) as fold change.

No targeted plasma metabolites differed significantly pre- and postbicarbonate. Plasma lactate, ketones, and pyruvate (n=11) were each quantitatively higher after sodium bicarbonate, but also were not statistically significant. In nontargeted platforms, 234 unique plasma metabolites and 195 unique urine metabolites were analyzed. Identifiable plasma and urine metabolites that differed after bicarbonate therapy at a nominal P value ≤0.1 are presented in Table 1. Probable contaminants were removed. After correcting P values for a false discovery rate (FDR) of 20%, only urinary citrate/isocitrate remained significant (FDR-adjusted P value=0.03). Using each timepoint tested, $\log_2$ peak areas for citrate/isocitrate correlated moderately with serum bicarbonate concentrations (r=0.43; P<0.01) but within-person changes in these parameters were not correlated (P=0.9).

Each of the nominally significant metabolites (Table 1) was entered in MetaboAnalyst 3.0 (http://www.metaboanalyst.ca/) to determine pathway effects. On the basis of urine metabolites, pathway analysis revealed a strong effect on the tricarboxylic acid (TCA) cycle (FDR-adjusted P value<0.001), with additional effects on propanoate metabolism (FDR-adjusted P value=0.01). There were marginally significant pathway effects on pyruvate (nominal P value=0.01; FDR-adjusted P value=0.14) and branched chain-amino acid metabolism (nominal P value=0.02; FDR-adjusted P value=0.19) on the basis of urine metabolites, and the TCA cycle on the basis of plasma metabolites (nominal P value=0.01; FDR-adjusted P value=0.66).

In this study, treatment with sodium bicarbonate resulted in increased urinary excretion of multiple organic anions, including citrate/isocitrate, fumarate, succinate, malate, and α-ketoglutarate. Citrate/isocitrate was the only individual metabolite significant at an FDR-adjusted level of significance; however, the more statistically powerful pathway analyses indicate a robust effect on the TCA cycle. In this context, higher

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Table 1. Change in selected metabolites in nontargeted analyses in plasma and urine

Metabolite Annotation[a]	Fold Change	Nominal *P* Value	Metabolite Description[b]
Plasma metabolites			
3-Indoleacetic acid	0.63	0.005	Tryptophan metabolite
β-Sitosterol	1.49	0.01	Dietary phytosterol
Methyl stearate[b]	1.50	0.01	Fatty acid methyl ester
Lauric acid	1.36	0.02	Medium-chain fatty acid
Sucrose and similar disaccharides	2.07	0.02	Simple sugars
Methyl palmitate[b]	1.38	0.02	Fatty acid methyl ester
Methyl oleate[b]	1.67	0.02	Fatty acid methyl ester
Methyl linolenate[b]	1.60	0.02	Fatty acid methyl ester
Glycerol 1-phosphate	1.86	0.04	Glycolysis intermediate
Decanoic acid	1.79	0.05	Median-chain fatty acid
Malic acid	1.46	0.05	Intermediate of TCA cycle
Nonanoic acid	1.46	0.06	Median-chain fatty acid
Maltose or similar disaccharide	1.79	0.06	Simple sugar
Citric acid/isocitric acid	1.40	0.07	Intermediate of TCA cycle
Glycerol	1.33	0.08	Backbone of triglyceride
Urea	1.40	0.08	End product of protein metabolism
α-Ketoglutaric acid	1.27	0.09	Intermediate of TCA cycle
p-Cresol	0.67	0.09	Phenol derived from bacterial metabolism
Dehydroascorbic acid	1.25	0.09	Oxidized vitamin C
2-Hydroxybutyric acid	1.35	0.09	α-Hydroxy fatty acid
Urine metabolites			
Citric acid/isocitric acid	2.71	<0.001	Intermediate of TCA cycle
Succinic acid	2.01	0.002	Intermediate of TCA cycle
3-Indoleacetic acid	2.01	0.005	Tryptophan metabolite
2-Ethyl-3-hydroxypropionic acid	1.42	0.01	β-Hydroxy fatty acid; related to BCAA metabolism
Methylmalonic acid	1.45	0.02	Dicarboxylic acid; anaplerotic substrate
Pimelic acid	0.71	0.02	Dicarboxylic acid
Glyoxylic acid	0.57	0.02	Carboxylic acid
α-Ketoglutaric acid	1.61	0.03	Intermediate of TCA cycle
3-Methyl-3-Hydroxyisobutanoic acid	1.41	0.03	β-Hydroxy fatty acid; increased in ketoacidosis
Fumaric acid	1.44	0.04	Intermediate of TCA cycle
2-Isopropylmalic acid	1.69	0.05	α-Hydroxy dicarboxylic acid; elevated in ketoacidosis
Hippuric acid	1.35	0.08	Acyl-glycine
Lactic acid	1.69	0.1	α-Hydroxy acid

TCA, tricarboxylic acid; BCAA, branched chain amino acid.
[a]Reported metabolites are identifiable metabolites from nontargeted analyses with a nominal *P* value ≤0.1.
[b]Plasma C12 hydrocarbon and aminomalonic acid, and urine 4-hydroxycyclohexanecarboxylic acid were removed as likely contaminants. Methyl esters may be artificially derived during the extraction protocol with methanol but are listed because they may represent change in the underlying fatty acid ester. Alternatively they may represent increased methylation reactions in the setting of higher plasma pH.

levels of circulating TCA intermediates (*e.g.*, citrate/isocitrate, α-ketoglutarate), as well as the directly measured organic anions lactate, ketones, and pyruvate, suggest broader systemic effects of bicarbonate administration despite these changes not being statistically significant. Anaplerotic pathways including branched chain-amino acid and propanoate and pyruvate metabolism, act to restore TCA cycle intermediates and were also affected by sodium bicarbonate in our patients. In light of published reports that implicate changes in the TCA cycle in diabetic and nondiabetic kidney disease (4,5), these results may suggest a mechanism of protection in CKD.

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Disclosures

None.

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