SUPPLEMENTARY MATERIAL

for

Derangement in nicotinamide adenine dinucleotide (NAD+) metabolism is observed during acute kidney injury among male agricultural workers at risk for Mesoamerican Nephropathy

Running Headline: AKI and NAD+ metabolism in workers at risk for MeN

Nathan H Raines MD, MPH[1,2]†

Dominic A Leone MS, MPH[3]

Juan Jose Amador MD, MPH[3]

Damaris Lopez-Pilarte ER, MPH[3]

Oriana Ramírez-Rubio MD, PhD, MPH[3,4]

Iris S Delgado MPH[3]

Lauren J Francey MS[1]

Jessica H Leibler DrPH, ScM[5]

John M Asara PhD[2,6]

Madeleine K Scammell DSc[3]

Samir M Parikh MD[7]

*Daniel R Brooks DSc[3]†

*David J Friedman MD[1,2]†

[1]Division of Nephrology, Department of Medicine, Beth Israel Deaconess Medical Center, Boston, MA 02215, USA

[2]Harvard Medical School, Boston, MA 02215, USA

[3]Department of Epidemiology, Boston University School of Public Health, Boston, MA 02118, USA

[4]ISGlobal, Barcelona Institute for Global Health, 08003 Barcelona, Spain

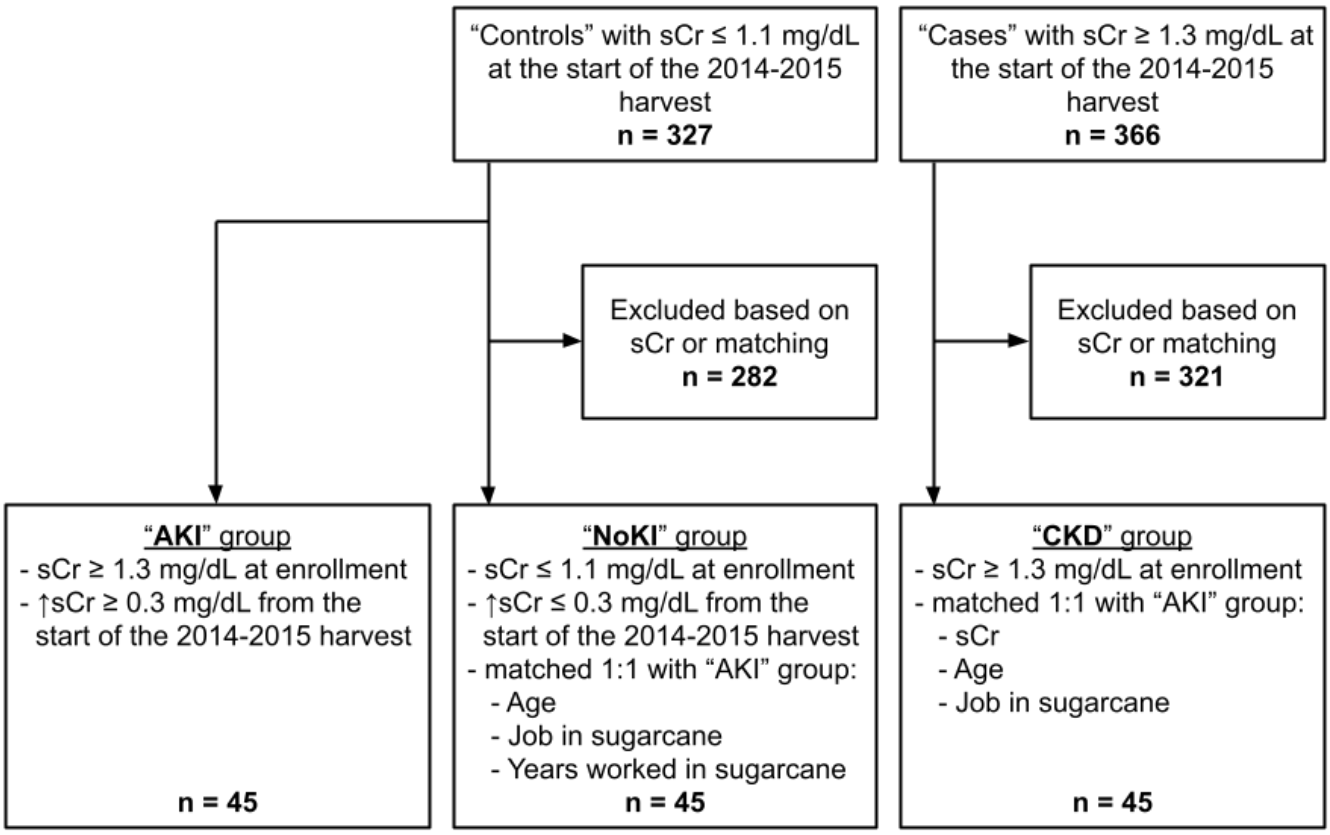
[5]Department of Environmental Health, Boston University School of Public Health, Boston, MA 02118, USA

[6]Division of Signal Transduction, Beth Israel Deaconess Medical Center

[7]Division of Nephrology, Department of Medicine, University of Texas Southwestern Medical School, Dallas, TX 75390, USA

*These authors contributed equally to this work (co-senior authors)

†Author to whom correspondence should be addressed



Supplementary Figure S1. Study Flow Diagram. sCr, serum creatinine; AKI, acute kidney injury; NoKI, no kidney injury; CKD, chronic kidney disease

Supplementary Table S1. Levels of metabolites in nicotinamide adenine dinucleotide (NAD+) biosynthetic pathways among kidney injury groups. Data shown are values for each of the acute kidney injury (AKI), no kidney injury (NoKI), and chronic kidney disease (CKD) groups. P values describe the comparison between the AKI and each of the NoKI and CKD groups. “Imputed” indicates the number of individuals in each group with values below the threshold of detection for each metabolite.

Metabolite	**AKI (n = 45)**	**NoKI (n = 45)**	**p**	**CKD (n = 45)**	**p**
NAD+ (positive) AUC, median (IQR)	571 (304 to 1502)	336 (242 to 538)	0.03	317 (236 to 452)	< 0.0001
Imputed, n (%)	0 (0)	1 (2)		3 (7)	
NAD+ (negative) AUC, median (IQR)	56 (15 to 117)	36 (3 to 62)	0.29	50 (3 to 123)	0.81
Imputed, n (%)	11 (24)	15 (33)		14 (31)	
Tryptophan AUC, median (IQR)	55262 (33576 to 91745)	93032 (70932 to 123040)	0.0002	63847 (48386 to 127222)	0.036
Imputed, n (%)	0 (0)	0 (0)		0 (0)	
Kynurenine AUC, median (IQR)	12490 (7373 to 21329)	12642 (7591 to 20004	0.85	16425 (10968 to 30981)	0.02
Imputed, n (%)	0 (0)	1 (2)		0 (0)	
Kynurenic acid AUC, median (IQR)	2266 (1614 to 3529)	2842 (2087 to 4804)	0.06	2979 (2284 to 4967)	0.02
Imputed, n (%)	0 (0)	0 (0)		0 (0)	
Quinolinate AUC, median (IQR)	5731 (3727 to 8741)	5278 (3628 to 8683)	0.91	7304 (4608 to 8817)	0.41
Imputed, n (%)	0 (0)	0 (0)		0 (0)	
Nicotinamide AUC, median (IQR)	48604 (42138 to 60600)	66792 (55421 to 106089)	0.0002	89115 (58702 to 122155)	< 0.0001
Imputed, n (%)	0 (0)	0 (0)		0 (0)	
Methylnicotinamide AUC, median (IQR)	12815 (9550 to 34595)	35104 (17970 to 77074)	0.02	33199 (17933 to 67019)	0.02
Imputed, n (%)	0 (0)	0 (0)		0 (0)	
Nicotinamide riboside	5157 (3004 to	5301 (3521 to	0.54	7289 (5335 to	0.09

AUC, median (IQR)	10547)	9922)		11524)	
Imputed, n (%)	0 (0)	0 (0)		0 (0)	
Nicotinic acid AUC, median (IQR)	167 (112 to 299)	129 (85 to 238)	0.81	132 (68 to 218)	0.13
Imputed, n (%)	1 (2)	3 (7)		3 (7)	

Supplementary Table S2: Supplementary Data File Dictionary. LC-MS/MS, liquid chromatography-coupled tandem mass spectrometry.

Variable	Description
PairID	Participant Identification
MatchGroup	Pairing group
KidneyGroup	Kidney disease category. AKI = acute kidney injury, NoAKI = no acute kidney injury, CKD = chronic kidney disease
sCr	Serum creatinine, in mg/dL
uCr	Urine creatinine, in mg/dL
Q/T	Urine quinolinate to tryptophan ratio, with each measured in LC-MS/MS peak area under curve
KynA/T	Urine kynurenic acid to tryptophan ratio, with each measured in LC-MS/MS peak area under curve
kynurenic acid	Urine LC-MS/MS peak area under curve, corrected for urine creatinine
kynureinine	Urine LC-MS/MS peak area under curve, corrected for urine creatinine
methylnicotinamide	Urine LC-MS/MS peak area under curve, corrected for urine creatinine
NAD+_nega	Urine LC-MS/MS peak area under curve, corrected for urine creatinine
NAD+_posi	Urine LC-MS/MS peak area under curve, corrected for urine creatinine
nicotinamide	Urine LC-MS/MS peak area under curve, corrected for urine creatinine
nicotinamide riboside	Urine LC-MS/MS peak area under curve, corrected for urine creatinine
nicotinate	Urine LC-MS/MS peak area under curve, corrected for urine creatinine
quinolinate	Urine LC-MS/MS peak area under curve, corrected for urine creatinine
tryptophan	Urine LC-MS/MS peak area under curve, corrected for urine creatinine

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	6, Tab. 1
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7-8
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8-9
Bias	9	Describe any efforts to address potential sources of bias	7,9
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	9-10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9-10
		(*b*) Describe any methods used to examine subgroups and interactions	n/a
		(*c*) Explain how missing data were addressed	9

		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	7, 10
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Fig. S1
		(b) Give reasons for non-participation at each stage	Fig. S1
		(c) Consider use of a flow diagram	Fig. S1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10, Tab. 2
		(b) Indicate number of participants with missing data for each variable of interest	Tab. S1
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	n/a
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	n/a
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	7, Tab. 2
		Cross-sectional study—Report numbers of outcome events or summary measures	n/a
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11, Fig 1.
		(*b*) Report category boundaries when continuous variables were categorized	n/a
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11, Fig. 1-3, Tab. S1
Discussion			
Key results	18	Summarise key results with reference to study objectives	12-13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13-14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-14

Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	222

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.