ARIC Manuscript Proposal #3421

PC Reviewed: 6/18/19  Status: _____  Priority: 2
SC Reviewed: _________  Status: _____  Priority: _____

1.a. Full Title: Validity and feasibility of toenail metal quantification using desktop XRF

b. Abbreviated Title (Length 26 characters):

2. Writing Group:
   Writing group members: Melinda C. Power, Marc G. Weisskopf, Aaron Specht, Eliseo Guallar, B. Gwen Windham

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. __AS___ [please confirm with your initials electronically or in writing]

First author:  Aaron Specht
   Address:  677 Huntington Ave, Boston, MA 02115

   Phone: (317)358-5258
   E-mail: aspecht@hsph.harvard.edu

ARIC author to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).
   Name:  Melinda C. Power
   Address:  950 New Hampshire Ave NW, Washington DC, 20052

   Phone: 202 994 7778
   E-mail: power@gwu.edu

3. Timeline: Pilot to begin immediately upon ARIC and CLUE II approval

4. Rationale:
While ageing is often associated with pathological processes, healthy ageing is possible, and large-scale prevention of cognitive and physical impairment would contribute to improved overall quality of life, reductions in associated healthcare costs and caregiver burden, as well as longer lives.1-7 The effects of metal exposures on age-related diseases and disorders have been understudied, despite plausible mechanisms that could link metal exposure to increased risk for these conditions. Metals play a role in inflammation, oxidative stress, or promotion of cardio-metabolic risk factors or cardiovascular disease6-10, which have been linked to mortality, declines in physical function, cognitive decline, and dementia risk.11-20 Because metal exposure is modifiable, understanding this association may lead to new opportunities for reducing age-related disease burden.
Thus far, relatively little attention has been given to the impact of exposures to metals besides lead (Pb) on physical function and mortality.\textsuperscript{21-23} While prior studies have identified associations between various trace metals and cognitive outcomes, most are cross-sectional studies which quantify associations with recent metal exposure.\textsuperscript{24-35} However, midlife exposures are likely more informative of dementia initiation and progression, as dementia pathogenesis takes years to decades. Similar latency between exposures and physical function or mortality are also likely. Therefore, findings of associations between time-appropriate exposures and age-related outcomes are needed. Work to quantify the effects of metals on related aspects of ageing will inform strategies for preventing diseases and conditions that are closely tied to quality of life and mortality in our aging population.

Midlife, or at least distant exposures are often most relevant to late-life health outcomes given the long preclinical periods or general accumulation of pathologies associated with many age-related diseases and disorders. However, few cohort studies have robust exposure assessment for metals or other environmental pollutants in midlife and late life assessment of cognitive or physical health. Retrospective quantification of midlife metal exposures is difficult, but potentially possible using biomarkers of exposure. While some metals can be quantified in serum or whole blood, valid measurements generally require that special care have been taken at sample collection (e.g. use of metal-free tubes and care to avoid contamination) that was not often implemented in cohorts with non-environmental focus. While some metals can be quantified in stored urine, this method does not generally yield measures of the metals we might think are most closely related to our health outcomes. Stored toenail samples, however, provide a unique opportunity to quantify midlife exposures to a substantial number of metals. While many existing techniques require toenail destruction as part of the quantification process, newer methods, such as the one we propose here, allow non-destructive, yet accurate quantification of toenail metal content.

Our long-term goal is to understand the contribution of environmental heavy metals on age-related disease and disorders. The overlap of ARIC and CLUE II at the Hagerstown, MD study sites allows a unique opportunity to consider such questions. Participants of CLUE II provided toenail samples in 1989, and a subset of CLUE II participants were also ARIC participants. Toenail metal content was previously samples from the subset of CLUE II-ARIC overlap participants using INAA, a non-destructive method. However, INAA can only quantify a limited number of metals, and those previously quantified are not those of most potential interest. Furthermore, INAA results in residual radioactivity of the toenails, which must then be stored safely until this radioactivity has subsided. We have identified an alternate, non-destructive approach for metal quantification – desktop X-ray fluorescence (XRF) that can measure a suite of 30 metals, including many metals of particular interest in relation to age-related diseases and disorders, and does not leave the toenails radioactive.\textsuperscript{36-41} Thus, the objective of this proposal is to conduct a pilot study to evaluate the feasibility and validity of assessing toenail metal content in stored toenails from midlife using a novel, non-destructive technique, desktop X-ray fluorescence.

### 5. Main Hypothesis/Study Questions:

**Aim 1:** Demonstrate feasibility of quantifying metals in toenail samples using desktop XRF

**Hypothesis 1:** Metal concentrations will exceed the limit of detection for the majority of metals of interest.

**Aim 2:** Demonstrate validity of metal quantification in toenail samples using desktop XRF

**Hypothesis 2:** Metal concentrations as assessed by INAA and desktop XRF will be highly correlated for the subset of metals quantified/quantifiable by both methods (Fe, Zn, Hg, As, Se, Br).

### 6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).
We propose to send as set of 15-20 toenail samples, chosen to represent the range of metal concentrations and sample mass available in the CLUE II/ARIC overlap participants with prior metal concentration by INAA to the lab of Marc Weisskopf and Aaron Specht at the Harvard School of Public Health, who will quantify the toenail metal concentrations using desktop XRF. The toenails will undergo a standard cleaning method using 1% Triton solution, three repeated deionized water rinses, followed by an acetone rinse, and three final deionized water rinses. Then the toenails will be freeze dried to remove any residual water prior to analysis. The toenails will be set between two 0.5 um mylar films in a standard 35 mm XRF sample cup, which will be placed in the desktop XRF sample changer for measurement. The desktop XRF measurement includes automatic rotation of the sample to ensure the sample is used in the measurement. The resultant counts for each element will be extracted via the XRF deconvolution method, which we can then compare to calibration standards to arrive at final concentrations. As done in previous studies of toenails and XRF, standards are derived from an epoxy resin doped material to mimic toenail properties for x-ray interactions and will be doped at various levels of elemental concentrations in order to identify a counts and concentration relationship using the standard deconvolution method. After the calibration is applied, final results for each element will be given in micrograms per gram toenail or part per million.

We will then tabulate the % above the limit of detection, as well as summary statistics for the metal concentrations across the samples (mean, median, range, standard deviation, histograms). Finally, we will run Pearson correlations and linear regression analyses to understand how well the prior and current metal concentrations align for the subset of metals quantified previously and as part of this pilot study.

7.a. Will the data be used for non-CVD analysis in this manuscript? __x__ Yes   ____ No

   b. If Yes, is the author aware that the file ICTDER03 must be used to exclude persons with a value RES_OTH = “CVD Research” for non-DNA analysis, and for DNA analysis RES_DNA = “CVD Research” would be used? __x__ Yes    ____ No

   (This file ICTDER has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript? ____ Yes    _x___ No

   8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER03 must be used to exclude those with value RES_DNA = “No use/storage DNA”? ____ Yes    ____ No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://www.cscc.unc.edu/aric/mantrack/maintain/search/dtSearch.html

   ___x___ Yes    _______ No
10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

#895 Heavy metal exposure and carotid atherosclerosis in the CLUE II/ARIC study
#885 Low toenail chromium as a risk factor for incident diabetes: results from the CLUE II study and the ARIC study

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?  Yes  No

11.b. If yes, is the proposal
   A. primarily the result of an ancillary study (list number* __________)
   B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* __________

*ancillary studies are listed by number at https://www2.cscc.unc.edu/aric/approved-ancillary-studies

12a. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is your responsibility to upload manuscripts to PubMed Central whenever the journal does not and be in compliance with this policy. Four files about the public access policy from http://publicaccess.nih.gov/ are posted in http://www.cscc.unc.edu/aric/index.php, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to PubMed central.


