Provisional Paper Title: Childhood lead exposure and long-term telomere erosion

Proposing Author: Aaron Reuben

Author's Email: aaron.reuben@duke.edu

P.I. Sponsor: Terrie Moffitt and Avshalom Caspi

Today's Date: September 10, 2020

Objective of the study:

Millions of adults alive today were exposed to high levels of lead as children. Lead is a persistent environmental pollutant that was once ubiquitous –in gasoline, paints and pipes. Adults now entering midlife were exposed to lead as children during the peak-era of leaded gasoline (mid-1960s through late-1980s). In 1976, 99.8% of American children had blood-lead levels above the current threshold for clinical attention,[1] and the average American's blood-lead level exceeded the current threshold three times over.[2] While lead-exposures declined sharply with the phase-out of lead-in-gasoline, millions worldwide remain at risk.[3]

Lead-exposure may shorten telomere length. Lead is known to harm most organs, and is particularly detrimental to children.[4] Emerging evidence suggests that early-life exposure may shorten telomeres.[5–8] Telomere attrition has consequently been proposed as one potential mechanism linking lead-induced toxicity to long-term harm.[5,9,10] The evidence is limited, however, and findings have been mixed. Three studies in children reported a negative association between BLLs and relative telomere length,[7,8,11] but two reported no association.[12,13] In adults, occupational lead-exposures have been associated with shorter telomeres[6,14] while general-population exposures have not.[5]

Existing evidence has important limitations. First, all studies were cross-sectional, making it impossible to evaluate long-term cumulative harm or gradual telomere erosion across time. Second, these studies were potentially underpowered to detect subtle effects; only two had N>200. Three, no study utilized archival lead-exposure data from the peak lead-exposure era (e.g.,1970s). While today's children usually have low lead-exposures, today's adults generally experienced high exposures as children. There is thus no evidence yet to inform the risk profile owing to childhood exposure for adults now entering later life.

The proposed study will evaluate associations between childhood lead-exposure and telomere length among lead-tested members of the Dunedin Study (N=579). Dunedin represents the only cohort where lead-exposure was unrelated to socioeconomic status.

While archived Dunedin blood-lead and relative leukocyte telomere length (LTL) data have been separately published in this cohort,[15,16] this will be the first study to bring them together. The proposed study would represent the largest comprehensive study of early life lead-exposure and long-term telomere attrition.

Data analysis methods:

AIM 1: Test the hypothesis that children with greater blood-lead level will display shorter LTL than their peers three decades later, at age 38.

Through correlations and multivariate regression we will test the association of childhood blood-lead level with LTL at age 38 in "baseline" models adjusted for sex and "fully adjusted" models adjusted for factors commonly included as confounds in studies of adult LTL or lead, including childhood SES, pack years smoked, BMI, and white blood cell count. If significant lead-LTL associations are found, follow-up tests will determine whether telomere differences co-occur with lead-related outcomes previously identified in the same cohort (e.g., cognitive deficits and decline, psychopathology, differences on MRI measures of brain structural integrity).

AIM 2: Test the hypothesis that children with greater blood-lead level will display greater LTL decline than age-peers across adulthood from age 26 to 38, suggesting an on-going enhanced cellular-aging process unfolding across time.

Through multivariate regression we will test the association of childhood blood-lead level with longitudinal LTL change from age 26-38. Residualized change will be modeled by including age-26-LTL in models regressing age-38-LTL onto lead following the same baseline and fully adjusted modeling approach specified under Aim 1. Sensitivity tests will determine whether significant longitudinal-LTL-differences are accompanied by declines on other biomarkers indicative of biological (as opposed to chronological) aging (e.g., the methylation Pace of Aging). Given uncertainty about the interpretation of telomere lengthening we will also conduct sensitivity tests excluding Study members whose telomeres lengthened from age 26 to 38 (~13% of the cohort).

Because lead exposure data are not available for all Dunedin Study members, we will also test for selective missingness and control for any possible selectivity in all analyses.

Variables needed at which ages:

Predictor
lead11ug_dl   Blood lead (uncorrected) at age 11

Outcomes
TeloBld26   Telomere length at 26, blood source only
TSratio_26   Telomere length at 26
TeloBld38   Telomere length at 38, blood source only
TSratio_38   Telomere length at 38

Covariates
sex
SESchildhd   Family SES averaged from birth to age 15,
source_26   Blood or buccal swab at 26
source_32   Blood or buccal swab at 32
source_38   Blood or buccal swab at 38
                     Whole white blood cell count at age 26
                     Individual counts of the 5 main white blood cells at age 26
                     White blood cell count at age 38
                     Individual counts of the 5 main white blood cells at age 38

PackYrLifTm26	Lifetime pack years to phase26
PackYrLifTm38	Lifetime pack years to phase38
bmip26	BMI phase 26
bmip38	BMI phase 38

Variables for sensitivity tests

Previously identified lead-related outcomes
infMem45	Informant reported memory difficulties at 45
infAtt45	Informant reported attention difficulties at 45
IQ79std	Full Scale IQ, mean of age 7 and 9, standardized
fsIQ45_STD	Full Scale IQ at 45, standardized
sa_tot	Mean cortical surface area at 45
bihippocampus	Bilateral hippocampal volume at 45
averagefa	Average fractional anisotropy at 45
brainage_centered	Brain age at 45
brainAGE	Brain age gap estimate at 45
P_BF45	P Factor, BF45, June2019
EXT_CF45	Externalizing Factor, CF45, June2019
INT_CF45	Internalizing Factor, CF45, June2019
THD_CF45	Thought Disorder Factor, CF45, June2019
BFagre45	Big five agreeableness @ 45, informants
BFcons45	Big five conscientiousness at 45, informants
BFneur45	Big five neuroticism at 45, informants

Measures of biological aging
| PoAm45 | Pace of Aging Methyl, p45, 4 May 2020 |

Significance of the Study (for theory, research methods or clinical practice):

Knowledge about lead-telomere associations could help lower the burden of disease. It will be years before most individuals lead-exposed in childhood are sufficiently aged for age-related disease endpoints to emerge. Shorter telomere length represents an intermediate measure, which has been plausibly linked to risk for dementia,[17] diabetes,[18] cancer,[19] and cardiovascular disease.[20] Identification of telomere erosion in adults lead-exposed as children would raise the possibility that interventions to slow telomere attrition could reduce burden of disease if targeted at this population. Follow-up studies can evaluate differences in telomere length by age 45 and via related measures of DNA methylation-based telomere length estimators.

References cited:

1. Pirkle JL, Brody DJ, Gunter EW, et al. The decline in blood lead levels in the United States: The National Health and Nutrition Examination Surveys (NHANES). *JAMA*. 1994;272(4):284-291. doi:10.1001/jama.1994.03520040046039

2. Annest J. Trends in the blood lead levels of the US population: The Second National Health

and Nutrition Examination Survey (NHANES II) 1976-1980. *Rutter M, Jones RR, eds Lead versus health Chichester, UK: Wiley*. Published online 1983:33-58.

3. Ettinger AS, Ruckart PZ, Dignam T. Lead poisoning prevention: The unfinished agenda. *Journal of Public Health Management and Practice*. 2019;25:S1. doi:10.1097/PHH.0000000000000902

4. Bellinger DC. Very low lead exposures and children's neurodevelopment. *Curr Opin Pediatr*. 2008;20(2):172-177. doi:10.1097/MOP.0b013e3282f4f97b

5. Zota AR, Needham BL, Blackburn EH, et al. Associations of cadmium and lead exposure with leukocyte telomere length: Findings from National Health and Nutrition Examination Survey, 1999–2002. *Am J Epidemiol*. 2015;181(2):127-136. doi:10.1093/aje/kwu293

6. Wu Y, Liu Y, Ni N, Bao B, Zhang C, Lu L. High lead exposure is associated with telomere length shortening in Chinese battery manufacturing plant workers. *Occup Environ Med*. 2012;69(8):557-563. doi:10.1136/oemed-2011-100478

7. Pawlas N, Płachetka A, Kozłowska A, Broberg K, Kasperczyk S. Telomere length in children environmentally exposed to low-to-moderate levels of lead. *Toxicol Appl Pharmacol*. 2015;287(2):111-118. doi:10.1016/j.taap.2015.05.005

8. Wai KM, Umezaki M, Kosaka S, et al. Impact of prenatal heavy metal exposure on newborn leucocyte telomere length: A birth-cohort study. *Environmental Pollution*. 2018;243:1414-1421. doi:10.1016/j.envpol.2018.09.090

9. Pottier G, Viau M, Ricoul M, et al. Lead exposure induces telomere instability in human cells. *PLoS One*. 2013;8(6). doi:10.1371/journal.pone.0067501

10. He L, Chen Z, Dai B, Li G, Zhu G. Low-level lead exposure and cardiovascular disease: the roles of telomere shortening and lipid disturbance. *The Journal of Toxicological Sciences*. 2018;43(11):623-630. doi:10.2131/jts.43.623

11. Herlin M, Broberg K, Igra AM, Li H, Harari F, Vahter M. Exploring telomere length in mother–newborn pairs in relation to exposure to multiple toxic metals and potential modifying effects by nutritional factors. *BMC Med*. 2019;17. doi:10.1186/s12916-019-1309-6

12. Lin S, Huo X, Zhang Q, et al. Short placental telomere was associated with cadmium pollution in an electronic waste recycling town in China. *PLOS ONE*. 2013;8(4):e60815. doi:10.1371/journal.pone.0060815

13. Alegría-Torres JA, Pérez-Rodríguez RY, García-Torres L, Costilla-Salazar R, Rocha-Amador D. Exposure to arsenic and lead in children from Salamanca México, effects on telomeric lengthening and mitochondrial DNA. *Environ Sci Pollut Res Int*. 2020;27(6):6420-6428. doi:10.1007/s11356-019-07108-4

14. Pawlas N, Płachetka A, Kozłowska A, et al. Telomere length, telomerase expression, and oxidative stress in lead smelters. *Toxicol Ind Health*. 2016;32(12):1961-1970. doi:10.1177/0748233715601758

15. Shalev I, Moffitt TE, Braithwaite AW, et al. Internalizing disorders and leukocyte telomere erosion: A prospective study of Depression, Generalized Anxiety Disorder and Post-Traumatic Stress Disorder. *Mol Psychiatry*. 2014;19(11):1163-1170. doi:10.1038/mp.2013.183

16. Reuben A, Caspi A, Belsky DW, et al. Association of childhood blood lead levels with cognitive function and socioeconomic status at age 38 years and with IQ change and socioeconomic mobility between childhood and adulthood. *JAMA*. 2017;317(12):1244-1251. doi:10.1001/jama.2017.1712

17. Honig LS, Kang MS, Schupf N, Lee JH, Mayeux R. Association of shorter leukocyte telomere repeat length with dementia and mortality. *Arch Neurol*. 2012;69(10):1332-1339. doi:10.1001/archneurol.2012.1541

18. Zhao J, Miao K, Wang H, Ding H, Wang DW. Association between telomere length and Type 2 Diabetes Mellitus: A meta-analysis. *PLOS ONE*. 2013;8(11):e79993. doi:10.1371/journal.pone.0079993

19. Ma H, Zhou Z, Wei S, et al. Shortened telomere length Is associated with increased risk of cancer: A meta-analysis. *PLOS ONE*. 2011;6(6):e20466. doi:10.1371/journal.pone.0020466

20. Muller M, Rabelink TJ. Telomere shortening: a diagnostic tool and therapeutic target for cardiovascular disease? *Eur Heart J*. 2014;35(46):3245-3247. doi:10.1093/eurheartj/ehu252

Data Security Agreement

Provisional Paper Title	Childhood lead exposure and long-term telomere attrition
Proposing Author	Aaron Reuben
Today's Date	August 31, 2020

Please keep one copy for your records and return one to the PI Sponsor
Please initial your agreement

ASR	I am current on Human Subjects Training (CITI (www.citiprogram.org) or equivalent)
ASR	My project is covered by Duke or Otago ethics committee OR I have /will obtain ethical approval from my home institution.

ASR	I will treat all data as "restricted" and store in a secure fashion.
	My computer or laptop is:
	    a) encrypted (recommended programs are FileVault2 for Macs, and Bitlocker for Windows machines)
	    b) password-protected
	    c) configured to lock-out after 15 minutes of inactivity AND
	    d) has an antivirus client installed as well as being patched regularly.

ASR	I will not "sync" the data to a mobile device.
ASR	In the event that my laptop with data on it is lost, stolen or hacked, I will immediately contact Professor Moffitt or Caspi. (919-684-6758, tem11@duke.edu, ac115@duke.edu)
ASR	I will not share the data with anyone, including my students or other collaborators not specifically listed on this concept paper.
ASR	I will not post data online or submit the data file to a journal for them to post. *Some journals are now requesting the data file as part of the manuscript submission process. The Dunedin Study Members have not given informed consent for unrestricted open access, so we have a managed-access process. Speak to Terrie or Avshalom for strategies for achieving compliance with data-sharing policies of journals.*
ASR	I will delete all data files from my computer after the project is complete. Collaborators and trainees may not take a data file away from the office. The data remains the property of the Study and cannot be used for further analyses without an approved concept paper for new analyses.

Signature: _____/s/ Aaron Samuel Reuben_____