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Supplemental Material

Arsenic Exposure and Epigenetic Aging: The Association with Cardiovascular Disease and All-Cause Mortality in the Strong Heart Study

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Figure S2. Simplified directed acyclic graph depicting the relationships between arsenic exposure, epigenetic age acceleration, cardiovascular disease, and other mediating variables. For simplicity, we are not showing the arrow between CVD risk factors and DNA-methylation based metrics of biological aging but it is well-established that CVD risk factors can also accelerate biological aging. Also for simplicity we are not showing the arrows between age, sex, and study center and other variables, including cardiovascular disease, and other covariates.

Additional Files- Excel Document and mediation_analysis_code.R

Table S1. Effect estimates (95% CI) of the association between urinary arsenic exposure and epigenetic aging measures (Hannum age acceleration and Horvath age acceleration), using multivariate linear regression in the Strong Heart Study (N=2323). In the model per IQR, arsenic was modeled as log_2 transformed and reported comparing an interquartile range (p25 vs. p75). The effect estimates compare epigenetic age acceleration values, reported in years based on the residual method.

Mean Difference	Model 1		Model 2		Model 3	
	Effect Estimate (95% CI)	P-value	Effect Estimate (95% CI)	P-value	Effect Estimate (95% CI)	P-value
Hannum						
IQR (5.24 vs. 14.42 μg/g creatinine)	0.31 (0.08, 0.55)	0.07	0.32 (0.08, 0.56)	0.07	0.23 (0.01, 0.45)	0.17
Q1 (< 5.24)	0.00 (ref.)	--	0.00 (ref.)	--	0.00 (ref.)	--
Q2 (5.24 - 8.56)	-0.15 (-0.73, 0.43)	0.62	-0.14 (-0.72, 0.43)	0.62	-0.35 (-0.89, 0.20)	0.21
Q3 (8.56 - 14.42)	0.29 (-0.32, 0.91)	0.35	0.30 (-0.32, 0.91)	0.35	0.04 (-0.54, 0.62)	0.89
Q4 (> 14.42)	0.44 (-0.23, 1.10)	0.20	0.45 (-0.22, 1.10)	0.19	0.24 (-0.40, 0.87)	0.47
Horvath						
IQR (5.24 vs. 14.42 μg/g creatinine)	0.01 (-0.23, 0.26)	0.94	0.06 (-0.18, 0.31)	0.74	0.04 (-0.20, 0.28)	0.84
Q1 (< 5.24)	0.00 (ref.)	--	0.00 (ref.)	--	0.00 (ref.)	--
Q2 (5.24 - 8.56)	-0.46 (-1.10, 0.15)	0.14	-0.45 (-1.10, 0.16)	0.15	-0.59 (-1.20, 0.00)	0.05
Q3 (8.56 - 14.42)	-0.15 (-0.80, 0.49)	0.64	-0.12 (-0.77, 0.52)	0.71	-0.28 (-0.91, 0.34)	0.37
Q4 (> 14.42)	-0.21 (-0.91, 0.49)	0.55	-0.14 (-0.84, 0.57)	0.71	-0.21 (-0.89, 0.47)	0.55

Model 1: adjusted for chronological age, eGFR, sex, center (Arizona, Oklahoma, and North/South Dakota), five genetic PCs

Model 2: additionally adjusted for smoking status (current/former/never), BMI

Model 3: additionally adjusted for Houseman cell proportions

P-values were taken from linear regression and compare epigenetic age acceleration means per one interquartile range increase on arsenic. CI, confidence interval; IQR, interquartile range; Q1, first quartile; Q2, second quartile; Q3, third quartile; Q4, fourth quartile; ref, reference; CVD, cardiovascular disease.

Table S2. Results of sensitivity analysis adjusting mediation models in Table 5 for Houseman cell proportions; number of incident cardiovascular disease (CVD), fatal CVD, or all-cause death cases per 100,000 person-years attributable to a doubling in urinary arsenic levels through (indirect effect) and not through (direct effect) changes in epigenetic age acceleration or pace of aging for each epigenetic aging measure (PhenoAge age acceleration, GrimAge age acceleration, and DunedinPACE), using mediation analysis by the product of coefficients approach as adapted by Lange and Hansen, in the Strong Heart Study (N=2323).

Outcome & Measure	Direct Effect (95% CI)	Indirect Effect (95% CI)	Total Effect (95% CI)	Relative IE (%)
CVD Incidence				
PhenoAge	321 (83, 558)	13.7 (-5.4, 41.3)	335 (96, 573)	4.1 (-2.1, 17.8)
GrimAge	290 (51, 529)	36.7 (10.4, 71.3)	327 (88, 565)	11.2 (2.8, 42.0)
DunedinPACE	291 (54, 528)	45.4 (10.1, 90.8)	337 (96, 577)	13.5 (2.8, 45.6)
CVD Mortality				
PhenoAge	156 (22, 290)	5.5 (-2.8, 19.2)	162 (27, 296)	3.4 (-2.8, 19.4)
GrimAge	134 (-1, 269)	25.0 (7.5, 47.3)	159 (23, 295)	15.7 (3.5, 73.2)
DunedinPACE	144 (9, 279)	17.5 (2.8, 38.3)	162 (26, 297)	10.8 (1.1, 49.9)
Total Mortality				
PhenoAge	590 (334, 844)	35.3 (-14.2, 92.4)	625 (364, 885)	5.6 (-2.5, 15.5)
GrimAge	527 (271, 783)	79.6 (26.5, 139.9)	607 (345, 868)	13.1 (4.5, 26.5)
DunedinPACE	563 (306, 820)	50.6 (11.4, 100.3)	614 (354, 873)	8.3 (1.9, 18.4)

Models adjusted for: eGFR, sex, smoking status (current/former/never), BMI (continuous), center (Arizona, Oklahoma, and North/South Dakota), genetic PCs, LDL cholesterol (continuous), HDL cholesterol (continuous), hypertension treatment (yes/no), systolic blood pressure (continuous), diabetes status, Houseman cell proportions
CVD, cardiovascular disease

Table S3. Interaction p-values between epigenetic aging measures and urinary As for CVD incidence, CVD mortality and all-cause mortality models.

	Epigenetic clock p-value	Interaction p-value*
CVD incidence		
PhenoAge	0.043	0.0037
GrimAge	0.039	0.92
DunedinPACE	0.78	0.073
CVD mortality		
PhenoAge	0.079	0.015
GrimAge	0.32	0.26
DunedinPACE	0.41	0.54
All-cause mortality		
PhenoAge	0.55	0.012
GrimAge	0.092	0.046
DunedinPACE	0.025	0.76

Models adjusted for chronological age, sex, center (Arizona, Oklahoma, and North/South Dakota), five genetic PCs, smoking status (current/former/never) and BMI.
* Interaction p-value between each epigenetic clock and log2 transformed arsenic.
CVD, cardiovascular disease

Table S4. Number of incident cardiovascular disease (CVD), fatal CVD, or all-cause death cases per 100,000 person-years attributable to a doubling in urinary arsenic levels through (indirect effect) and not through (direct effect) changes in PhenoAge epigenetic age acceleration, considering an interaction between arsenic and PhenoAge.

	Natural direct effect	Natural indirect effect
Not adjusted for Houseman cell counts		
CVD incidence	161.3	-31.7
CVD mortality	71.6	-16.2
All-cause mortality	494.4	11.5
Adjusted for Houseman cell counts		
CVD incidence	438.9	-12.3
CVD mortality	208.2	-7.7
All-cause mortality	756.9	1.1

Models adjusted for chronological age, sex, center (Arizona, Oklahoma, and North/South Dakota), five genetic PCs, smoking status (current/former/never) and BMI.
CVD, cardiovascular disease

Table S5. Number of incident cardiovascular disease (CVD), fatal CVD, or all-cause death cases per 100,000 person-years attributable to a doubling in urinary arsenic levels through (indirect effect) and not through (direct effect) changes in epigenetic age acceleration for each epigenetic aging measure (Hannum age acceleration, and Horvath age acceleration), using mediation analysis by the product of coefficients approach as adapted by Lange and Hansen, in the Strong Heart Study (N=2323).

Outcome & Measure	Direct Effect (95% CI)	Indirect Effect (95% CI)	Total Effect (95% CI)	Relative IE (%)
CVD Incidence				
Hannum	363 (129, 597)	-3.0 (-22.7, 13.3)	360 (126, 594)	-0.8 (-8.4, 4.4)
Horvath	361 (127, 595)	1.8 (-10.4, 16.9)	363 (129, 596)	0.5 (-3.4, 5.9)
CVD Mortality				
Hannum	171 (47, 295)	-5.1 (-18.7, 3.3)	166 (42, 290)	-3.1 (-18.8, 2.5)
Horvath	169 (44, 293)	-2.3 (-12.2, 4.3)	166 (42, 290)	-1.4 (-10.9, 3.3)
Total Mortality				
Hannum	728 (475, 981)	30.1 (-5.1, 77.4)	758 (502, 1015)	4.0 (-0.7, 10.5)
Horvath	745 (493, 996)	15.1 (-9, 49.3)	760 (506, 1013)	2.0 (-1.2, 6.6)

Models adjusted for: chronological age, eGFR, sex, smoking status (current/former/never), BMI, center (Arizona, Oklahoma, and North/South Dakota), genetic PCs, LDL cholesterol, HDL cholesterol, systolic blood pressure, hypertension treatment, diabetes status
CVD, cardiovascular disease

Table S6. Results of further adjustment of mediation models in Table S5 for CVD risk factors; number of incident cardiovascular disease (CVD), fatal CVD, or all-cause death cases per 100,000 person-years attributable to a doubling in urinary arsenic levels through (indirect effect) and not through (direct effect) changes in epigenetic age acceleration for each epigenetic aging measure (Hannum age acceleration, and Horvath age acceleration), using mediation analysis by the product of coefficients approach as adapted by Lange and Hansen, in the Strong Heart Study (N=2323).

Outcome & Measure	Direct Effect (95% CI)	Indirect Effect (95% CI)	Total Effect (95% CI)	Relative IE (%)
CVD Incidence				
Hannum	361 (119, 603)	-1.0 (-15.4, 11.6)	360 (118, 602)	-0.3 (-5.4, 4.0)
Horvath	361 (119, 602)	-0.6 (-16.5, 14.6)	360 (119, 601)	-0.2 (-5.8, 5.1)
CVD Mortality				
Hannum	177 (43, 310)	-3.3 (-14.7, 3.7)	173 (39, 307)	-1.9 (-13.8, 2.7)
Horvath	175 (41, 309)	-4.3 (-16.9, 3.3)	171 (36, 304)	-2.5 (-16.7, 2.6)
Total Mortality				
Hannum	697 (440, 954)	16.2 (-12.6, 53.7)	713 (454, 972)	2.3 (-1.9, 7.8)
Horvath	706 (450, 961)	14.1 (-5.6, 46.1)	720 (463, 977)	2.0 (-0.8, 6.6)

Models adjusted for: chronological age, eGFR, sex, smoking status (current/former/never), BMI, center (Arizona, Oklahoma, and North/South Dakota), genetic PCs, LDL cholesterol, HDL cholesterol, systolic blood pressure, hypertension treatment, diabetes status
CVD, cardiovascular disease

Table S7. Hazard ratios (95% CI) for CVD incidence, CVD mortality, and all-cause mortality comparing the interquartile ranges of epigenetic aging measures (PhenoAge age acceleration, GrimAge age acceleration, and DunedinPACE), using Cox proportional-hazards models in the Strong Heart Study (N=2323, missing=0).

	Model 1		Model 2		Model 3		Model 4	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
CVD Incidence								
PhenoAge	1.11 (1.02, 1.21)	0.01	1.10 (1.01, 1.20)	0.03	1.07 (0.98, 1.17)	0.11	1.10 (1.00, 1.21)	0.04
GrimAge	1.32 (1.21, 1.45)	2.07E-9	1.34 (1.20, 1.48)	3.86E-8	1.29 (1.16, 1.43)	1.72E-6	1.33 (1.19, 1.48)	8.35E-7
DunedinPACE	1.39 (1.28, 1.51)	1.19E-14	1.31 (1.20, 1.43)	1.69E-9	1.24 (1.13, 1.36)	3.39E-6	1.32 (1.19, 1.46)	1.08E-7
CVD Mortality								
PhenoAge	1.25 (1.07, 1.46)	0.004	1.23 (1.06, 1.44)	0.008	1.16 (1.00, 1.36)	0.05	1.11 (0.94, 1.31)	0.20
GrimAge	1.70 (1.45, 2.00)	1.44E-10	1.81 (1.51, 2.17)	1.37E-10	1.78 (1.48, 2.14)	7.25E-10	1.77 (1.45, 2.16)	1.46E-8
DunedinPACE	1.60 (1.38, 1.86)	5.03E-10	1.53 (1.31, 1.78)	8.86E-8	1.46 (1.24, 1.72)	5.14E-6	1.48 (1.24, 1.76)	1.69E-5
Total Mortality								
PhenoAge	1.48 (1.36, 1.61)	<2.22E-16	1.47 (1.35, 1.60)	<2.22E-16	1.39 (1.28, 1.52)	3.21E-14	1.31 (1.20, 1.44)	2.82E-9
GrimAge	1.80 (1.65, 1.97)	<2.22E-16	1.86 (1.68, 2.05)	<2.22E-16	1.79 (1.62, 1.98)	<2.22E-16	1.71 (1.53, 1.90)	<2.22E-16
DunedinPACE	1.55 (1.43, 1.68)	<2.22E-16	1.53 (1.41, 1.67)	<2.22E-16	1.41 (1.30, 1.55)	1.11E-14	1.36 (1.24, 1.50)	2.54E-10

Model 1: adjusted for chronological age, sex, center (Arizona, Oklahoma, and North Dakota and South Dakota), genetic PCs

Model 2: additionally adjusted for smoking status (current/former/never), BMI

Model 3: additionally adjusted for eGFR, LDL cholesterol, HDL cholesterol, systolic blood pressure, hypertension treatment, diabetes status

Model 4: additionally adjusted for Houseman cell proportions

HR, hazard ratio; CI, confidence interval; CVD, cardiovascular disease

Table S8. Hazard ratios (95% CI) for CVD incidence, CVD mortality, and all-cause mortality comparing the interquartile ranges of epigenetic aging measures (Hannum age acceleration and Horvath age acceleration), using Cox proportional-hazards models in the Strong Heart Study (N=2323, missing=0).

	Model 1		Model 2		Model 3		Model 4	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
CVD Incidence								
Hannum	1.00, (0.92, 1.08)	0.97	0.99, (0.91, 1.07)	0.79	1.01, (0.93, 1.09)	0.90	1.02, (0.93, 1.11)	0.70
Horvath	1.04, (0.96, 1.12)	0.35	1.03, (0.95, 1.11)	0.46	1.02, (0.95, 1.10)	0.55	1.02, (0.95, 1.11)	0.55
CVD Mortality								
Hannum	0.96, (0.84, 1.11)	0.61	0.96, (0.83, 1.11)	0.57	0.96, (0.83, 1.10)	0.55	0.94, (0.81, 1.09)	0.43
Horvath	0.99, (0.86, 1.13)	0.86	0.98, (0.85, 1.13)	0.78	0.96, (0.84, 1.10)	0.55	0.96, (0.84, 1.11)	0.59
Total Mortality								
Hannum	1.19, (1.10, 1.28)	3.85E-6	1.19, (1.11, 1.28)	3.16E-6	1.15, (1.07, 1.24)	1.75E-04	1.12, (1.03, 1.21)	0.006
Horvath	1.13, (1.05, 1.22)	0.002	1.14, (1.06, 1.23)	7.25E-4	1.11, (1.03, 1.20)	0.007	1.10, (1.02, 1.19)	0.02

Model 1: adjusted for chronological age, sex, center (Arizona, Oklahoma, and North/South Dakota), five genetic PCs

Model 2: additionally adjusted for smoking status (current/former/never), BMI

Model 3: additionally adjusted for eGFR, LDL cholesterol, HDL cholesterol, systolic blood pressure, hypertension treatment, diabetes status

Model 4: additionally adjusted for Houseman cell proportions

HR, hazard ratio; CI, confidence interval; CVD, cardiovascular disease

Table S9. Subdistribution hazard ratios obtained from the Fine Gray model for the competing risks by death analysis for CVD incidence and mortality.

	HR (95% CI)	P-value
CVD incidence		
PhenoAge	1.04 (0.93, 1.15)	0.51
GrimAge	1.16 (1.02, 1.32)	0.034
DunedinPACE	1.25 (1.12, 1.39)	0.0001
CVD mortality		
PhenoAge	1.02 (0.86, 1.20)	0.83
GrimAge	1.49 (1.22, 1.82)	9.61E-05
DunedinPACE	1.34 (1.12, 1.61)	0.0019

Models adjusted for chronological age, sex, center (Arizona, Oklahoma, and North/South Dakota), five genetic PCs, smoking status (current/former/never), BMI, eGFR, LDL cholesterol, HDL cholesterol, systolic blood pressure, hypertension treatment, diabetes status and Houseman cell proportions.

Figure S1. Forest plot of epigenetic age distributions (Hannun, Horvath, and PhenoAge) and pace of aging (DunedinPACE, years of biological aging/chronological years), by participants' baseline characteristics. (N=2323, missing=0). Corresponding data is in Excel Table S3.

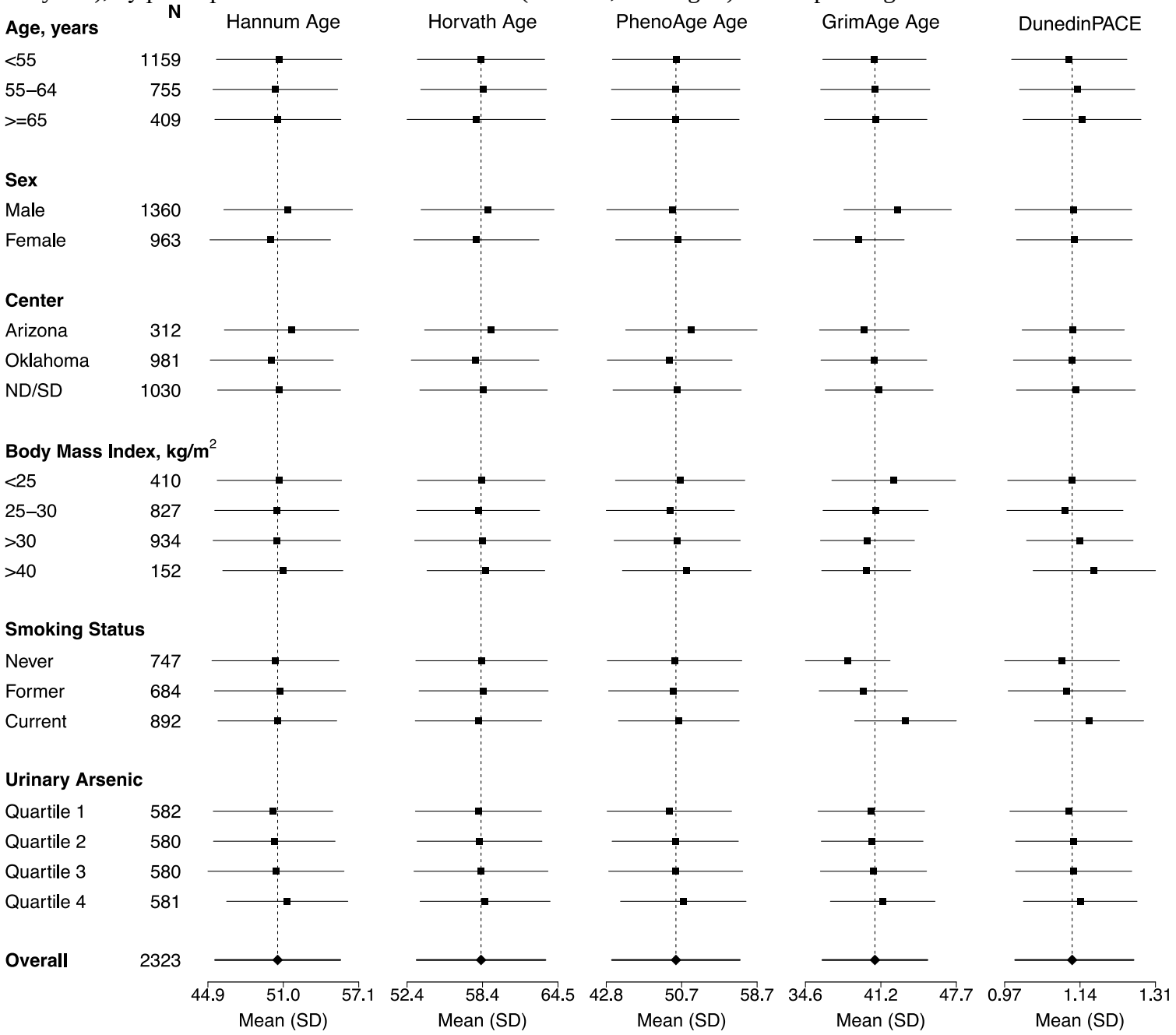

Figure S2. Simplified directed acyclic graph depicting the relationships between arsenic exposure, epigenetic age acceleration, cardiovascular disease, and other mediating variables. For simplicity, we are not showing the arrow between CVD risk factors and DNA-methylation based metrics of biological aging but it is well-established that CVD risk factors can also accelerate biological aging. Also for simplicity we are not showing the arrows between age, sex, and study center and other variables, including cardiovascular disease, and other covariates.