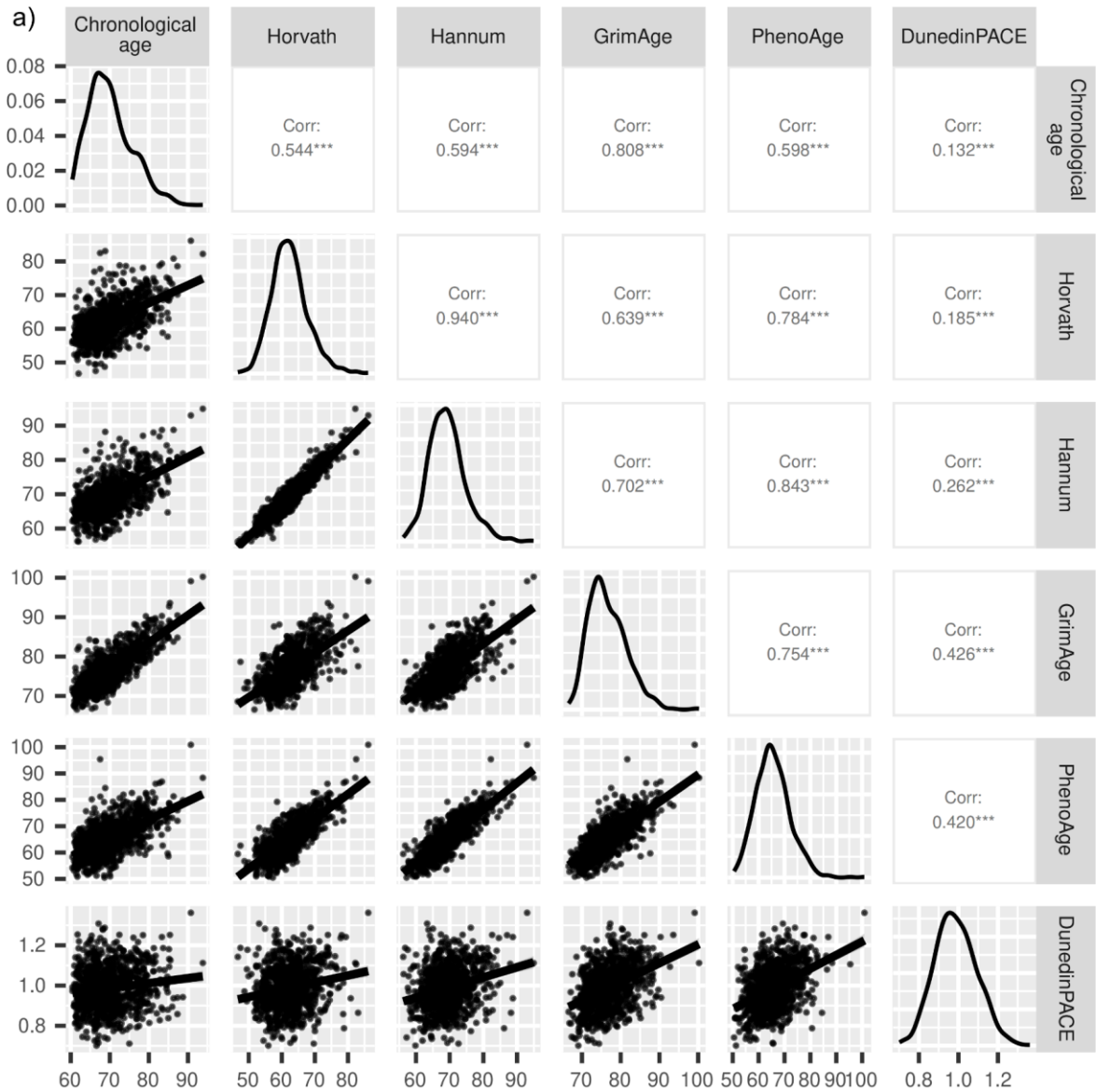


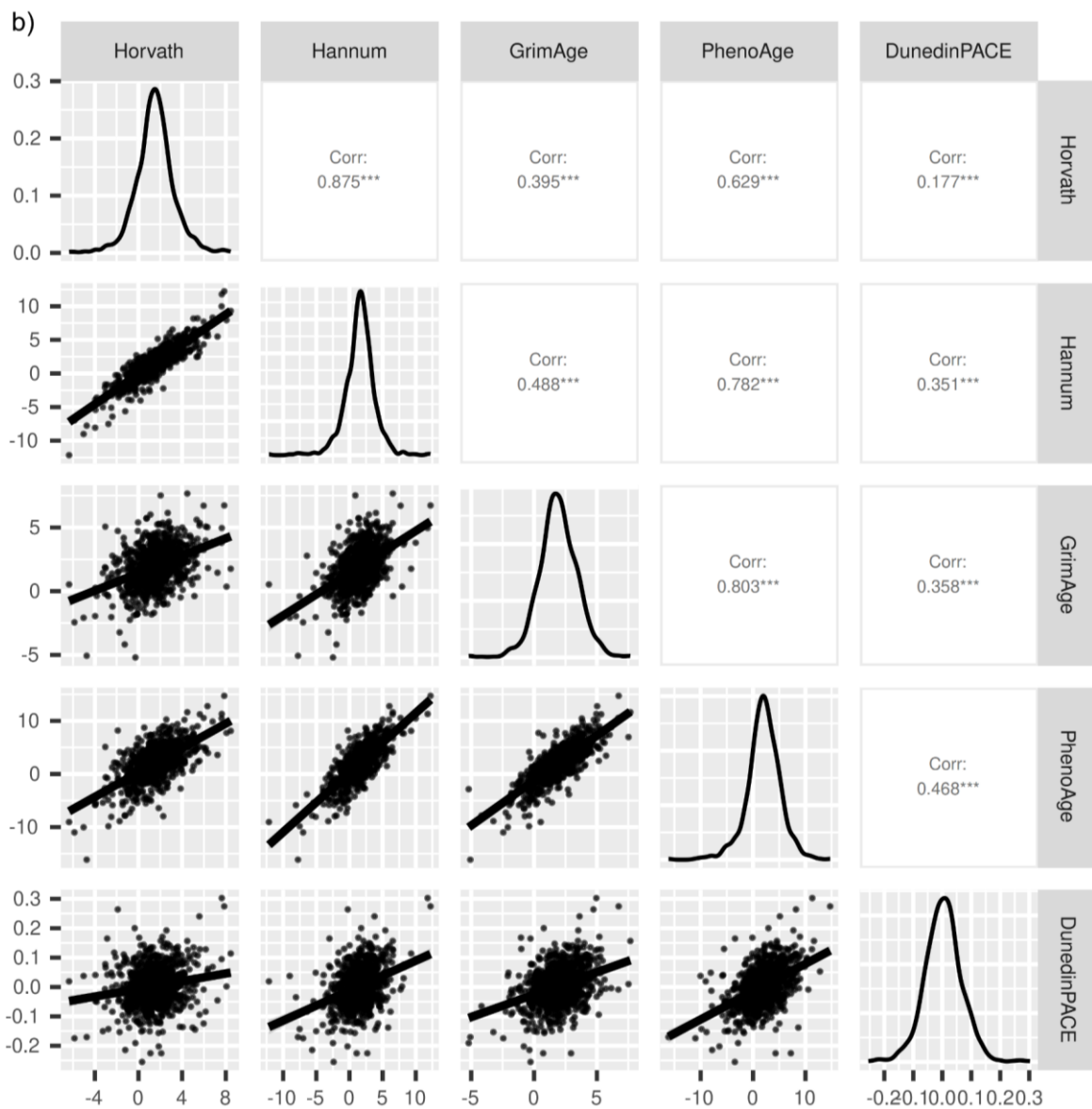
Effects of daily multivitamin–multimineral and cocoa extract supplementation on epigenetic aging clocks in the COSMOS randomized clinical trial

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Supplementary Figure 1. Correlation between epigenetic clocks of biological aging

a, Spearman correlation between epigenetic clocks of biological aging at baseline. b, Spearman Correlation between 2-year changes in epigenetic clocks of biological aging. The line represents the linear regression fit, and each point represents an individual, with a total of n = 958. Pearson's r correlation coefficient and the corresponding nominal P value are shown in each panel.





Supplementary Table 1. Mean values and between-group differences of change in epigenetic clocks of biological aging from baseline to year 1 and year 2 using Mixed Models for Repeated Measures (Figures 2-3 Source Data)

Age Clock	Year	Change from baseline		Difference (Active versus placebo)	Cohen's <i>d</i>	P value
		Placebo	Active			
Multivitamin						
PCHorvath1	1	0.952 (0.747, 1.157)	0.767 (0.562, 0.972)	-0.185 (-0.397, 0.027)	-0.034 (-0.074, 0.006)	0.087
	2	1.635 (1.422, 1.848)	1.596 (1.383, 1.808)	-0.039 (-0.265, 0.187)	-0.007 (-0.05, 0.036)	0.734
PCHannum	1	1.003 (0.745, 1.261)	0.739 (0.482, 0.997)	-0.264 (-0.53, 0.003)	-0.052 (-0.106, 0.001)	0.053
	2	1.75 (1.482, 2.019)	1.619 (1.35, 1.887)	-0.132 (-0.418, 0.155)	-0.026 (-0.083, 0.031)	0.368
PCPhenoAge	1	1.362 (1.007, 1.717)	1.011 (0.657, 1.365)	-0.351 (-0.718, 0.016)	-0.063 (-0.129, 0.003)	0.061
	2	2.414 (2.039, 2.789)	1.971 (1.596, 2.346)	-0.443 (-0.848, -0.037)	-0.08 (-0.153, -0.006)	0.033
PCGrimAge	1	1.117 (0.953, 1.281)	0.985 (0.822, 1.149)	-0.132 (-0.302, 0.038)	-0.04 (-0.091, 0.011)	0.128
	2	2.046 (1.872, 2.219)	1.837 (1.663, 2.01)	-0.209 (-0.397, -0.021)	-0.062 (-0.118, -0.005)	0.030
DunedinPACE	1	0.004 (-0.003, 0.011)	0 (-0.008, 0.007)	-0.004 (-0.012, 0.003)	-0.042 (-0.116, 0.032)	0.267
	2	0.007 (-0.001, 0.014)	0.001 (-0.006, 0.009)	-0.006 (-0.014, 0.002)	-0.054 (-0.13, 0.022)	0.161
Cocoa extract						
PCHorvath1	1	0.805 (0.594, 1.017)	0.916 (0.717, 1.115)	0.111 (-0.102, 0.323)	0.019 (-0.021, 0.059)	0.309
	2	1.526 (1.306, 1.745)	1.701 (1.494, 1.908)	0.175 (-0.052, 0.403)	0.034 (-0.009, 0.077)	0.131
PCHannum	1	0.846 (0.58, 1.111)	0.899 (0.649, 1.15)	0.054 (-0.214, 0.321)	0.009 (-0.044, 0.063)	0.695
	2	1.62 (1.343, 1.897)	1.745 (1.484, 2.005)	0.124 (-0.164, 0.412)	0.025 (-0.033, 0.082)	0.398
PCPhenoAge	1	1.11 (0.744, 1.475)	1.271 (0.927, 1.616)	0.162 (-0.207, 0.531)	0.028 (-0.039, 0.094)	0.390
	2	2.004 (1.617, 2.391)	2.371 (2.007, 2.735)	0.367 (-0.04, 0.774)	0.066 (-0.008, 0.139)	0.078
PCGrimAge	1	1.001 (0.832, 1.169)	1.104 (0.945, 1.263)	0.104 (-0.067, 0.274)	0.029 (-0.022, 0.08)	0.235
	2	1.858 (1.679, 2.037)	2.022 (1.854, 2.191)	0.164 (-0.025, 0.353)	0.049 (-0.008, 0.106)	0.089
DunedinPACE	1	-0.001 (-0.008, 0.007)	0.004 (-0.003, 0.011)	0.005 (-0.003, 0.013)	0.045 (-0.03, 0.119)	0.238
	2	0.003 (-0.005, 0.01)	0.005 (-0.002, 0.012)	0.002 (-0.006, 0.01)	0.023 (-0.053, 0.099)	0.545

Mixed Models for Repeated Measures included treatment group and time point of follow-up measurements (year 1 and year 2) as fixed effects. Other covariates treated as fixed effects were the baseline biological aging from that clock, the follow-up time point by baseline age clock interaction, the treatment by follow-up time point interaction, chronological age at baseline, sex, recruitment source, and the other randomized arm. Individual-specific effects enter the model as a random effect with a normal distribution and expected value 0. An unstructured covariance matrix for the residuals was used. Cohen's *d* was estimated by scaling the repeated measures of age-difference values (i.e. epigenetic clock age - chronological age) using the standard deviation of age-difference values at baseline for the first two generations of epigenetic clocks; DunedinPACE was directly scaled using the standard deviation at baseline. Statistical significance with two-tailed P values was determined through the interactions of intervention and time in the linear mixed-effect models, without adjustment for multiple comparisons.

Supplementary Table 2. Change in epigenetic clocks of biological aging by Multivitamin-multimineral assignment and baseline biological aging acceleration (Figure 4 Source Data)

	Placebo		Multivitamin		Between-group difference	P value	P _{interaction}
	Raw mean (SD)	Change from baseline	Raw mean (SD)	Change from baseline			
PCHorvath							0.797
Decelerated	N=252		N=250				
Baseline	59.16 (3.70)	-	58.98 (3.97)	-	-		
Year 1	60.19 (4.08)	1.126 (0.847,1.406)	59.77 (4.04)	0.935 (0.656,1.214)	-0.191 (-0.498, 0.115)	0.222	
Year 2	60.74 (3.89)	1.754 (1.475,2.033)	60.65 (4.16)	1.751 (1.472,2.03)	-0.003 (-0.307, 0.302)	0.985	
Yearly change	-	0.803 (0.696,0.91)	-	0.798 (0.667,0.929)	-0.005 (-0.157,0.147)	0.949	
Accelerated	230		N=226				
Baseline	65.96 (4.97)	-	65.88 (4.49)	-	-		
Year 1	66.65 (5.26)	0.789 (0.474,1.103)	66.44 (4.82)	0.617 (0.308,0.925)	-0.172 (-0.463, 0.119)	0.247	
Year 2	67.31 (5.18)	1.533 (1.197,1.869)	67.21 (5.10)	1.463 (1.133,1.794)	-0.069 (-0.406, 0.267)	0.687	
Yearly change	-	0.713 (0.605,0.820)	-	0.678 (0.546,0.811)	-0.034 (-0.187,0.119)	0.661	
PCHorvath AgeDev							0.778
Decelerated	N=252		N=250				
Baseline	-3.58 (2.78)	-	-3.43 (2.70)	-	-		
Year 1	-3.25 (3.08)	0.422 (0.141,0.702)	-3.33 (2.88)	0.229 (-0.050,0.508)	-0.193 (-0.5, 0.114)	0.218	
Year 2	-3.19 (3.05)	0.491 (0.212,0.770)	-3.05 (2.88)	0.490 (0.211,0.769)	-0.002 (-0.306, 0.303)	0.992	
Yearly change	-	0.184 (0.077,0.291)	-	0.181 (0.050,0.312)	-0.003 (-0.154,0.149)	0.972	
Accelerated	N=230		N=226				
Baseline	3.63 (3.05)	-	3.43 (3.36)	-	-		
Year 1	3.63 (3.42)	0.072 (-0.242,0.387)	3.33 (3.68)	-0.096 (-0.404,0.212)	-0.168 (-0.459, 0.123)	0.257	
Year 2	3.79 (3.29)	0.264 (-0.072,0.6)	3.59 (4.02)	0.196 (-0.135,0.527)	-0.068 (-0.404, 0.268)	0.692	
Yearly change	-	0.097 (-0.011,0.204)	-	0.062 (-0.071,0.194)	-0.035 (-0.188,0.119)	0.657	
PCHannum							0.17
Decelerated	N=246		N=247				
Baseline	66.05 (3.63)	-	66.14 (4.06)	-	-		
Year 1	67.06 (4.08)	1.147 (0.79,1.504)	66.93 (4.43)	1.034 (0.688,1.38)	-0.113 (-0.497, 0.271)	0.565	
Year 2	67.85 (3.90)	1.983 (1.624,2.341)	68.08 (4.46)	2.041 (1.693,2.389)	0.059 (-0.326, 0.443)	0.765	
Yearly change	-	0.902 (0.766,1.037)	-	0.934 (0.768,1.1)	0.032 (-0.159,0.224)	0.74	
Accelerated	N=236		N=229				

Baseline	72.86 (5.18)	-	72.51 (4.56)	-	-		
Year 1	73.63 (5.59)	0.861 (0.476,1.245)	72.92 (5.11)	0.463 (0.065,0.861)	-0.398 (-0.764, -0.031)	0.034	
Year 2	74.17 (5.60)	1.518 (1.107,1.929)	73.58 (5.42)	1.216 (0.791,1.64)	-0.303 (-0.725, 0.12)	0.161	
Yearly change	-	0.7 (0.564,0.836)	-	0.54 (0.372,0.708)	-0.16 (-0.355,0.034)	0.107	
PCHannum AgeDev							0.168
Decelerated	N=246		N=247				
Baseline	-3.44 (2.61)	-	-3.28 (2.57)	-	-		
Year 1	-3.19 (2.97)	0.364 (0.006,0.721)	-3.23 (3.13)	0.253 (-0.093,0.599)	-0.111 (-0.495, 0.274)	0.573	
Year 2	-2.94 (2.99)	0.588 (0.229,0.946)	-2.76 (3.04)	0.648 (0.299,0.996)	0.06 (-0.325, 0.445)	0.76	
Yearly change		0.219 (0.083,0.355)		0.253 (0.087,0.419)	0.034 (-0.158,0.225)	0.73	
Accelerated	N=236		N=229				
Baseline	3.46 (3.06)	-	3.32 (3.12)	-	-		
Year 1	3.46 (3.51)	0.076 (-0.308,0.461)	2.96 (3.66)	-0.323 (-0.721,0.075)	-0.399 (-0.766, -0.032)	0.033	
Year 2	3.45 (3.66)	0.123 (-0.288,0.533)	3.09 (3.97)	-0.182 (-0.607,0.242)	-0.305 (-0.726, 0.117)	0.157	
Yearly change	-	0.020 (-0.116,0.156)	-	-0.140 (-0.308,0.028)	-0.16 (-0.354,0.035)	0.108	
PCPhenoAge							0.106
Decelerated	N=249		N=254				
Baseline	61.55 (4.78)	-	61.72 (4.83)	-	-		
Year 1	63.22 (5.25)	1.873 (1.411,2.336)	63.04 (5.46)	1.612 (1.153,2.071)	-0.261 (-0.76, 0.237)	0.304	
Year 2	64.31 (5.11)	2.975 (2.49,3.46)	64.33 (5.69)	2.844 (2.36,3.329)	-0.131 (-0.67, 0.409)	0.635	
Yearly change	-	1.37 (1.181,1.559)	-	1.296 (1.065,1.526)	-0.075 (-0.34, 0.191)	0.582	
Accelerated	N=233		N=222				
Baseline	69.82 (5.81)	-	69.71 (5.41)	-	-		
Year 1	70.57 (6.48)	0.797 (0.238,1.357)	70.03 (6.09)	0.344 (-0.215,0.903)	-0.453 (-0.995, 0.089)	0.102	
Year 2	71.37 (6.67)	1.799 (1.211,2.388)	70.61 (6.58)	1.002 (0.413,1.591)	-0.797 (-1.398, -0.197)	0.010	
Yearly change	-	0.847 (0.651,1.043)	-	0.453 (0.211,0.696)	-0.394 (-0.675, -0.112)	0.006	
PCPhenoAge AgeDev							0.107
Decelerated	N=249		N=254				
Baseline	-4.24 (3.15)	-	-3.98 (3.09)	-	-		
Year 1	-3.47 (3.85)	0.933 (0.47,1.395)	-3.53 (3.75)	0.672 (0.213,1.131)	-0.261 (-0.759, 0.237)	0.305	
Year 2	-3.08 (4.07)	1.300 (0.815,1.785)	-3.02 (3.84)	1.166 (0.682,1.65)	-0.134 (-0.674, 0.406)	0.626	
Yearly change	-	0.553 (0.364,0.741)	-	0.479 (0.249,0.709)	-0.074 (-0.339,0.192)	0.587	
Accelerated	N=233		N=222				
Baseline	4.04 (3.28)	-	4.17 (3.86)	-	-		

Year 1	3.85 (4.14)	-0.140 (-0.7,0.419)	3.56 (4.94)	-0.583 (-1.142,-0.024)	-0.443 (-0.984, 0.099)	0.11	
Year 2	4.06 (4.42)	0.139 (-0.45,0.727)	3.45 (5.38)	-0.660 (-1.249,-0.071)	-0.799 (-1.4, -0.198)	0.009	
Yearly change	-	0.033 (-0.164,0.229)	-	-0.359 (-0.602,-0.116)	-0.392 (-0.674,-0.11)	0.007	
PCGrimAge							0.018
Decelerated	N=269		N=262				
Baseline	74.51 (3.86)	-	74.82 (4.10)	-	-		
Year 1	75.80 (3.85)	1.379 (1.186,1.572)	75.97 (4.26)	1.271 (1.077,1.465)	-0.108 (-0.318, 0.103)	0.317	
Year 2	76.71 (3.99)	2.316 (2.11,2.522)	77.04 (4.36)	2.292 (2.084,2.500)	-0.023 (-0.257, 0.21)	0.844	
Yearly change		1.103 (1.021,1.185)		1.090 (0.989,1.191)	-0.013 (-0.13,0.104)	0.827	
Accelerated	N=213		N=214				
Baseline	79.23 (5.04)	-	78.91 (4.33)	-	-		
Year 1	80.10 (5.22)	0.817 (0.517,1.118)	79.64 (4.46)	0.661 (0.364,0.958)	-0.156 (-0.434, 0.121)	0.271	
Year 2	80.91 (5.17)	1.736 (1.422,2.051)	80.21 (4.71)	1.298 (0.987,1.609)	-0.439 (-0.746, -0.131)	0.005	
Yearly change	-	0.906 (0.804,1.009)		0.671 (0.545,0.796)	-0.236 (-0.38,-0.091)	0.001	
PCGrimAge AgeDev							0.019
Decelerated	N=269		N=262				
Baseline	-2.26 (1.59)	-	-2.13 (1.43)	-	-		
Year 1	-1.88 (1.71)	0.459 (0.267,0.652)	-1.84 (1.69)	0.351 (0.158,0.543)	-0.109 (-0.318, 0.101)	0.309	
Year 2	-1.65 (1.89)	0.675 (0.470,0.880)	-1.54 (1.77)	0.652 (0.445,0.859)	-0.023 (-0.256, 0.21)	0.85	
Yearly change	-	0.302 (0.220,0.384)	-	0.290 (0.189,0.390)	-0.012 (-0.129,0.104)	0.833	
Accelerated	N=213		N=214				
Baseline	2.21 (1.80)	-	2.46 (2.18)	-	-		
Year 1	2.20 (2.15)	-0.112 (-0.415,0.190)	2.29 (2.48)	-0.259 (-0.558,0.040)	-0.147 (-0.426, 0.132)	0.303	
Year 2	2.42 (2.16)	0.097 (-0.219,0.413)	2.21 (2.69)	-0.347 (-0.659,-0.035)	-0.444 (-0.752, -0.136)	0.005	
Yearly change	-	0.107 (0.004,0.209)	-	-0.127 (-0.253,-0.001)	-0.233 (-0.379,-0.088)	0.002	
DunedinPACE							0.122
Decelerated	N=270		N=274				
Baseline	0.913 (0.061)	-	0.913 (0.061)	-	-		
Year 1	0.927 (0.080)	0.009 (-0.001,0.018)	0.927 (0.079)	0.010 (0,0.019)	0.001 (-0.009, 0.011)	0.846	
Year 2	0.928 (0.075)	0.011 (0.001,0.02)	0.928 (0.071)	0.011 (0.001,0.020)	0 (-0.010, 0.010)	0.96	
Yearly change	-	0.008 (0.004,0.012)	-	0.008 (0.003,0.012)	0 (-0.005,0.005)	0.913	
Accelerated	N=212		N=202				
Baseline	1.081 (0.064)	-	1.088 (0.064)	-	-		
Year 1	1.071 (0.080)	-0.003 (-0.015,0.008)	1.066 (0.089)	-0.014 (-0.026,-0.002)	-0.011 (-0.023, 0.002)	0.087	

Year 2	1.076 (0.079)	0 (-0.012,0.012)	1.067 (0.085)	-0.012 (-0.024,0)	-0.012 (-0.025, 0.001)	0.066	
Yearly change	-	-0.003 (-0.008,0.001)		-0.010 (-0.016,-0.005)	-0.007 (-0.013,0)	0.042	

AgeDev denotes age deviation, which was estimated by residualizing epigenetic clocks of biological aging with respect to chronological age.

Accelerated DNAm age was defined as participants with corresponding age deviation residuals>0 at baseline for the first two generations of epigenetic clocks. For DunedinPACE, accelerated DNAm age was defined as participants with DunedinPACE >1 at baseline.

Yearly changes in epigenetic clocks of biological aging and their between-group differences were estimated using linear mixed-effect models including treatment, follow-up time (0, 1, 2), and their interaction term and adjusting for chronological age at baseline, sex, recruitment source, baseline biological aging measures from that clock, the other randomized assignment, and a random intercept of each individual. Statistical significance for the consistency between subgroups, as determined by two-tailed P values, was assessed through the three-way interactions of intervention, time, and the stratified variables, without adjustment for multiple comparisons.

Changes from baseline to year 1 and year 2 were estimated using mixed models for repeated measures including treatment group, time point of follow-up measurements (year 1 and year2), and their interactions as fixed effects and also adjusting for baseline biological aging from that clock, the follow-up time point by baseline age clock interaction, chronological age at baseline, sex, recruitment source, the other randomized arm, and a random intercept of each individual.