

Abbreviations List:

PA: Physical Activity

DNAm: DNA Methylation

BMI: Body Mass Index

PIR: Poverty Income Ratio

HorvathAge: Horvath's Epigenetic Age

HannumAge: Hannum's Epigenetic Age

SkinBloodAge: Skin and Blood Epigenetic Age

LinAge: Lin's Epigenetic Age

WeidnerAge: Weidner's Epigenetic Age

VidalBravoAge: Vidal-Bravo Epigenetic Age

ZhangAge: Zhang's Epigenetic Age

PhenoAge: Phenotypic Age

For HorvathAge (Table S1), PA was significantly inversely associated in both males ($\beta = -0.01$, 95% CI: -0.015 to -0.004) and females ($\beta = -0.009$, 95% CI: -0.015 to -0.004). This association was particularly strong in non-Hispanic whites ($\beta = -0.013$, 95% CI: -0.017 to -0.008) and participants with a poverty income ratio (PIR) of 1–3 ($\beta = -0.016$, 95% CI: -0.024 to -0.008), as well as those with a BMI between 25 and 30 ($\beta = -0.012$, 95% CI: -0.022 to -0.003) and former smokers ($\beta = -0.01$, 95% CI: -0.018 to -0.001).

Table S1. Subgroup analysis of the association between physical activity and DNA methylation-predicted HorvathAge

Subgroups	β (95% CI)	p-value	p for interaction
Gender			0.898
Male	-0.01(-0.015,-0.004)	<0.001	
Female	-0.009(-0.015,-0.004)	<0.001	
Race/ethnicity			< 0.001
Non-hispanic White	-0.013(-0.017,-0.008)	<0.0001	
Non-hispanic Black	0.004(-0.004,0.013)	0.298	
Mexican American	-0.001(-0.011,0.010)	0.902	
Other race/ethnicity	0.003(-0.008,0.014)	0.446	
Marital status			0.059
Never married	0.006(-Inf,Inf)	0.296	
Married/living with partner	-0.009(-0.013,-0.004)	<0.001	
Widowed/ divorced	-0.016(-0.030,-0.002)	0.029	
Poverty income ratio			0.021
< 1	0(-0.006,0.007)	0.923	
[1,3)	-0.016(-0.024,-0.008)	<0.001	
≥ 3	-0.007(-0.012,-0.002)	0.012	
Education			0.798
Below high school	-0.008(-0.033,0.016)	0.491	
High school	-0.01(-0.018,-0.003)	0.006	
College or above	-0.007(-0.016,0.002)	0.107	
BMI (kg/m ²)			0.676
< 25	-0.006(-0.017,0.004)	0.209	
[25, 30)	-0.012(-0.022,-0.003)	0.010	
≥ 30	-0.009(-0.018,-0.001)	0.029	
Smoking status			0.916
Never smoking	-0.008(-0.016,0.001)	0.079	
Former smoking	-0.01(-0.018,-0.001)	0.032	
Current smoking	-0.01(-0.018,-0.002)	0.021	
Alcohol use status			0.075
None	-0.009(-0.016,-0.002)	0.010	
Moderate alcohol use	-0.012(-0.018,-0.005)	0.001	
High alcohol use	0.005(-0.008,0.017)	0.447	

Similarly, for HannumAge (Table S2), PA was associated with lower age predictions in both males ($\beta = -0.007$, 95% CI: -0.013 to -0.001) and females ($\beta = -0.01$, 95% CI: -0.018 to -0.001). Non-Hispanic whites ($\beta = -0.011$, 95% CI: -0.017 to -0.005), participants with a PIR of 1–3 ($\beta = -0.016$, 95% CI: -0.023 to -0.008), and moderate alcohol consumers ($\beta = -0.01$, 95% CI: -0.017 to -0.002) demonstrated particularly strong negative associations with HannumAge.

Table S2. Subgroup analysis of the association between physical activity and DNA methylation-predicted HannumAge.

Subgroups	β (95% CI)	p-value	p for interaction
Gender			0.452
Male	-0.007(-0.013,-0.001)	0.023	
Female	-0.01(-0.018,-0.001)	0.023	
Race/ethnicity			< 0.0001
Non-hispanic White	-0.011(-0.017,-0.005)	<0.001	
Non-hispanic Black	0.007(-0.002,0.017)	0.116	
Mexican American	-0.002(-0.010,0.006)	0.587	
Other race/ethnicity	0.006(-0.006,0.018)	0.211	
Marital status			0.015
Never married	0.011(-Inf,Inf)	0.176	
Married/living with partner	-0.007(-0.012,-0.002)	0.009	
Widowed/ divorced	-0.013(-0.026,-0.001)	0.042	
Poverty income ratio			0.003
< 1	0.002(-0.006,0.010)	0.577	
[1,3)	-0.016(-0.023,-0.008)	<0.001	
≥ 3	-0.003(-0.010,0.003)	0.289	
Education			0.675
Below high school	0.003(-0.024,0.029)	0.816	
High school	-0.008(-0.017,0.000)	0.049	
College or above	-0.005(-0.014,0.004)	0.280	
BMI (kg/m ²)			0.834
< 25	-0.006(-0.019,0.006)	0.326	
[25, 30)	-0.01(-0.021,0.001)	0.065	
≥ 30	-0.005(-0.016,0.006)	0.333	
Smoking status			0.962
Never smoking	-0.006(-0.016,0.004)	0.229	
Former smoking	-0.008(-0.017,0.002)	0.126	
Current smoking	-0.007(-0.017,0.002)	0.106	
Alcohol use status			0.182
None	-0.007(-0.015,0.002)	0.115	
Moderate alcohol use	-0.01(-0.017,-0.002)	0.013	
High alcohol use	0.005(-0.008,0.017)	0.434	

In the case of SkinBloodAge (Table S3), PA was significantly associated with lower predicted ages in both males ($\beta = -0.01$, 95% CI: -0.015 to -0.004) and females ($\beta = -0.009$, 95% CI: -0.015 to -0.004). The effect was most pronounced in non-Hispanic whites ($\beta = -0.013$, 95% CI: -0.017 to -0.008) and participants with a PIR of 1–3 ($\beta = -0.016$, 95% CI: -0.024 to -0.008). Notably, those with a BMI between 25 and 30 also showed a significant reduction in SkinBloodAge ($\beta = -0.012$, 95% CI: -0.022 to -0.003).

Table S3. Subgroup analysis of the association between physical activity and DNA methylation-predicted SkinBloodAge.

Subgroups	β (95% CI)	p-value	p for interaction
Gender			0.898
Male	-0.01(-0.015,-0.004)	<0.001	
Female	-0.009(-0.015,-0.004)	<0.001	
Race/ethnicity			< 0.0001
Non-hispanic White	-0.013(-0.017,-0.008)	<0.0001	
Non-hispanic Black	0.004(-0.004,0.013)	0.298	
Mexican American	-0.001(-0.011,0.010)	0.902	
Other race/ethnicity	0.003(-0.008,0.014)	0.446	
Marital status			0.059
Never married	0.006(-Inf,Inf)	0.296	
Married/living with partner	-0.009(-0.013,-0.004)	<0.001	
Widowed/ divorced	-0.016(-0.030,-0.002)	0.029	
Poverty income ratio			0.021
< 1	0(-0.006,0.007)	0.923	
[1,3)	-0.016(-0.024,-0.008)	<0.001	
≥ 3	-0.007(-0.012,-0.002)	0.012	
Education			0.798
Below high school	-0.008(-0.033,0.016)	0.491	
High school	-0.01(-0.018,-0.003)	0.006	
College or above	-0.007(-0.016,0.002)	0.107	
BMI (kg/m ²)			0.676
< 25	-0.006(-0.017,0.004)	0.209	
[25, 30)	-0.012(-0.022,-0.003)	0.010	
≥ 30	-0.009(-0.018,-0.001)	0.029	
Smoking status			0.916
Never smoking	-0.008(-0.016,0.001)	0.079	
Former smoking	-0.01(-0.018,-0.001)	0.032	
Current smoking	-0.01(-0.018,-0.002)	0.021	
Alcohol use status			0.075
None	-0.009(-0.016,-0.002)	0.010	
Moderate alcohol use	-0.012(-0.018,-0.005)	0.001	
High alcohol use	0.005(-0.008,0.017)	0.447	

For LinAge (Table S4), PA was negatively associated with predicted age in both males ($\beta = -0.011$, 95% CI: -0.018 to -0.004, $p = 0.005$) and females ($\beta = -0.016$, 95% CI: -0.023 to -0.008, $p < 0.001$). Non-Hispanic whites ($\beta = -0.015$, 95% CI: -0.022 to -0.008) and those with a BMI of 30 or greater ($\beta = -0.016$, 95% CI: -0.026 to -0.005) exhibited significant reductions in LinAge.

Table S4. Subgroup analysis of the association between physical activity and DNA methylation-predicted LinAge.

Subgroups	β (95% CI)	p-value	p for interaction
Gender			0.312
Male	-0.011(-0.018,-0.004)	0.005	
Female	-0.016(-0.023,-0.008)	<0.001	
Race/ethnicity			0.023
Non-hispanic White	-0.015(-0.022,-0.008)	<0.001	
Non-hispanic Black	0.002(-0.009,0.013)	0.712	
Mexican American	-0.004(-0.015,0.007)	0.434	
Other race/ethnicity	-0.006(-0.020,0.008)	0.320	
Marital status			0.387
Never married	-0.009(-Inf,Inf)	0.256	
Married/living with partner	-0.01(-0.017,-0.004)	0.004	
Widowed/ divorced	-0.019(-0.036,-0.001)	0.037	
Poverty income ratio			0.002
< 1	-0.009(-0.024,0.006)	0.200	
[1,3)	-0.026(-0.035,-0.018)	<0.0001	
≥ 3	-0.004(-0.013,0.005)	0.407	
Education			0.127
Below high school	-0.008(-0.041,0.025)	0.609	
High school	-0.016(-0.024,-0.008)	<0.001	
College or above	-0.004(-0.013,0.006)	0.403	
BMI (kg/m ²)			0.724
< 25	-0.012(-0.025,0.000)	0.043	
[25, 30)	-0.009(-0.019,0.001)	0.063	
≥ 30	-0.016(-0.026,-0.005)	0.006	
Smoking status			0.237
Never smoking	-0.006(-0.015,0.002)	0.147	
Former smoking	-0.015(-0.023,-0.008)	<0.001	
Current smoking	-0.013(-0.025,-0.002)	0.023	
Alcohol use status			0.612
None	-0.014(-0.025,-0.003)	0.017	
Moderate alcohol use	-0.012(-0.021,-0.003)	0.009	
High alcohol use	-0.002(-0.027,0.023)	0.855	

For WeidnerAge (Table S5), a similar trend was observed, with significant inverse associations between PA and age predictions in males ($\beta = -0.01$, 95% CI: -0.017 to -0.002) and non-Hispanic whites ($\beta = -0.013$, 95% CI: -0.020 to -0.005). Additionally, participants with a PIR of 1–3 ($\beta = -0.016$, 95% CI: -0.028 to -0.004) and current smokers ($\beta = -0.017$, 95% CI: -0.027 to -0.007) exhibited notable reductions in WeidnerAge.

Table S5. Subgroup analysis of the association between physical activity and DNA methylation-predicted WeidnerAge.

Subgroups	β (95% CI)	p-value	p for interaction
Gender			0.663
Male	-0.01(-0.017,-0.002)	0.013	
Female	-0.006(-0.021,0.008)	0.377	
Race/ethnicity			< 0.001
Non-hispanic White	-0.013(-0.020,-0.005)	0.002	
Non-hispanic Black	-0.006(-0.015,0.004)	0.218	
Mexican American	0(-0.019,0.020)	0.980	
Other race/ethnicity	0.012(0.002,0.022)	0.027	
Marital status			0.207
Never married	0.011(-Inf,Inf)	0.268	
Married/living with partner	-0.01(-0.016,-0.004)	0.002	
Widowed/ divorced	-0.012(-0.037,0.013)	0.336	
Poverty income ratio			0.051
< 1	0.003(-0.004,0.009)	0.439	
[1,3)	-0.016(-0.028,-0.004)	0.010	
≥ 3	-0.006(-0.014,0.002)	0.118	
Education			0.798
Below high school	-0.018(-0.044,0.007)	0.139	
High school	-0.008(-0.018,0.001)	0.084	
College or above	-0.008(-0.020,0.004)	0.204	
BMI (kg/m ²)			0.52
< 25	-0.005(-0.022,0.013)	0.577	
[25, 30)	-0.008(-0.018,0.002)	0.124	
≥ 30	-0.014(-0.024,-0.004)	0.008	
Smoking status			0.084
Never smoking	0(-0.010,0.010)	0.995	
Former smoking	-0.01(-0.022,0.001)	0.079	
Current smoking	-0.017(-0.027,-0.007)	0.002	
Alcohol use status			0.857
None	-0.006(-0.019,0.007)	0.350	
Moderate alcohol use	-0.01(-0.020,0.000)	0.044	
High alcohol use	-0.008(-0.031,0.015)	0.459	

PA also had a significant effect on VidalBraloAge (Table S6), particularly in males ($\beta = -0.005$, 95% CI: -0.009 to 0.000) and non-Hispanic whites ($\beta = -0.007$, 95% CI: -0.012 to -0.003). Individuals with a high school education ($\beta = -0.007$, 95% CI: -0.012 to -0.001) and a BMI of 30 or greater ($\beta = -0.008$, 95% CI: -0.014 to -0.003) also showed significant reductions in predicted age. Table S6. Subgroup analysis of the association between physical activity and DNA methylation-predicted VidalBraloAge.

Subgroups	β (95% CI)	p-value	p for interaction
Gender			0.495
Male	-0.005(-0.009,0.000)	0.039	
Female	-0.007(-0.015,0.000)	0.051	
Race/ethnicity			0.017
Non-hispanic White	-0.007(-0.012,-0.003)	0.003	
Non-hispanic Black	0.001(-0.007,0.009)	0.734	
Mexican American	-0.002(-0.012,0.008)	0.719	
Other race/ethnicity	0.002(-0.004,0.007)	0.474	
Marital status			0.381
Never married	0(-Inf,Inf)	0.885	
Married/living with partner	-0.006(-0.010,-0.002)	0.002	
Widowed/ divorced	-0.003(-0.015,0.009)	0.660	
Poverty income ratio			0.224
< 1	-0.001(-0.008,0.007)	0.857	
[1,3)	-0.009(-0.017,-0.001)	0.032	
≥ 3	-0.004(-0.008,0.001)	0.115	
Education			0.119
Below high school	-0.016(-0.034,0.001)	0.066	
High school	-0.007(-0.012,-0.001)	0.015	
College or above	-0.001(-0.007,0.006)	0.850	
BMI (kg/m ²)			0.422
< 25	-0.005(-0.012,0.003)	0.200	
[25, 30)	-0.003(-0.009,0.003)	0.383	
≥ 30	-0.008(-0.014,-0.003)	0.005	
Smoking status			0.799
Never smoking	-0.004(-0.009,0.001)	0.085	
Former smoking	-0.006(-0.012,0.000)	0.037	
Current smoking	-0.005(-0.011,0.002)	0.149	
Alcohol use status			0.043
None	-0.008(-0.014,-0.002)	0.009	
Moderate alcohol use	-0.004(-0.010,0.002)	0.166	
High alcohol use	0.005(-0.002,0.011)	0.147	

For ZhangAge (Table S7), PA was inversely associated with predicted age in both males ($\beta = -0.003$, 95% CI: -0.006 to -0.001) and females ($\beta = -0.004$, 95% CI: -0.006 to -0.001). Non-Hispanic whites ($\beta = -0.005$, 95% CI: -0.007 to -0.003) and individuals with a BMI between 25 and 30 ($\beta = -0.005$, 95% CI: -0.009 to -0.002) showed strong negative associations with ZhangAge.

Table S7. Subgroup analysis of the association between physical activity and DNA methylation-predicted ZhangAge.

Subgroups	β (95% CI)	p-value	p for interaction
Gender			0.72
Male	-0.003(-0.006,-0.001)	0.010	
Female	-0.004(-0.006,-0.001)	0.004	
Race/ethnicity			< 0.001
Non-hispanic White	-0.005(-0.007,-0.003)	<0.001	
Non-hispanic Black	0.002(-0.001,0.006)	0.191	
Mexican American	-0.002(-0.005,0.002)	0.351	
Other race/ethnicity	0.001(-0.004,0.005)	0.635	
Marital status			0.033
Never married	0.002(-Inf,Inf)	0.337	
Married/living with partner	-0.003(-0.005,-0.001)	0.004	
Widowed/ divorced	-0.006(-0.011,-0.001)	0.014	
Poverty income ratio			0.015
< 1	-0.001(-0.003,0.002)	0.697	
[1,3)	-0.007(-0.011,-0.003)	<0.001	
≥ 3	-0.002(-0.004,0.001)	0.137	
Education			0.649
Below high school	-0.004(-0.013,0.005)	0.383	
High school	-0.004(-0.008,-0.001)	0.019	
College or above	-0.002(-0.006,0.001)	0.228	
BMI (kg/m ²)			0.536
< 25	-0.002(-0.006,0.002)	0.261	
[25, 30)	-0.005(-0.009,-0.002)	0.004	
≥ 30	-0.003(-0.008,0.002)	0.223	
Smoking status			0.715
Never smoking	-0.002(-0.006,0.002)	0.230	
Former smoking	-0.004(-0.007,0.000)	0.036	
Current smoking	-0.004(-0.008,-0.001)	0.010	
Alcohol use status			0.127
None	-0.004(-0.007,-0.001)	0.024	
Moderate alcohol use	-0.004(-0.007,-0.001)	0.006	
High alcohol use	0.002(-0.003,0.007)	0.412	

Finally, for PhenoAge (Table S8), PA was associated with lower predicted ages in both males ($\beta = -0.008$, 95% CI: -0.013 to -0.003) and females ($\beta = -0.009$, 95% CI: -0.018 to -0.001). Significant reductions were observed in non-Hispanic whites ($\beta = -0.012$, 95% CI: -0.017 to -0.006), individuals with a high school education ($\beta = -0.011$, 95% CI: -0.018 to -0.004), and current smokers ($\beta = -0.009$, 95% CI: -0.016 to -0.001).

Table S8. Subgroup analysis of the association between physical activity and DNA methylation-predicted PhenoAge.

Subgroups	β (95% CI)	p-value	p for interaction
Gender			0.738
Male	-0.008(-0.013,-0.003)	0.003	
Female	-0.009(-0.018,-0.001)	0.032	
Race/ethnicity			0.005
Non-hispanic White	-0.012(-0.017,-0.006)	<0.001	
Non-hispanic Black	0.007(-0.004,0.018)	0.199	
Mexican American	-0.002(-0.017,0.014)	0.833	
Other race/ethnicity	0.003(-0.011,0.017)	0.566	
Marital status			0.452
Never married	0.001(-Inf,Inf)	0.646	
Married/living with partner	-0.008(-0.014,-0.002)	0.009	
Widowed/ divorced	-0.008(-0.025,0.008)	0.313	
Poverty income ratio			0.122
< 1	-0.001(-0.012,0.009)	0.766	
[1,3)	-0.014(-0.023,-0.006)	0.002	
≥ 3	-0.004(-0.012,0.003)	0.248	
Education			0.164
Below high school	-0.01(-0.041,0.021)	0.502	
High school	-0.011(-0.018,-0.004)	0.004	
College or above	-0.001(-0.009,0.006)	0.680	
BMI (kg/m ²)			0.812
< 25	-0.006(-0.015,0.004)	0.246	
[25, 30)	-0.008(-0.017,0.000)	0.054	
≥ 30	-0.01(-0.020,0.000)	0.046	
Smoking status			0.988
Never smoking	-0.008(-0.018,0.001)	0.084	
Former smoking	-0.008(-0.016,0.000)	0.057	
Current smoking	-0.009(-0.016,-0.001)	0.026	
Alcohol use status			0.475
None	-0.008(-0.015,-0.002)	0.015	
Moderate alcohol use	-0.009(-0.016,-0.001)	0.021	
High alcohol use	0(-0.015,0.015)	0.987	