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Socioeconomic Position and DNA Methylation Age Acceleration across the Lifecourse

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Running head: Socioeconomic position and DNA methylation age

Keywords: Epigenomics, Aging, Socioeconomic Factors

Abbreviations: DNAm age: DNA methylation age, UKHLS: UK Household Longitudinal Study, BHPS: British Household Panel Survey

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Abstract

Accelerated DNA methylation age is linked to all-cause mortality and environmental factors, but studies of associations with socioeconomic position are limited. Studies generally use small selected samples, and it is unclear how findings with two commonly used methylation age calculations (Horvath and Hannum) translate to general population samples including younger and older adults. In 1099 UK adults aged 28-98y in 2011-12, we assessed the relationship of Horvath and Hannum DNA methylation age acceleration with a range of social position measures: current income and employment, education, income and unemployment across a 12-year period, and childhood social class. Accounting for confounders, participants less advantaged in childhood were epigenetically 'older' as adults: compared to participants with professional/managerial parents, Hannum age was 1.07 years higher (95% confidence interval (CI):0.20-1.94) for those with parents in semi-skilled/unskilled occupations, and 1.85 years higher (95%CI:0.67-3.02) for participants without a working parent at age 14. No other robust associations were seen. Results accord with research implicating early life circumstances as critical for DNA methylation age in adulthood. Since methylation age acceleration as measured by the Horvath and Hannum estimators appears strongly linked to chronological age, research examining associations with the social environment must take steps to avoid age-related confounding.

Keywords: aging; epigenomics; socioeconomic factors

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Epigenetics is the study of chemical modifications to DNA, and the histone proteins bound to it, which play an important regulatory role in gene expression without changing the heritable DNA sequence. The most widely studied epigenetic modification in relation to human health and disease is DNA methylation. In recent years a number of mathematical models predicting age from DNA methylation profiles, or 'epigenetic clocks' have been developed including those by Horvath(1) and Hannum(2). Utilizing age-related changes in DNA methylation which occur through the lifespan, these allow calculation of a person's DNA methylation age (DNAm age) based on methylation at a small number of selected sites (<0.1% of sites available from microarrays used to profile DNA methylation, <0.001% of CpG sites in the human genome(3)). DNAm can therefore be considered a measure of 'biological age' (2).

Although DNAm age and chronological age are highly correlated, the relationship varies between individuals, such that some people are in DNA methylation terms 'older' than their chronological age would predict, and others 'younger'. This variation is often described in terms of Δage or 'delta age', the difference in years between a person's DNAm age and chronological age. Individuals with unexpectedly high DNAm age are said to show DNA methylation 'age acceleration' (although, since Δage describes a difference rather than rate of change, a more appropriate term might be elevation). Inter-personal variation in Δage is associated with functioning among elderly people - walk speed, lung function and cognition(4) - and all-cause mortality(5, 6). Individuals with higher Horvath or Hannum DNAm age are at increased risk of age-related mortality, and individuals with younger methylation profiles at lower risk. Strikingly, this applies even within pairs of twins(7). Meanwhile, DNAm age acceleration has been linked with environment factors including

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economic hardship(8), lifetime stress(9), dietary factors(10), pollution(11) and education(12). This suggests DNAm age acceleration may reflect processes contributing to social differences in morbidity and mortality, opening up new pathways of inquiry in health inequalities research. A mediating role of stress(13) is plausible given existing research on socioeconomic disadvantage, stress and adverse ageing profiles(14).

Another area of ongoing research concerns the applicability of the clocks to population samples with a different age range to that on which the clocks were calibrated. The Horvath clock, based on 8,000 samples encompassing different tissues from participants aged 0-100 of different ethnicities(1) and the Hannum clock, calculated from blood of 666 White or Hispanic American adults(2), obtain linear relationships between chronological age and DNAm age in their samples. However, it is unclear how well this relationship holds in populations with different age distributions. A recent analysis by Zhang(15), using a German sample aged 50-75y, suggests DNAm age calculated using the clocks may predict chronological age less well at older ages. If the relationship of chronological and DNAm age is not constant during adulthood, this must be considered in analysis of possible 'accelerators' which are age-patterned, to avoid age-related confounding.

To investigate possible contribution of DNAm age acceleration to socioeconomic inequalities in health, we examine the relationship of DNAm age with a range of socioeconomic measures in 1099 UK men and women aged 28-98. We investigate Horvath age and Hannum age, the most widely used measures of DNAm age, in parallel. Since socioeconomic position has multiple dimensions whose associations with health may differ(16), and previous epigenetic studies suggest accumulation processes may exert particular effects(9), we consider contemporaneous factors (current employment status and

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income) cumulative measures (income and total unemployment over 12 years) and factors from earlier in life (educational qualifications, parental social class at age 14). We also investigate whether the relationship between DNAm age and chronological age changes across the adult lifespan.

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METHODS

Participants

The British Household Panel Survey began in 1991, and in 2010 was incorporated into the larger UK Household Longitudinal Study(17). Since 1991 annual interviews have collected sociodemographic information, and in 2011-12, blood samples for BHPS participants were collected at a nurse visit in the participant’s home. Respondents were eligible to give a blood sample if they were aged 16+, were not pregnant, and met other conditions detailed in the user guide (18). Methylation was profiled in DNA extracted from whole blood for 1193 individuals eligible for and consented to blood sampling and genetic analysis, who participated at all annual interviews between 1999 and 2011, and whose time between blood sample collection and processing did not exceed 3 days. Eligibility requirements for genetic analyses meant the epigenetic sample was restricted to participants of white ethnicity. 18 individuals were excluded following laboratory quality control checks. The current analysis excluded 76 participants for whom inverse-probability weights could not be calculated, and outliers whose Δage exceeded 3sd from the mean (n=6 for Horvath analyses, n=5 for Hannum analyses). Pairwise deletion for missing data resulted in a minimum sample size of 932 for a summary measure of net household income over the 12-year period.

Measures

DNA Methylation

500ng of whole blood DNA from 1193 individuals were treated with sodium bisulfite using the EZ96 DNA methylation kit (Zymo Research, CA, USA) following manufacturer's standard protocol. DNA methylation was assessed using Illumina Infinium HumanMethylationEPIC

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BeadChips (Illumina Inc, CA, USA)(19). DNA methylation levels were quantified on an Illumina HiScan System. Raw signal intensities were parsed into R and converted into beta values using the Bioconductor Bigmelon package(20).

Outliers were identified and removed using `wateRmelon::outlyx`, low quality samples (<85% bisulfite conversion) identified and removed using `wateRmelon::bscon`, and data normalized using `wateRmelon::dasen`. Difference between normalized and raw data were estimated using `wateRmelon::qual`. Observations with a root mean square difference and standard deviation of difference >0.05 were removed. After removal of outlying/poor quality observations, data were subjected to `wateRmelon::pfilter` and re-normalised using `wateRmelon::dasen` leaving 857071 probes and 1175 individuals for analysis.

DNAm age was calculated through linear functions using wateRmelon::agep, supplying different sets of coefficients for Horvath or Hannum calculations following:

$$Age_{Sample} = m_{probe\ 1}\beta_{probe1} + m_{probe\ 2}\beta_{probe\ 2} + \ldots + m_{probe\ n}\beta_{probe\ n} + c$$

Where m is the coefficient of the specified probe, β is the measurement of DNA methylation for a specified probe and given individual and c is the intercept defined by the author's model. As both clocks were designed for an earlier micro-array design, missing probes (17 for Horvath, 6 for Hannum, listed in Web Table 1) were not included in calculations.

Horvath and Hannum Δage were calculated as the difference between DNAm and chronological age, and included in linear regression models as the dependent variable.

Socioeconomic measures

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Measures of current socioeconomic position were based on participants' self-report data from the annual interview preceding the 2011 nurse visit, while lifetime measures used information given during the previous 12 years. For current income, quartiles of equivalized net household income in 2011 were calculated separately within 5-year age bands, given substantial age-band differences in household income (two-sided $P<0.01$), which increased to age 60 before decreasing sharply around retirement age. Equivalently, a summary income measure over the 12-year period considered years spent in the lowest age-band specific quartile (categorized as 0, 1-2, 3-6, or 7+ for roughly equal groups). Current employment status was categorized as employed/self-employed/unemployed/retired/looking after home or family/long-term sick or disabled/other; unlike for other measures, this analysis was restricted to participants of working age (≤65y). Reports of current and former employment and non-employment spells from each annual wave were used to construct 12-year activity histories for each participant, from which aggregated unemployment in months was calculated; this was categorized as none, ≤12 months, and >12 months. Highest educational qualification was categorized as university degree/qualifications below degree/no qualifications. Following Fiorito(12), this was standardized within groups of gender and five-year age band to account for generational differences in education; this results in a continuous score between 0 and 1, with higher scores indicating less education relative to others within the same age band and gender. Childhood social class was based on parents' Registrar General's Social Classification when participants were 14 years old, and categorized as professional/managerial occupations, skilled non-manual occupations, skilled manual occupations, or semi-skilled/unskilled occupations. The social class of the father was used

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except where this was not available, when the mother's was used instead. Where neither parent was in work, or both were deceased, participants were assigned to a separate group.

Covariates

All analyses included as covariates sex, chronological age and age^2, to capture possible non-linearity in age-related confounding, and smoking and adiposity. All analyses were adjusted for batch and blood cell composition estimates, calculated using the Houseman Reference based algorithm implemented in the estimateCellCounts function packaged in minfi(21, 22).

Smoking was categorized as never/ex-/current, ≤10 per day/current, 11-20 per day/current,>10 per day. Adiposity was indexed using WHO classifications of body mass index: underweight (<18.5kg/m^2), recommended weight (18.5-24.9), overweight (25.0-29.9), class I obese (30.0-34.9) and class II obese (30.0-34.9). Due to substantial missingness, alcohol consumption in the past week and psychological distress were examined in sensitivity analyses only. These considered drinking frequency (most days/3-4 days/1-2 days/not in the past week) and drinking heaviness (not in the past week/under the recommended limit/1-2x the recommended limit/≥2x the recommended limit). Psychological distress was assessed at the interview preceding the nurse visit using the 12-item General Health Questionnaire, scored continuously from 0-36.

Analyses were conducted in STATAv15 (Stata Corporation, College Station, Texas). Analyses used inverse-probability weights to account for both unequal initial sampling probabilities and differential attrition and nonresponse (Web Appendix), accounting for survey design effects using the *svyset* command.

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RESULTS

Description of the sample

The analytic sample included for DNA methylation analysis were indirectly selected on age, since DNA methylation was only profiled for individuals who had participated as adult (16+) survey respondents annually since 1999. Compared to White/European BHPS participants who took part in the nurse assessment and were in the eligible age range (28+y) but were not in the analytic sample, participants included in analyses did not differ significantly (at $p<0.05$) on sex, mean body mass index, aggregated unemployment or childhood social class. They were however older (58.4 vs 53.9), less educated (28.8% vs 30.8% with a degree, 17.6% vs 14.4% with no qualifications) and with lower equivalized net household income (£1599.2 vs £1978.6 per month). They were less likely to be employed (41.0% vs 50.8%), more likely to be retired (40.8 vs 29.4%), and less likely to be current smokers (15.7% vs 20.2%), all at $p<0.05$. Childhood social class predicted adult socioeconomic position measures (Web Table 2).

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Correlation of chronological and DNA methylation age across the adult lifespan

Across the sample, chronological age correlated highly with Horvath DNA methylation age (r=0.90) and Hannum DNA methylation age (r=0.94). Horvath and Hannum DNA methylation were also highly correlated (r=0.92). However, scatterplots of DNA methylation with chronological age (Figure 1 and Figure 2) showed that the relationship of DNA methylation age and chronological age differed substantially by chronological age. Using the Horvath clock, the youngest participants were substantially older in terms of DNA methylation than chronologically, whereas for the older participants the reverse was true. Using the Hannum clock, DNA methylation and chronological age correlated well for younger participants but older participants were substantially younger in DNA methylation terms than chronologically. In weighted regression models adjusted for sex, batch and blood cell composition, coefficients of Δage against chronological age were negative and significant for both clocks, and did not differ by sex (Horvath: -0.39, (95% confidence interval(95%CI):-0.42,-0.36) (all participants), -0.39(95%CI:-0.41,-0.36) (men), -0.39(95%CI:-0.42,-0.37) (women); Hannum: -0.38(95%CI:-0.40,-0.36) (all participants), -0.37 (95%CI:-0.39,-0.35) (men), -0.38 (95%CI:-0.40,-0.36) (women)). Addition of quadratic age terms did not indicate substantial non-linearity (age^2 coeff=-.0007, two-sided *P*=0.39; coeff=-.0003, two-sided *P*=0.67 for Horvath and Hannum, respectively).

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Association of DNA methylation age acceleration with socioeconomic factors

Horvath DNA methylation age

Using the Horvath clock (Table 2), in adjusted models no significant associations were seen between Δage and current income, income over the 12-year period, educational qualifications, or aggregated unemployment. Participants not working due to sickness/disability were 1.89y ‘older’ (95%CI:0.40,3.37) than employed counterparts, with no other employment status differences observed. Only childhood social class showed clear elevations for less advantaged groups (Table 2). Compared to participants with professional/managerial parents, Δage for participants with parents in skilled non-manual occupations was 1.42y higher (95%CI:0.24,2.59), and for participants with no parent in work or both deceased at 14y, 2.40y higher (95%CI:0.60,4.19). Treating childhood social class as continuous showed a significant increasing Δage across groups (per-category Δage change 0.33y (95%CI:0.06-0.59). Addition of alcohol measures or psychological distress reduced sample sizes and hence precision, but did not affect conclusions (Table 3).

Hannum DNA methylation age

Using the Hannum clock (Table 2), no differences were seen for employment status, current income, income over the 12-year period, or aggregated unemployment. In contrast, there was a significant association of lower education and higher Hannum age (Δage 0.98y, 95%CI:0.03,1.93), comparing the least to the most educated individuals within age-and-gender bands, and clear stepwise associations were seen with childhood social class. Compared to participants with professional/managerial parents, Δage for participants with parents in skilled manual occupations was 0.68y higher (95%CI:0.11,1.25), for participants

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with parents in semi-skilled/unskilled occupations 1.07y higher (95%CI:0.20,1.94), and for participants with no parent in work or both deceased at 14y, 1.85y higher (95%CI: 0.67,3.02). Including childhood social class as continuous confirmed a significant association of increasing Δage across groups (per-category Δage change 0.39y, (95%CI:0.17-0.61)). For childhood social class, addition of alcohol measures or psychological distress reduced sample sizes and hence precision, but did not affect conclusions (Table 3). For education, associations were partially explained by psychological distress (adjusted Δage 0.78y, 95%CI:-0.15,1.70), and fully explained by childhood social class (adjusted Δage 0.56y, 95%CI:-0.15,1.70). In contrast, adjustment for education barely affected associations for childhood social class (Table 3).

Results for Horvath and Hannum age were very similar unadjusted for smoking and body mass index (Web Table 3). Additional adjustment for processing time of blood samples (1 day/2 days/3 days) did not affect results for either clock.

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DISCUSSION

In 1099 men and women aged 28-98, we assessed associations of a range of socioeconomic position measures with DNAm age acceleration, to investigate possible contribution of DNAm age acceleration to socioeconomic inequalities in health. We show that Δage is primarily associated with socioeconomic position in childhood, rather than later in life. Documenting a negative relationship of Δage and chronological age, we show that associations of Δage with the social environment may be vulnerable to substantial age-related confounding.

Correlation of chronological and DNA methylation age across the adult lifespan

Chronological age correlated highly with DNA methylation age derived using both Horvath and Hannum estimators. However, we found Δage to be robustly and negatively correlated with chronological age. Using the Horvath and Hannum estimators, older individuals were almost exclusively 'young' for their age, and using the Horvath clock younger individuals were almost exclusively 'old' for their age. Precisely because DNA methylation age predicts mortality, the typically low Δage of older individuals may partly result from survival bias. However, it is unclear how survival bias can explain the unexpectedly positive Horvath Δage among younger participants. Since participants for whom methylation was profiled needed to meet a range of criteria, including consent to genetic analysis and participation in 12 annual surveys, an influence of other forms of bias was possible. However, these patterns persisted after applying inverse-probability weights for nonresponse. This suggests relationships of chronological age and methylation at sites included in the clocks may differ between populations, which has important implications for research using the clocks to investigate exposures or outcomes which are age-patterned. Researchers do not always

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adjust for chronological age when Δage is the outcome(23), but we find this can produce spurious associations of Δage with factors which are age-correlated. Since unemployment is disproportionately experienced by people early in their working lives, striking Δage elevations occur with aggregated unemployment: with Horvath, 3.16y (95%CI: 1.81-4.52) for <12 months and 3.66y (95%CI: 1.68-5.64) for ≥12 months; with Hannum, 3.30y, (95%CI:2.11-4.50) for <12 months and 2.82y, (95%CI:1.42-4.21) for ≥12 months. These elevations disappear completely adjusting for chronological age.

Association of DNA methylation age acceleration with socioeconomic factors

In general, we observed accelerated epigenetic ageing in relation to social disadvantage in childhood. Compared to participants with professional/managerial parents, a clear pattern was seen of increasing Hannum Δage for less advantaged groups; we also saw elevations in Horvath Δage for some less-advantaged groups. Since smoking and adiposity may be mediators or confounders of these associations, results from health behavior-adjusted models may be regarded as conservative. In any case, these differences were not explained by smoking, adiposity or alcohol consumption, suggesting mechanisms independent of health behaviors are involved. That associations of Hannum Δage and low education were explained by childhood social class, but not vice versa, supports early life as a critical period for establishment of DNA methylation age trajectories. Since there is some evidence that methylation sites in the Hannum clock may be more subject to stress-related processes(24), clearer patterns with the Hannum clock may implicate stress as a key factor in the social differences observed, or reflect use of a DNAm age measure designed for whole blood, the same tissue used in this analysis. That psychological distress did not explain associations may indicate that processes are largely independent of perceived distress, or reflect

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limitations of subjective psychological wellbeing measures in the study of socioeconomic inequalities.

Associations with childhood social class accord with results from the 1958 British Birth Cohort(25), and research suggesting that childhood may critical for establishment of DNA methylation age trajectories(26), and other aspects of DNA methylation(27). Since we were unable to examine particular aspects of the childhood environment which may plausibly impact DNA methylation ageing – such as diet, housing quality or psychosocial stress – further research will be required to identify which factors in childhood are most relevant to DNA methylation ageing. It is also possible that childhood disadvantage is acting as a proxy for in utero conditions. Of note, since participants who at 14y had parents in professional occupations were younger (mean 55.4y) than those whose parents were in semi-skilled/unskilled occupations (mean 63.3y), or without a parent in work (mean 58.8), any residual confounding by age itself is likely to have led to underestimation of associations with childhood social class. Thus, our estimates may be regarded as conservative.

Although childhood social class predicted adult socioeconomic position measures (Web Table 2), we saw no association with either current equivalized income, or equivalized income over a 12-year period. This contrasts with results from a study of African-American women(8) but accords with a study of older Italians(10). Since our income measures were based on detailed, annually-reported information, discrepancies are unlikely to result from the quality of the income data. To investigate contribution of sample gender composition, income analyses were repeated with a gender interaction term, but found no evidence of female-specific associations. It is possible that greater economic hardship experienced by participants of American samples (8) played a role. Our sample contained only white

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participants, but associations may differ by ethnicity; they may also differ between countries with different welfare provision. Results for education and Horvath Δage are consistent with two recent analyses reporting no robust associations (6, 28). Results for education and Hannum Δage are consistent with previously reported associations (6, 12, 28) but suggest they may be partly explained by education acting as a proxy for unmeasured conditions earlier in life.

Strengths and limitations

This study has several considerable strengths: based on a national study, it comprises a large sample size, with representation from almost the entire adult range. We applied inverse-probability weights to models, thus minimizing the impact of nonresponse bias, and annually-repeated data collection minimized the impact of recall error on summary measures of income and unemployment across 12 years. We were able to consider diverse dimensions of socioeconomic position, finding that associations with education were explained by childhood social class. However, we could not examine conditions in early childhood or in utero, where effects on DNAm age trajectories are plausibly stronger than at 14y. The fairly crude measure of mental health available may not have adequately captured contribution of psychological processes to DNA methylation age acceleration, and the sample was restricted to white individuals, meaning results may not be generalizable to other ethnic groups. As with previous studies, the prediction of mortality by DNA methylation age acceleration means survival bias may have produced underestimates of the impact of social exposures in older age groups.

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Conclusion

In a large British sample aged 28-98, DNA methylation age measured by the Horvath and Hannum clocks was associated with childhood social class, but not measures of social position later in life, consistent with a lasting influence of early life conditions on DNA methylation age trajectories. Across the adult age range, population mean values of DNA methylation age 'acceleration' varied substantially with chronological age. Studies examining associations with exposures and outcomes which themselves are age patterned should take this into account to avoid age-related confounding.

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Figure Legends:

Figure 1:

Horvath DNA methylation age (years) by chronological age (years) in UK Household Longitudinal Study participants, 2011-12 (N=1093). For men (A) and women (B), the solid line represents the line of best fit, and the dashed line is a y=x line. Δage (years) is the difference between DNA methylation age and chronological age. If mean Δage were constant with age, observations would be roughly symmetrical about the y=x line. Instead, Δage decreases with chronological age

Figure 2:

Hannum DNA methylation age (years) by chronological age (years) in UK Household Longitudinal Study participants, 2011-12 (N=1094). For men (A) and women (B), the solid line represents the line of best fit, and the dashed line is a y=x line. Δage (years) is the difference between DNA methylation age and chronological age. If mean Δage were constant with age, observations would be roughly symmetrical about the y=x line. Instead, Δage decreases with chronological age

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Table 1: Descriptive Characteristics of the Analytic Sample: UK Household Longitudinal Study, Wave 3: (2011-2012). N=1099

	N	(%)
Gender		
Male	466	42.4
Female	633	57.6
Current employment status		
Employed	450	41.0
Self-employed	93	8.5
Unemployed	23	2.1
Retired	448	40.8
Looking after home or family	47	4.3
Long-term sick or disabled	31	2.8
Other	7	0.6
Highest Educational Qualification		
Degree	315	28.7
Qualifications below degree	586	53.3
No qualifications	193	17.6
Missing	5	0.5
Total unemployment, 1999-2011		
None	923	84.0
<12 months	114	10.4
≥12 months	60	5.5
Missing	2	0.2
Childhood social class[a]		
Professional/Managerial	281	25.6
Skilled non-manual	104	9.5
Skilled manual	406	36.9
Semi-skilled/unskilled	192	17.5
No parent in work/both deceased	48	4.4
Missing	68	6.2
Smoking		
Never	594	54.1
Ex-smoker	332	30.2
Current, up to 10/day	60	5.5
Current, 11-20/day	90	8.2
Current, >20/day	23	2.1
Body mass index[b]		
Underweight (<18.5kg/m^2)	6	0.6
Recommended (18.5-24.9 kg/m^2)	303	27.6
Overweight (25.0-29.9 kg/m^2)	449	40.9
Obese class I (30.0-34.9 kg/m^2)	227	20.7
Obese class II (35+ kg/m^2)	114	10.4

Age (years): mean (SD) 58.4 (14.9), range 28-98

[a]Parental Registrar General's Social Classification when participant was aged 14

[b]Weight (kg)/height (m)^2

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Table 2: Association[a] of Socioeconomic Factors with DNA Methylation Age Acceleration, UK Household Longitudinal Study participants, 2011−12 (N=1094)

	Horvath[a]			Hannum[a]		
Quartiles of equivalized net household income	Δage(years)	95%CI	Model N	Δage(years)	95%CI	Model N
Highest	0	Referent	1093	0	Referent	1094
3	−0.88	−1.84, 0.07		−0.36	−1.04, 0.31	
2	0.44	−0.49, 1.37		0.12	−0.62, 0.86	
Lowest	−0.68	−1.61, 0.25		−0.17	−1.00, 0.65	
Current employment status (participants aged <65)						
Employed	0	Referent	716	0	Referent	717
Self−employed	0.81	−0.39, 2.01		0.07	−0.93, 1.06	
Unemployed	−0.97	−2.99, 1.05		−0.88	−2.14, 0.37	
Retired	−0.65	−1.86, 0.57		−0.33	−1.33, 0.66	
Looking after home or family	0.98	−0.35, 2.31		0.52	−0.53, 1.57	
Long−term sick or disabled	1.89	0.40, 3.37		−0.37	−1.83, 1.08	
Other	1.35	−0.78, 3.48		0.92	−1.69, 3.52	
Years in the lowest age−specific income quartile, 1999−2011						
0 (ref)	0	Referent	932	0	Referent	933
1−2	0.34	−0.55, 1.23		0.68	−0.13, 1.48	
3−6	−0.46	−1.28, 0.36		−0.01	−0.64, 0.62	
7+	−0.73	−1.54, 0.08		−0.24	−0.94, 0.46	
Total unemployment, 1999−2011						

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None	0	Referent	1091	0	Referent	1092
<12 months	−0.72	−1.70, 0.26		−0.45	−1.22, 0.32	
≥12 months	−0.26	−1.76, 1.25		−0.92	−1.85, 0.01	
Highest educational qualification[c]						
Least vs. most educated	0.26	−0.97, 1.49	1088	0.98[b]	0.03, 1.93	1089
Childhood social class[d]						
Professional/Managerial	0	Referent	1025	0	Referent	1026
Skilled non−manual	1.42[b]	0.24, 2.59		0.33	−0.51, 1.17	
Skilled manual	0.44	−0.30, 1.19		0.68[b]	0.11, 1.25	
Semi−skilled/unskilled	0.85	−0.08, 1.79		1.07[b]	0.20, 1.94	
No parent in work/both deceased	2.40[b]	0.60, 4.19		1.85[b]	0.67, 3.02	

Abbreviations: CI=confidence interval

[a]Adjusted for chronological age, age^2, gender, blood cell count, batch, smoking, body mass index.

[b]Associations significant at $p<0.05$

[c]Standardized within categories of gender and 5−year age−band. Range 0−1, higher scores indicate lower education

[d]Parental Registrar General's Social Classification when participant was aged 14

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Table 3: Sensitivity Analyses for Childhood Social Class and Education. UK Household Longitudinal Study participants, 2011-12

	Sensitivity Analysis 1: Adjustment for Drinking Frequency[a]		Sensitivity Analysis 2: Adjustment for Drinking Heaviness[b]		Sensitivity Analysis 3: Adjustment for Psychological Distress[c]		Sensitivity Analysis 4: Mutual Adjustment: Childhood Social Class and Education[d]	
	Δage(years)	95%,CI	Δage(years)	95%,CI	Δage(years)	95%,CI	Δage(years)	95%,CI
Childhood Social Class: Horvath[e]								
Skilled non−manual	1.35[f]	0.09, 2.60	1.41[f]	0.14, 2.68	1.35[f]	0.14, 2.57	1.42[f]	0.24, 2.59
Skilled manual	0.24	−0.57, 1.04	0.41	−0.39, 1.21	0.49	−0.28, 1.25	0.45	−0.31, 1.20
Semi−skilled/unskilled	0.41	−0.62, 1.45	0.58	−0.46, 1.63	1.12[f]	0.17, 2.07	0.84	−0.15, 1.84
No parent in work/both deceased	2.47[f]	0.61, 4.33	2.52[f]	0.61, 4.43	2.40[f]	0.62, 4.18	2.42[f]	0.62, 4.21
Childhood Social Class: Hannum[g]								
Skilled non−manual	0.36	−0.48, 1.21	0.43	−0.44, 1.30	0.18	−0.67, 1.04	0.30	−0.54, 1.13
Skilled manual	0.75[f]	0.12, 1.39	0.75[f]	0.12, 1.39	0.49	−0.11, 1.09	0.62[f]	0.06, 1.19
Semi−skilled/unskilled	0.86	−0.09, 1.81	0.85	−0.11, 1.81	1.11[f]	0.25, 1.97	0.96[f]	0.06, 1.87
No parent in work/both deceased	1.86[f]	0.60, 3.13	1.80[f]	0.51, 3.09	1.83[f]	0.63, 3.04	1.77[f]	0.58, 2.96
Highest educational qualification: Hannum[h]								
Lowest vs. highest education	1.08[f]	0.06, 2.10	0 .97	−0.07, 2.02	0.78	−0.15, 1.70	0.56	−0.48, 1.60

Abbreviations: CI: confidence interval

[a]Adjusted for drinking frequency, chronological age and age2, sex, white blood cell composition, batch, smoking and body mass index categories.

[b]Adjusted for drinking heaviness, chronological age and age2, sex, white blood cell composition, batch, smoking and body mass index categories.

[c]Adjusted for psychological distress, chronological age and age2, sex, white blood cell composition, batch, smoking and body mass index categories.

[d]Mutual adjustment for childhood social class and highest educational qualification, chronological age and age2, sex, white blood cell composition, batch, smoking and body mass index categories.

[e]Childhood Social Class: Parental Registrar General's Social Classification when participant was aged 14. Analytic samples for Sensitivity Analyses 1−4: N=924 N=904 N=964 N=1025

[f]Associations significant at $p<0.05$

[g]Childhood Social Class: Parental Registrar General's Social Classification when participant was aged 14. Analytic samples for Sensitivity Analyses 1−4: N=925 N=905 N=965 N=1026

[h]Standardized within categories of gender and 5−year age−band. Range 0−1, higher scores indicate lower education. Analytic samples for Sensitivity Analyses 1−4: N=977 N=957 N=1026 N=1025

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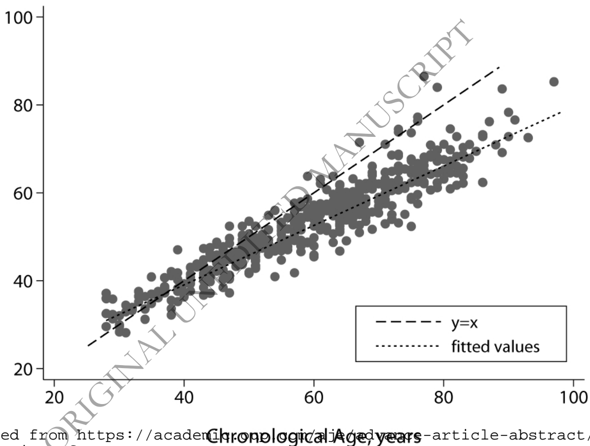

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