

The effects of social status on biological aging as measured by white-blood-cell telomere length

L. F. Cherkas,¹ A. Aviv,² A. M. Valdes,¹ J. L. Hunkin,¹
J. P. Gardner,² G. L. Surdulescu,¹ M. Kimura²
and T. D. Spector¹

¹Twin Research & Genetic Epidemiology Unit, St Thomas' Hospital, London SE1 7EH, UK

²The Center of Human Development and Aging, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, NJ 07103-2714, USA

Summary

Low socio-economic status (SES) is associated with a shortened life expectancy, but its effect on aging is unknown. The rate of white-blood-cell (WBC) telomere attrition may be a biological indicator of human aging. We tested the hypothesis that SES is associated with telomere attrition independent of known risk factors influencing the aging process. We studied 1552 female twins. A venous blood sample was taken from each twin and isolated WBCs used for extraction of DNA. Terminal restriction fragment length (TRFL) was measured. Questionnaire data were collected on occupation, education, income, smoking, exercise, height and weight. Standard multiple linear regression and multivariate analyses of variance tested for associations between SES and TRFL, adjusting for covariates. A discordant twin analysis was conducted on a subset to verify findings. WBC telomere length was highly variable but significantly shorter in lower SES groups. The mean difference in TRFL between nonmanual and manual SES groups was 163.2 base pairs (bp) of which 22.9 bp (~14%) was accounted for by body mass index, smoking and exercise. Comparison of TRFL in the 17 most discordant SES twin pairs confirmed this difference. Low SES, in addition to the harmful effects of smoking, obesity and lack of exercise, appears to have an impact on telomere length.

Key words: Aging; social; somatic cells; status; telomere.

Correspondence

Professor Tim Spector, Twin Research & Genetic Epidemiology Unit, St Thomas' Hospital, London SE1 7EH, UK. Tel.: 020 7188 6765; fax: 020 7188 6718; e-mail: tim.spector@kcl.ac.uk

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Introduction

Socio-economic status (SES) has a major impact on health (WHO, 2002; Adams & White, 2004; Marmot, 2004, 2005; Sapolsky, 2005). There is a wealth of evidence that lower SES is associated with increased risks of cardiovascular, respiratory, rheumatic and psychiatric diseases; low birthweight; infant mortality; and mortality from all causes (Sapolsky, 2005). These and lifestyle factors such as smoking, physical activity and diet increase the propensity to aging-related diseases (WHO, 2002). SES has an impact on life expectancy as well as health (Marmot, 2004). As aging confers the greatest risk of death, a lower SES may diminish life expectancy not only by predisposing to aging-related diseases, but also because it may influence the aging process itself.

Aging is the progressive loss of metabolic and physiologic functions (Kirkwood & Austad, 2000), but its trajectory is not uniform, suggesting considerable variation in the biology of aging. This variation may arise from a host of genetic and environmental factors that impact on oxidative stress (Finkel & Holbrook, 2000) and inflammation (Finch & Crimmins, 2004). In this narrow sense, aging may be defined as the life history of the individual, expressed in the cumulative burden of oxidative stress and inflammation. White-blood-cell (WBC) telomere dynamics (telomere length and attrition rate) *in vivo* appears to chronicle these processes and, as such, provides an account of biological aging above and beyond chronological age (Aviv, 2004).

Telomeres consist of DNA repeat sequences, which together with telomere-binding proteins, cap and protect chromosome ends (Wong & Collins, 2003). In cultured human somatic cells, telomere length is shortened with each cycle of replication and such attrition ultimately leads to a loss of replicative capacity (replicative senescence). As telomere length in proliferative somatic cells is inversely correlated with age, it appears that in humans, telomere length also registers cell replication *in vivo* (Aviv, 2004).

We tested the hypothesis that SES is associated with telomere attrition independent of known risk factors influencing the aging process. To this end, using 1552 unselected female twins, we examined the relationship between SES (as measured in the UK by employment-derived social class groups) and WBC terminal restriction fragment length (TRFL), and assessed the contribution of known socio-economically distributed risk factors for ill-health, including body mass index (BMI), smoking and exercise to this association. By examining within-pair telomere differences for a small subgroup of atypical twins, currently discordant for SES, we were able to test further our hypothesis while reducing the confounding effects of birth cohort, genetic variation and upbringing (Williams *et al.*, 2005).

Table 1 Descriptive statistics by social class grouping and correlations of covariates with social class and age-adjusted telomere length

Variables	Test for trend with TRFL*	Social status					Test for trend with SES
		Nonmanual			Manual		
		I 22.30% n = 291	II 38.20% n = 498	IIIN 18.60% n = 243	IIIM 8.10% n = 105	IV–V 12.70% n = 166	
Age-adjusted TRFL	N/A	7.11	7.13	7.15	7.03	6.93	$P < 0.024$
Age (years)	N/A	45.73	44.37	47.09	47.25	47.49	$P < 0.002$
SE		0.62	0.47	0.68	1.03	0.82	
BMI (kg m ⁻²)	$P < 0.045$	24.22	24.85	24.9	25.32	25.96	$P < 0.0001$
SE		0.27	0.21	0.21	0.47	0.38	
Current smokers	$P < 0.03^{**}$	12.30%	20.20%	18.20%	21.10%	23.50%	$P < 0.0067$
Exercise (1–4)	$P < 0.005$	2.73	2.59	2.47	2.52	2.54	$P < 0.002$
SE		0.04	0.03	0.05	0.08	0.06	
Educational attainment (1–12)	$P < 0.25$	9.18	7.5	4.91	4.95	3.96	$P < 0.0001$
SE		0.2	0.15	0.23	0.37	0.27	

*Age adjusted; **tests for trend current smokers vs. ex-smokers vs. nonsmokers. SE, standard error.

Results

TRFL, although highly variable with a range from 5.15 kb to 9.38 kb, was negatively correlated with chronological age, getting shorter at an extrapolated annual average rate of 19.8 bp per year (SE ± 1.7). All of the lifestyle risk factors considered were significantly associated, in the expected direction, with SES (Table 1). BMI and smoking status were negatively correlated, while increasing physical activity and higher SES were positively correlated with age-adjusted TRFL (Table 1).

Figure 1 shows the mean age-adjusted TRFL for each SES group (I to IV/V). There was an overall decreasing trend in TRFL with lower SES ($P < 0.024$). We divided the SES into nonmanual (white-collar) workers (I–IIIN) and manual (blue-collar) workers (IIIM–V) and showed a significant mean difference of 163.2 bp (SE 37.9 bp) between the two groups ($P < 0.01$) (Fig. 2). After adjusting for age, BMI, smoking status, and physical activity, a significant difference of 140.3 bp (SE 38.0 bp) in TRFL remained ($P < 0.04$). After further adjustment for paternal and maternal age, the mean difference in TRFL between blue- and white-collar workers was essentially unchanged at 126.4 bp ($P < 0.047$).

One possibility is that the SES association is due mainly to poverty and resulting deprivation. However, our data on family income, although incomplete (69%), showed no link between income and WBC TRFL. The average income of those classified as nonmanual was between £20 000 and £24 999, and for the manual workers was between £15 000 and £19 999. The use of income as a marker of SES is less discriminatory for health outcomes in the UK than either education- or occupation-based social class, as opposed to in the USA (Duncan *et al.*, 2002; Marmot, 2004). Ignorance about health-related risks is another potential explanation, but despite an association between education and SES, we found that the trend between educational attainment and telomere length was not significant. This may be because once achieved, education level cannot be affected by subsequent changes in SES.

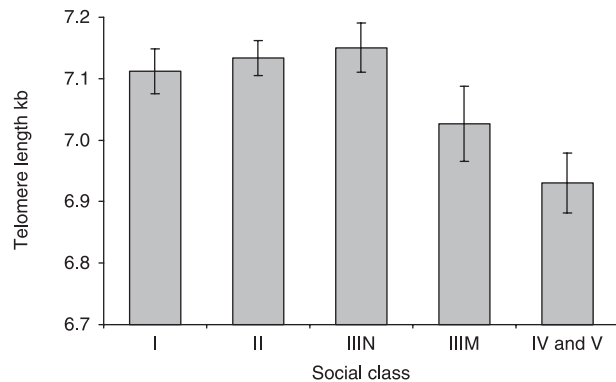


Fig. 1 Mean age-adjusted telomere length and standard error by social class grouping (P for trend = 0.0024).

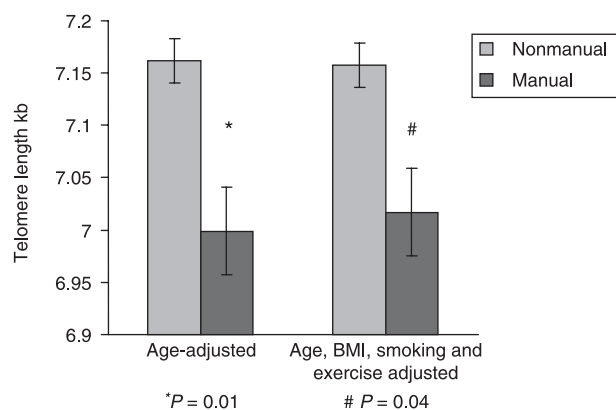


Fig. 2 Mean telomere length and standard error by manual vs. nonmanual social class groupings.

TRFL has been previously noted to be heritable (familial) based on twin and family studies (Jeanclos *et al.*, 2000; Nawrot *et al.*, 2004). Therefore, genetics could be a mediator or confounder of the relationship. From a previous study – of which the current

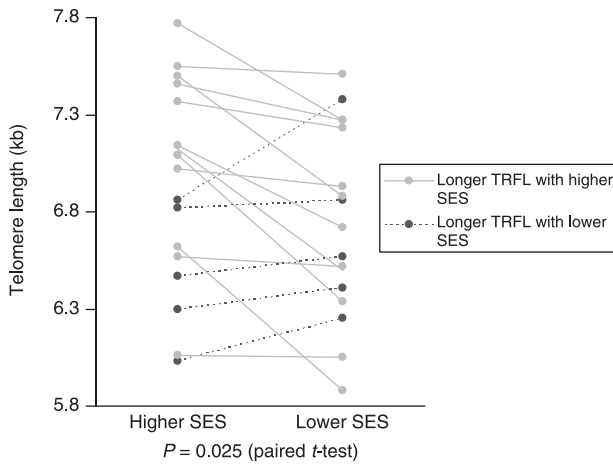


Fig. 3 Comparison of TRFL within 17 twin pairs most discordant for SES.

sample is a subgroup – we estimated heritability to be ~36% (95% CI 18–48%) (Andrew *et al.*, 2006). To reduce the influence of genetic factors and to clarify the importance of nongenetic and nonshared factors on TRFL, we looked at TRFL differences in pairs of twins who were raised together until at least the age of 16 and were discordant for SES. Comparison of 17 pairs of twins (all dizygotic and aged between 32 and 68 years) who were most discordant for SES revealed that in 12 pairs the individual with the higher SES also had a longer TRFL (Fig. 3). Summing the 17 discordant pairs, there was an average difference in TRFL between the twin in class I vs. their co-twin in class IV or V of 187 bp ($P < 0.025$). This difference was still significant after adjustment for confounders (BMI, smoking and exercise) (177 bp, $P < 0.043$).

Discussion

Socio-economic status influences health and mortality (WHO, 2002; Adams & White, 2004; Marmot, 2004, 2005; Sapolsky, 2005), but its impact on the aging process itself is unknown. WBC telomere attrition is accelerated with an increase in body mass and a rise in insulin resistance (Gardner *et al.*, 2005). In the same population as reported here, not only increased body mass but also leptin levels and smoking were found to be significantly associated with shorter WBC telomeres (Valdes *et al.*, 2005). In addition, WBC telomeres are shorter in individuals with coronary artery disease (Ogami *et al.*, 2004) and dementia (Panossian *et al.*, 2003). Such disorders are associated with increased levels of oxidative stress and inflammation – expressed by increased WBC turnover (Valdes *et al.*, 2005). Moreover, in one study, WBC telomere length forecasts death so that elderly individuals with shorter telomere length have a higher mortality risk than their peers with longer telomeres (Cawthon *et al.*, 2003). Other studies have not confirmed these findings (Martin-Ruiz *et al.*, 2005; Bischoff *et al.*, 2006). However, these two later studies were conducted in extreme elderly populations (with mean ages

of 90 and 81, respectively), so results could be subject to a healthy survivor bias.

Here we report that individuals of a lower SES have shorter telomeres than their peers of a higher SES. In fact, the mean difference in TRFL between nonmanual (I–IIIN) and manual (IIIM–V) workers was 163.2 bp (which represents eight times the average annual loss in telomere length based on the rate of TRFL attrition for the entire cohort). After accounting for the influences of body size, smoking and physical activity, we found women of the lower SES (manual workers) had WBC telomeres shorter on average by 140.3 bp compared with those of a higher SES (nonmanual or white-collar workers). This implies that individuals in lower SES experience a more intense exposure to risk factors (other than obesity, smoking and lack of exercise) that accelerate WBC telomere attrition. Such findings are compatible with the concept linking SES with health and longevity (Adams & White, 2004; Marmot, 2004). By studying twin pairs who lived together during childhood and then became discordant for SES – either through their own or their spouses' occupation – we confirmed the effect of SES on TRFL in the whole group. These discordant twin pairs exhibited an average intrapair difference in TRFL of 177 bp, suggesting substantial influences of social hierarchy, not only on health as shown in humans and primates (Sapolsky, 2005), but also on telomere attrition.

Recent evidence suggests that psychosocial factors may actually have greater impact on health than lifestyle variables such as smoking, drinking, diet, activity and susceptibility to infections (Sapolsky, 2005). In this context, the Whitehall II study of UK civil servants showed a clear health gradient with employment grade. Psychosocial work characteristics such as job strain, in particular low control at work and effort-reward imbalance (leading to low self-esteem), were strong determinants of self-reported coronary events among men (Kuper & Marmot, 2003). With respect to SES, exposure to adverse psychosocial environments is not only likely to be greater among lower socio-economic groups, but these individuals are also more vulnerable to associated harmful effects (Siergrist & Marmot, 2004). Psychological stress is therefore one obvious contributor to the observed reduction in telomere length with lower SES in this study. Several recent studies support this association. Perceived stress levels were found to have a significant effect on telomere length in a group of 39 'caregiving' mothers (Epel *et al.*, 2004) and also on oxidative DNA damage in female workers (Irie *et al.*, 2001). Furthermore, oxidative DNA damage was found in women suffering from psychological depression (Irie *et al.*, 2003).

Although all volunteers for this study were female, SES was based on the higher occupational level of either the twin or their partner, so we believe that the conclusions can be generalized to the whole population. Furthermore, the twins were similar to age-matched population singletons in terms of disease-related and lifestyle characteristics (Andrew *et al.*, 2001). As with any study of SES, the measure used is open to criticism, as different socio-economic measures in different populations and contexts result in different estimates of the effect of SES on health (Duncan *et al.*, 2002). There is no global gold standard for estimating

SES status in women; however, the use of The Registrar General's Social Classification is the accepted standard method used in the UK and any random misclassification due to this method would have reduced the size or significance of any real relationship.

Finally, age-adjusted WBC telomere length is highly variable among humans. Our data are cross-sectional; therefore, do not take into account movement between classes over lifetime. For future studies, a longitudinal design would be most informative to detect interindividual variation in telomere length both over time and across different social groups and to clarify causality. Several such prospective studies to date have produced conflicting results as to the association between telomere length and subsequent morbidity or mortality in the very elderly (Cawthon *et al.*, 2003; Martin-Ruiz *et al.*, 2005; Bischoff *et al.*, 2006). Nonetheless, our study, performed on a large cohort, indicates that differences in WBC telomere length between low and high SES individuals cannot be explained by variations in genes, smoking, BMI and physical activity. Rather, in and of itself, SES appears to have an impact on WBC telomere dynamics.

Experimental procedures

Subjects

We studied 1552 Caucasian female twins, comprising 749 dizygotic (DZ) and 27 monozygotic (MZ) pairs. All were volunteers from the St Thomas' (TwinsUK) Adult Twin Registry, ascertained from the general population and shown to be comparable to age-matched population singletons (Andrew *et al.*, 2001). These unselected DZ and MZ twins have been recruited since 1992 using twin registers and national media campaigns and used in a wide variety of studies (www.twin-research.ac.uk). Zygosity was determined by a validated questionnaire (Peeters *et al.*, 1998) and confirmed by multiplex DNA fingerprinting in cases of uncertainty. Historically the cohort is predominantly female and TRF measurements were performed preferentially on DZ twins as part of an ongoing genetic linkage study. Individuals in this cohort (ages 18–75) underwent detailed clinical assessment and completed questionnaires concerning a wide range of health and lifestyle issues. All provided informed consent approved by The St Thomas' Hospital Research Ethics Committee.

Measurements

Subjects completed a questionnaire detailing their educational level (12-point scale ranging from no qualification to university degree), and their own and their partner's occupation. The grouping of occupations was based on Goldthorpe & Hope (1974) and classifications based on the National Statistics Socio-Economic Classification (NS-SEC, 2002).

Subjects were assigned to an SES I, II, IIIN, IIIM, IV or V (as used routinely in UK studies; Marmot, 2004), based on the higher occupational level of either the twin or their partner, in accordance with the new UK NS social-economic measure. According to this system: I, professional occupations; II, managers & administrators

and associate professional & technical workers; IIIN, clerical and secretarial nonmanual skilled occupations; IIIM, crafts & manual related skilled occupations; IV, partly skilled occupations; and V, unskilled occupations. In addition, subjects indicated if they were not working (e.g. housewife, student, unemployed, disabled or retired). Two hundred forty-nine (249) subjects could not be included in the analysis because both they and their partner were in this latter category.

Smoking – ascertained from a self-reported measure – was recorded as a semiquantitative variable with three values: 0 = never smoked, 1 = ex-smoker, and 2 = current smoker. Height and weight – from which BMI was calculated as weight in kg/height in m² – were recorded as previously described (Valdes *et al.*, 2005). Current physical activity during leisure time was recorded on a 4-point scale: 1 = inactive, 2 = light, 3 = moderate, and 4 = heavy. This classification system was significantly correlated with more detailed activity assessments reported in previous exercise research on a subset of these individuals several years earlier based on the Allied Dunbar Health Survey (Etherington *et al.*, 1996) as previously described. Household income data were also collected on a subsample of respondents willing to disclose this information (69%).

A venous blood sample was taken from each twin following an overnight fast. The isolated WBCs were used for extraction of DNA. Terminal restriction fragment length (TRFL), an index of telomere length, was measured by Southern blot method as previously described (Benetos *et al.*, 2001). The laboratory conducting the TRFL measurements was completely blinded to all characteristics of the WBC donors. Results of TRFL measurements, identified only by coded ID numbers, were electronically transmitted and merged with the covariate data.

Statistical analyses (cross-sectional analysis)

We used standard multiple linear regression techniques to correlate the TRFL with parameters of interest and, using multivariate analyses of variance, tested associations between categorical semiquantitative variables and TRFL, adjusting for age and other covariates. In order to explore linear trends, we stratified by SES I, II, IIIN, IIIM, IV and V. For the purpose of analysis, SES IV and V were merged, as there were fewer than 50 in the lowest group (unskilled manual workers). Using multivariate analysis of variance, we computed least-squared TRFL means and the corresponding standard errors for specific groups of SES or physical activity level, adjusted for age and, where indicated, for other covariates. To adjust for non-independence between twins in a pair, bootstrap sets were generated selecting a random twin from each pair using analysis of variance and the *P*-value of the mean test statistic from 100 replicates was used to confirm statistical significance. S-Plus 6.0 (Insightful Corp) software was used.

Discordant twin pair analysis

To further test our hypothesis, we examined within twin pair TRFL differences for 17 pairs of twins who were raised together

until at least 16 years but are currently discordant for SES (I vs. IV/V). All such twins were non-identical. This comparison reduces the effect of random genetic and environmental variation as non-identical twins (DZ) share on average 50% of their genes and most of their early environmental influences and education. Overall mean and standard error of within pair TRFL difference were calculated.

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