We estimated the possible contribution of cases missed because field reports were incomplete, as follows: 40 of 66 (61%) of all cases of PCHI identified in this study were confirmed before the age of 18 months (data not shown) and the mean rate of emigration of the general population of 0–15-year olds out of the four study districts was 1·4% per annum,[9] thus over 95% of true cases in this birth cohort have probably been identified in our study. Furthermore, the similar figures for prevalence of 121 and 124 cases per 100 000 population born during respective periods with UNS and without UNS suggests that any under-ascertainment did not bias the estimate of the size of the effect of UNS.

The US Preventive Services Task Force[4] has stated that better evidence about the effectiveness of UNS is needed and could be obtained via population-based studies that begin with inception cohorts and carefully report outcomes in all possible patients, as well as rates of loss to follow-up. Our report fits this description and is the strongest available evidence of the added benefit of UNS in the early detection of PCHI. Assessment of the effect of early intervention on the speech and language of the children and the costs incurred by their families and by health providers is in progress and will be the subject of future reports. Policymakers should consider these data, as well as those presented here, when contemplating the introduction of UNS.

Contributors

The design and application for funding of this study was developed from an idea by C R Kennedy with help from M J Campbell; A R D Thornton, L Kimm, and D C McCann oversaw the conduct of the earlier and later stages of the study, respectively, with help from all the other authors. M J Campbell, assisted by C R Kennedy and D C McCann, undertook statistical analysis. All authors contributed to the preparation of the manuscript.

Conflict of interest statement

We declare that we have no conflict of interest.

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Obesity, cigarette smoking, and telomere length in women

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Obesity and smoking are important risk factors for many age-related diseases. Both are states of heightened oxidative stress, which increases the rate of telomere erosion per replication, and inflammation, which enhances white blood cell turnover. Together, these processes might accelerate telomere erosion with age. We therefore tested the hypothesis that increased body mass and smoking are associated with shortened telomere length in white blood cells. We investigated 1122 white women aged 18–76 years and found that telomere length decreased steadily with age at a mean rate of 27 bp per year. Telomeres of obese women were 240 bp shorter than those of lean women (p=0·026). A dose-dependent relation with smoking was recorded (p=0·017), and each pack-year smoked was equivalent to an additional 5 bp of telomere length lost (18%) compared with the rate in the overall cohort. Our results emphasise the pro-ageing effects of obesity and cigarette smoking.

Telomeres cap the ends of chromosomes and protect them from degradation and end-to-end fusion. Telomeres of cultured somatic cells undergo erosion with each cycle of replication, and oxidative stress enhances this process.[1]

Both obesity and cigarette smoking are important risk factors in many age-related diseases, and are associated with increased oxidative stress and inflammation.[2,3] The latter process is marked by increased white blood cell (WBC) turnover. Telomere attrition (expressed in WBCs)

can serve as a marker of the cumulative oxidative stress and inflammation and, consequently, show the pace of biological ageing. We therefore expected obese individuals and smokers to have shortened telomeres. To investigate this hypothesis we studied WBC telomere length in 1122 healthy white women aged 18–72 years, examining the relations with both smoking and obesity-related phenotypes.

Participants were female twins (45 monozygotic and 516 dizygotic pairs) from the TwinsUK Adult Twin Registry, a group previously developed to study the heritability and genetics of diseases with a higher prevalence among women. These women were recruited from the general population through national media campaigns in the UK, and were similar to age-matched population singletons in terms of disease-related and lifestyle characteristics (www.twinsuk.ac.uk).[4] In our cohort, body-mass index (BMI) was >30 in 119 (11%) women and <20 in 85 (8%). None of the participants had clinical diabetes. 531 (47%) women had never smoked, 369 (33%) were ex-smokers, 203 (18%) were still smoking, and smoking status was unknown for 19 (2%). Smoking history was recorded with a standardised questionnaire. Smoking exposure was measured as pack-years=number of cigarette packs smoked per day×number of years smoking.

A venous blood sample was taken after an overnight fast. We extracted DNA from WBCs, and measured the concentration of leptin in serum with a radioimmunoassay (Linco, St Charles, MO, USA). We measured the mean of the terminal telomere restriction fragment (TRF) lengths, an index of telomere length, with the Southern blot method.[5] Written and oral informed consent was obtained from all participants. The St Thomas' Hospital Research Ethics Committee approved the study.

Standard linear regression techniques were used to correlate the TRF length with age and the age-adjusted TRF with individual factors. Log-transformed leptin values were used for both the age-adjusted and unadjusted linear regressions. The associations between categorical variables and telomere length, adjusting for age or other covariates, were assessed using analyses of variance. To adjust for non-independence between twins in a pair, bootstrap sets were generated by 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. No significant difference in the variables studied was noted between monozygotic and dizygotic twins.

Telomere length decreased steadily with age at a mean rate of 27 bp per year (SD 50·2; figure A) and a highly significant negative correlation was detected (table). The proportion of the variance in telomere length accounted for by age was 20·6%. Squared and cubed age terms were also added to the model and had no significant

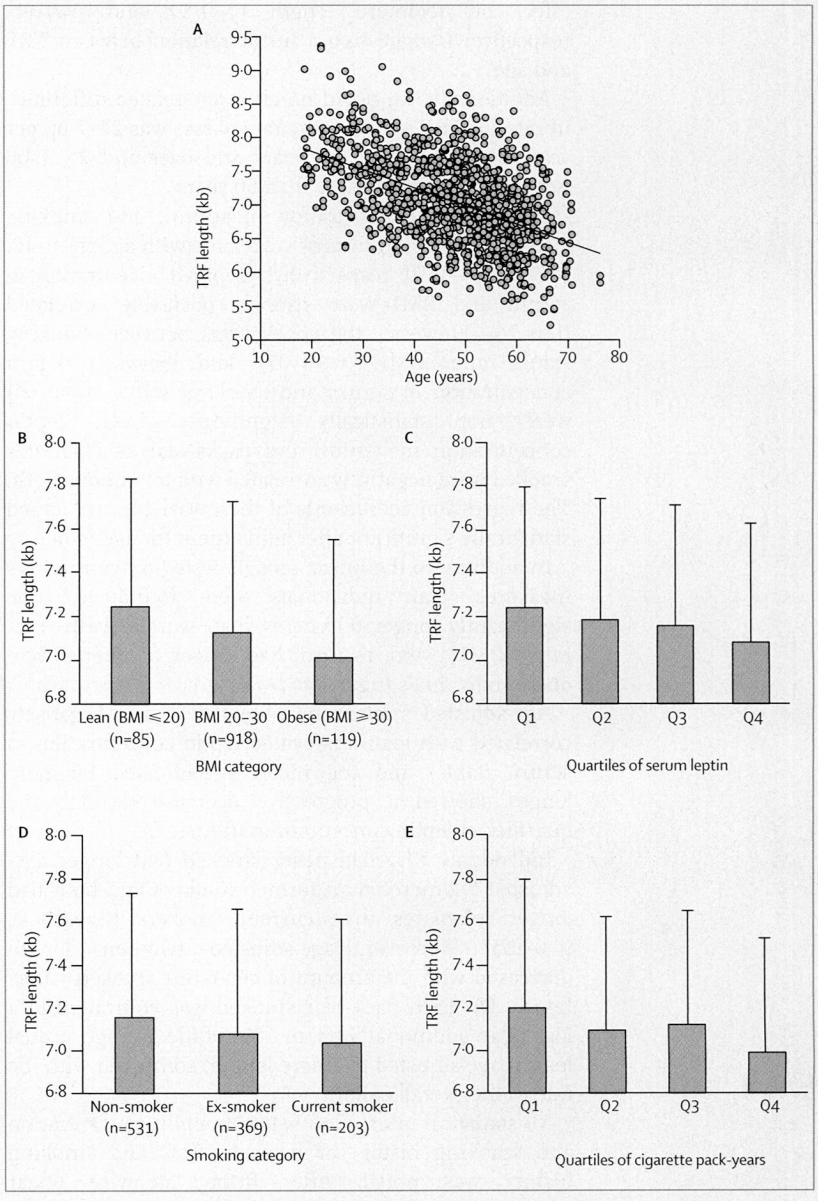

Figure: **Relation between telomere length and (A) age, (B) BMI, (C) leptin, (D) smoking history, and (E) cigarette pack-years**
Data for B–E are age-adjusted mean TRF with SD.

	Mean (SD)	Correlation with TRF	p	Age-adjusted correlation with TRF	p*
TRF (kb)	7·06 (0·67)				
Age (years)	47·77 (12·11)	−0·455	<0·0001	n/a	n/a
BMI (kg/m²)	25·05 (4·69)	−0·126	<0·0001	−0·077	0·031
Serum leptin (ng/mL)	16·26 (12·50)	−0·124	<0·0001	−0·088	0·019
Smoking status†	n/a	−0·031	ns	−0·087	0·017
Cigarette pack-years‡	8·15 (14·31)	−0·214	<0·0001	−0·110	0·045

ns=not significant. *Statistical significance of regression coefficient from 100 bootstrap replicates. †Coded as 0=never smoked, 1=ex-smokers, 2=current smokers. ‡Among ex-smokers and current smokers only.

Table 1: **Descriptive statistics of study subjects and correlations with telomere terminal restriction fragment (TRF) length before and after adjusting for chronological age**

effect on telomere length (p=0·92 and p=0·98, respectively) suggesting a linear relation between TRF and age.

Additionally, we noted no clear age-related difference in rates of TRF loss; average rate of loss was 27·7 bp per year in women aged 50 years and over and 25·7 bp per year in those younger than 50 years.

BMI, leptin concentration in serum, and smoking status were all significantly correlated with age (r= 0·12, r=0·13, r=–0·10, respectively). Leptin concentration in serum and BMI were strongly positively correlated (r=0·76). However, the correlations between smoking status and BMI (r=–0·05) and between leptin concentration in serum and smoking status (r=–0·06) were not statistically significant. BMI, leptin concentration in serum, and packs-year of cigarettes smoked were negatively correlated with telomere length. The regression coefficients of these variables remained statistically significant after adjustment for age (table).

In addition to the linear models tested on continuous measures, lean individuals were found to have significantly longer telomeres than women with mid-range BMIs, who, in turn, had longer telomeres than obese individuals (figure, B; p=0·026).

Age-adjusted telomere length was negatively correlated with log-transformed leptin concentration in serum (table) and the mean age-adjusted telomere length showed a progressive decrease through the quartiles of leptin concentration (figure, C).

Individuals who had never smoked had longer age-adjusted telomeres than former smokers and both had longer telomeres than current smokers (figure, D; p=0·02). Moreover, age-adjusted telomere length decreased with the amount of cigarettes smoked (table; figure, D). Each pack-year smoked was equivalent to a loss of an additional 5 bp, or 18% of the average annual loss in age-adjusted telomere length, compared with the rate in the overall cohort.

No statistical interaction between leptin concentration and smoking history or between BMI and smoking history was noted. After fitting stepwise linear regression, age, smoking (p<0·0004), and leptin (p<0·006) remained significantly associated with telomere length, but BMI did not, suggesting that the mechanisms by which obesity affects telomere length might be better represented by leptin concentration than by BMI. We conclude that both obesity and smoking are associated with shortened WBC telomere length in women. Additionally, telomere length was inversely correlated with the serum concentration of leptin—a marker and regulator of body fat that itself may have some pro-inflammatory properties known to increase oxidative stress.[6]

Our findings suggest that obesity and cigarette smoking accelerate human ageing. Our cross-sectional data underscore the considerable variation in telomere length between individuals. Thus large cohorts are needed to capture the effects of inflammation and oxidative stress.[1] However, in view of the hypothesis that telomere length in vivo represents cellular turnover and exposure to oxidative and inflammatory damage, the difference in telomere length between being lean and being obese corresponds to 8·8 years of ageing; smoking (previous or current) corresponds on average to 4·6 years of ageing; and smoking a pack per day for 40 years corresponds to 7·4 years of ageing. Our results emphasise the potential wide-ranging effects of the two most important preventable exposures in developed countries—cigarettes and obesity.

Contributors

A M Valdes participated in the statistical analysis and in the preparation of the manuscript. T Andrew participated in the processing and statistical analysis of the data. E Oelsner and L F Cherkas collected and verified the clinical information of the study participants. J Gardner and M Kimura did the telomere assays and participated in the processing of the data. T D Spector and A Aviv designed and coordinated the study and participated in the preparation of the manuscript.

Conflict of interest statement

We declare that we have no conflict of interest.

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