

Homocysteine levels and leukocyte telomere length

J.B. Richards^a, A.M. Valdes^a, J.P. Gardner^b, B.S. Kato^a, A. Siva^c,
M. Kimura^b, X. Lu^b, M.J. Brown^c, A. Aviv^{b,1}, T.D. Spector^{a,*,1}

^a Centre for Twin Research and Genetic Epidemiology Unit, St. Thomas' Hospital, King's College London School of Medicine, London SE1 7EH, UK

^b The Center of Human Development and Aging, University of Medicine and Dentistry of New Jersey, Newark, NJ, USA

^c Clinical Pharmacology Unit, Addenbrooke's Hospital, University of Cambridge, Cambridge, UK

Received 30 August 2007; received in revised form 20 December 2007; accepted 21 December 2007

Available online 15 February 2008

Abstract

Objective: Elevated plasma homocysteine is a risk factor for vascular diseases, possibly due to homocysteine-mediated increase in oxidative stress and inflammation. As leukocyte telomere length (LTL) registers the cumulative oxidative stress and inflammation, we examined the relationship between homocysteine and LTL.

Methods: LTL was measured using the Southern blot method. The relationship between LTL and homocysteine levels was considered for confounding with the following covariates: age, sex, smoking, obesity, physical activity, menopause, hormone replacement therapy use and creatinine clearance.

Results: 1,319 healthy subjects were recruited from a population-based cohort. LTL was negatively correlated with plasma homocysteine levels, after adjustment for smoking, obesity, physical activity, menopause, hormone replacement therapy use and creatinine clearance. The difference in multiply-adjusted LTL between the highest and lowest tertile of homocysteine levels was 111 base pairs ($p = 0.004$), corresponding to 6.0 years of telomeric aging. This relationship was further accentuated by decreased concentrations of serum folate and increased levels of C-reactive protein.

Conclusions: Increased homocysteine levels are associated with shortened LTL, further supporting the tenet that LTL is an index of cardiovascular risk.

© 2008 Elsevier Ireland Ltd. All rights reserved.

Keywords: Telomeres; Homocysteine; Oxidative stress; Inflammation; Cardiovascular disease

1. Introduction

Classical homocystinuria (cystathionine-beta synthase deficiency) is a rare disease characterized by a marked increase in plasma homocysteine levels and early onset of many age-related diseases, such as severe premature atherosclerosis, thromboembolic disease and osteoporotic fractures [1]. In normal subjects without homocystinuria, plasma homocysteine levels are increased in essential hypertension [2], cardiovascular disease (CVD) [3–6], osteoporosis [7] and dementia [8,9].

Shortened leukocyte telomere length (LTL) has been observed in a similar spectrum of diseases and conditions marked by increased oxidative stress and inflammation, including hypertension and atherosclerotic CVD [10–13]. In addition, LTL is shortened in osteoarthritis [14], dementia [15], obesity, insulin resistance [16,17], cigarette smoking [16,18] and hypovitaminosis D [19].

The common threads that link elevated plasma homocysteine levels with shortened LTL may be oxidative stress and inflammation.

In the vasculature, homocysteine increases oxidative stress [20,21] which may partially explain the association between homocysteine and CVD. Homocysteine is associated with a decrease in both the number and function of endothelial progenitor cells [22,23], which might help to repair

* Corresponding author. Tel.: +44 207 188 6765; fax: +44 207 188 6718.

E-mail address: tim.spector@kcl.ac.uk (T.D. Spector).

¹ These authors contributed equally to the work.

vessel damage and prevent vascular events [24,25]. In cultured somatic cells, including endothelial cells, oxidative stress accelerates telomere attrition per cell division [26–30]. Importantly, homocysteine accelerates telomere attrition by increasing telomere loss per replication of vascular endothelial cells. This process is largely reversed by catalase, indicating that the homocysteine effect on telomere dynamics is mediated by oxidative stress [31]. In addition, in endothelial progenitor cells, homocysteine might inhibit telomerase [32], the reverse transcriptase that adds back telomere repeats onto chromosomal ends [33,34].

As oxidative stress provokes inflammation and vice versa, it is difficult to dissociate *in vivo* between these two processes, but LTL registers the cumulative burden of both. This is because oxidative stress might shorten the lifespan of hematopoietic stem cells (HSCs) [35]. Thus, oxidative stress would enhance telomere attrition not only because it causes a greater telomere loss per replication [28–32], but also due to the increased replication of HSCs to maintain the HSC pool. In addition, increased oxidative stress and inflammation would shorten the biological life of peripheral leukocytes. It is anticipated, therefore, that if homocysteine increases oxidative stress and inflammation, an inverse relationship might exist between LTL and plasma homocysteine. We have tested this hypothesis in a population-based cohort of men and women across a wide age spectrum. We also explored the effects of folate and C-reactive protein (CRP), an index of inflammation [36], on this relationship.

2. Methods

2.1. Study population

Our study population consisted of members of the Twin-sUK cohort (www.twinsuk.ac.uk), which is an adult twin registry investigating many age-related phenotypes, including, but not limited to: CVD, arthritis, osteoporosis, eye disease and obesity. This study population has been previously shown to be representative of singleton populations and the United Kingdom population in general [37]. The study was approved by the Guy's and St. Thomas' Hospital Ethics Committee. Participants provided written informed consent.

2.2. Phenotypic variables

The body mass index (BMI) of each subject was calculated. Physical activity was recorded as inactive, light, moderate or heavy exercise during leisure time. This previously validated measure of activity correlated well with an in-depth measure of physical activity in the Dunbar Health Survey [38]. Subjects were asked if they were currently smoking cigarettes daily.

2.3. Biochemical measurements

Fasting plasma homocysteine was measured by a rapid high-performance liquid chromatographic assay for total homocysteine [39]. The between-batch coefficient of variation (CV) for this method is 6.6%. A competitive binding radioimmunoassay was used to measure serum folate levels (BioRad). Serum C-reactive protein (CRP) levels were measured using an ELISA method. The lower limit of detection of this assay is 0.15 mg/l and has a CV of 8.7% at 0.5 mg/l. Fasting plasma creatinine was measured using the Vitros 950 analyser (Johnson & Johnson). The inter-assay CV for plasma creatinine was 1.1% at 81 $\mu\text{mol/l}$. Glomerular Filtration Rate (GFR) was calculated using the formula: $[(140 - \text{age}) - \text{weight}] / \text{plasma creatinine}$, multiplied by 0.85 for females [40].

2.4. Leukocyte telomere length measurement

Leukocyte DNA was extracted from freshly frozen whole blood. The mean LTL was assessed using the terminal restriction fragment length (TRFL) which was measured using Southern blot analysis as described previously [41]. Briefly, each sample was digested using restriction enzymes and resolved on 0.5% agarose gels. DNA was then depurinated and denatured. After transfer to a positively charged nylon membrane, hybridization with digoxenin 3'-end-labeled telomeric probes was conducted overnight. Probes were then detected using a digoxenin luminescent detection procedure (Roche). Autoradiograph scanning was performed to delineate a histogram for each TRFL signal and mean TRFL was calculated from this histogram. Each DNA sample was resolved in duplicate (on different gels). If the difference between the duplicates was >5%, a third measurement was performed and the mean of two results <5% apart was taken. This occurred in <5% of the samples. The CV of the TRFL assay in this study was 1.4%. The laboratory conducting the TRFL measurements was blinded to all characteristics of the leukocyte donors, who were identifiable only by coded ID numbers.

2.5. Statistical methods

The normality of variables was assessed and CRP and homocysteine levels were subsequently log-transformed. LTL was adjusted for covariates which were selected by employing the Bayesian Information Criterion (BIC). The variables considered as potential confounders were age, sex, BMI, GFR, smoking status, menopausal and HRT status for women, CRP, LTL-measurement batch effects and physical activity levels. Since we were interested in the relationship between homocysteine levels and LTL, we assessed potential confounders by analyzing the change in the regression coefficient for homocysteine across the top five models as generated by the BIC. If a variable had no effect on LTL and there was no confounding with homocysteine, the variable

was removed from the model. A quadratic term for age and interaction terms between CRP and homocysteine and folate and homocysteine were also considered, but did not achieve statistical significance and were not included in subsequent analyses.

The relationship between homocysteine levels and LTL was further assessed by dividing the population into tertiles of homocysteine levels and calculating the mean multiply-adjusted LTL for each homocysteine tertile. To further assess the relationship between LTL, homocysteine and CRP, again, the multiply-adjusted LTL (but unadjusted for CRP) for each homocysteine tertile was calculated, and each tertile was subsequently divided into those subjects with a CRP level greater and less than 2.0 mg/l, which is regarded as a lower limit for clinically detectable inflammation [36]. To assess the contribution of homocysteine to the model predicting LTL, we performed nested regression. The addition of homocysteine to the covariates selected by the BIC, improved the model substantially ($p=0.0003$, for the F -statistic for the model including homocysteine). For all regression analyses, since twins are not necessarily independent observations, we controlled for familial aggregation by using the robust regression cluster option in Stata/SE 9.2. All statistical analysis was performed using Stata/SE 9.2 (College Station, TX, USA) with the addition of the BIC software component [42].

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

3. Results

3.1. General characteristics of the cohort

Table 1 summarizes characteristics of the 1319 subjects (91.5% women) included in the study cohort. The majority of the subjects were non-smokers and approximately half of the study sample reported moderate or heavy physical activity.

3.2. Relationships between homocysteine, age, LTL, folate and CRP

LTL was negatively correlated with age (Pearson correlation coefficient $r=-0.33$, $p<0.0001$), with an extrapolated annual rate of LTL decrease of 18.5 base pairs per year. LTL (Fig. 1A) and age-adjusted LTL (Fig. 1B) were negatively correlated with plasma homocysteine level, which increased with age. CRP was positively correlated ($r=0.09$, $p=0.0036$), while serum folate level was negatively correlated ($r=-0.29$, $p<0.0001$) with plasma homocysteine level.

In linear regression analysis, increased plasma homocysteine was associated with a decreased age-adjusted LTL (β coefficient for log-transformed homocysteine = -0.21 , 95% confidence interval [CI]: -0.33 , -0.09). This relationship was similar when serum CRP levels, GFR, physical activity and menopausal/estrogen status were included in the

Table 1
Selected characteristics of study population

	N = 1319
Age (years) ^a	49.0 (12.5)
Women	1207 (91.5%)
Leukocyte telomere length (kb) ^a	6.91 (0.69)
Homocysteine ($\mu\text{mol/l}$) ^a	
Lowest tertile	6.0 (0.85)
Middle tertile	8.1 (0.59)
Highest tertile	11.8 (4.2)
Serum folate (nmol/l) ^a	12.4 (6.1)
Serum CRP (mg/l) ^a	3.3 (7.0)
BMI (kg/m^2) ^a	25.6 (4.6)
Physical activity	
Inactive or light	720 (55%)
Moderate or heavy	553 (45%)
Menopausal and HRT status for women	
Pre-menopausal	241 (18.3%)
Menopausal and never HRT	797 (60.4%)
Menopausal and former HRT	149 (11.3%)
Menopausal and current HRT	132 (10.0%)
Glomerular filtration rate (ml/min) ^a	87.1 (25.6)
Smoking status	
Non-smoker	1059 (80.3%)
Smoker	260 (19.7%)

BMI = body mass index; CRP = C-reactive protein; HRT = hormone replacement therapy.

^a Mean and [S.D.] or count and [%].

regression model ($\beta = -0.26$, 95% CI: -0.38 , -0.14), indicating that these covariates did not confound the relationship between homocysteine and LTL. Increased serum folate was associated with an increase in age-adjusted LTL ($\beta = 0.01$, 95% CI: 0.004, 0.017), while increased CRP was associated with a decrease in age-adjusted LTL ($\beta = -0.02$, 95% CI: -0.04 , -0.001).

After consideration of all listed covariates using the BIC, the relationship between LTL and homocysteine was adjusted for age, sex and measurement batch-effects. Increasing tertiles of homocysteine were associated with progressively shorter multiply-adjusted LTL (p -value for trend = 0.007) (Fig. 2). The multiply-adjusted difference in LTL between the

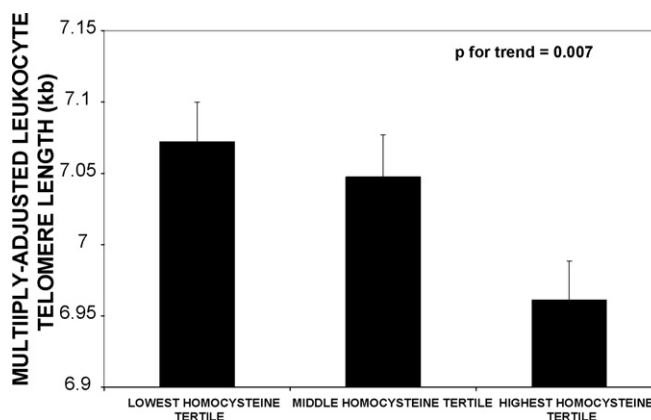


Fig. 2. Multiply-adjusted association between tertiles of homocysteine levels and leukocyte telomere length (kb). (adjusted for age, sex and measurement-batch effects. vertical bars represent S.E.).

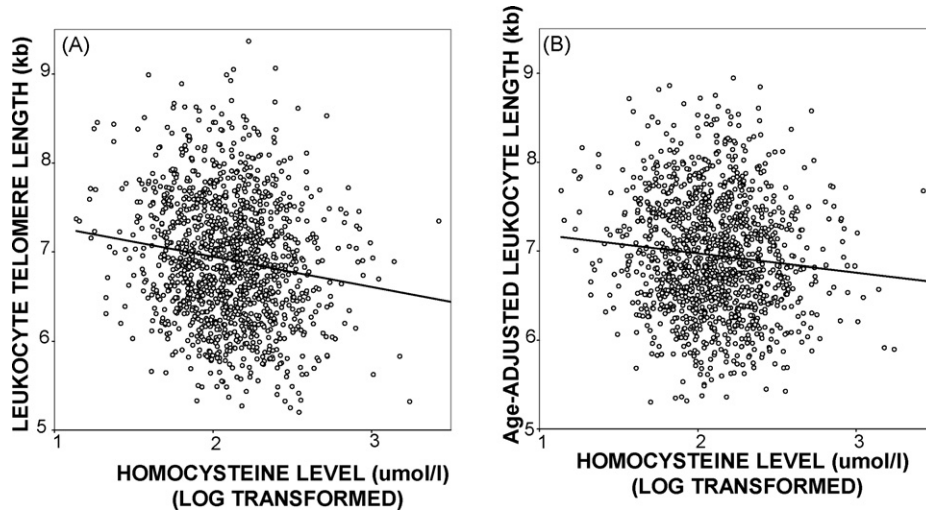


Fig. 1. Relationship between (A) homocysteine levels and leukocyte telomere length (kb) ($n = 1319$, Pearson correlation coefficient = -0.15 , $p < 0.0001$) and (B) homocysteine levels and age-adjusted leukocyte telomere length (kb) ($n = 1319$, Pearson correlation coefficient = -0.10 , $p = 0.0002$).

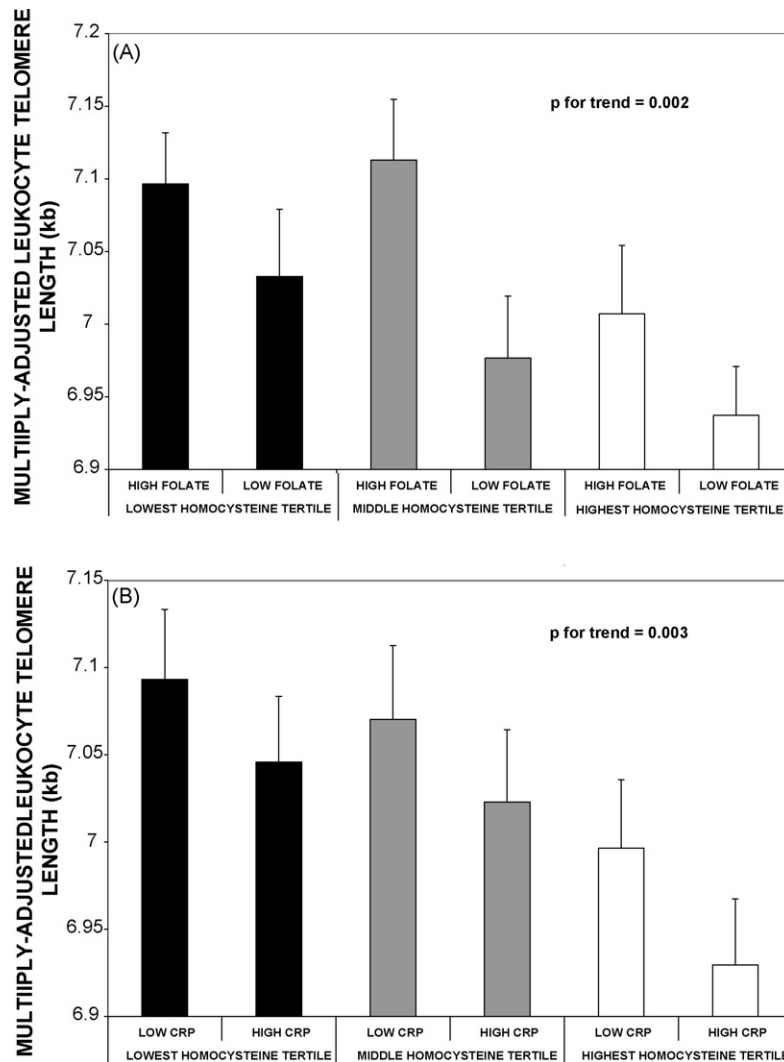


Fig. 3. (A) Multiply-adjusted association between tertiles of homocysteine levels and leukocyte telomere length (kb), stratified by serum folate level. (B) Multiply-adjusted association between tertiles of homocysteine levels and leukocyte telomere length (kb). Stratified by serum CRP level (adjusted for age, sex and measurement-batch effects). Vertical bars Represent S.E. High and low folate levels delineated by Median folate level).

highest and lowest tertile of plasma homocysteine was 111 base pairs (95% CI: 35, 189, $p=0.004$), which is equivalent to 6 years of average telomeric attrition. Within each tertile of plasma homocysteine levels, individuals with higher serum folate levels had a longer LTL (p -value for trend=0.002) (Fig. 3A). On the other hand, within each tertile of homocysteine, individuals with higher levels of serum CRP had a shorter LTL (p -value for trend=0.003) (Fig. 3B). When assessing extreme groups, the difference in LTL between individuals with high folate levels in the lowest tertile of homocysteine levels and those subjects with low folate in the highest tertile of homocysteine levels was 159 base pairs (95% CI: 64, 255, $p=0.001$) which is equivalent to 8.6 years of average telomeric attrition (Fig. 3A). Similarly, the difference in LTL between individuals with low CRP levels in the lowest tertile of homocysteine levels and those subjects with high CRP levels in the highest tertile of homocysteine levels was 164 base pairs (95% CI: 55, 271, $p=0.003$) or 8.9 years of average telomeric attrition (Fig. 3B). These results indicate the relationship between homocysteine and LTL is further modified by CRP and folate levels.

4. Discussion

In this large, population-based study, LTL was inversely associated with plasma homocysteine levels and this relationship was accentuated by lower serum folate and higher serum CRP levels. The difference in multiply-adjusted LTL between the highest and lowest tertile of homocysteine was equivalent to a cumulative loss of telomere length over a period of 6 years. In addition, our findings point to an easily modifiable risk factor, folate, a low level of which is associated with shorter LTL. By reducing homocysteine levels, folate supplementation may attenuate telomere attrition—a proposition that can be tested experimentally.

LTL is highly variable among newborns [43,44] and adults [56]. Accordingly, in most studies, age itself accounts for less than 15% (<11% in this study) of LTL variability among individuals. Cross-sectional telomere length therefore displays a high inter-individual variability, underscoring the need to study large numbers of subjects to detect modest effects, and the proportion of variance in LTL accounted for by homocysteine levels reflects this high inter-individual variability.

Although we cannot infer causality from the inverse associations between LTL and homocysteine, it is very unlikely that shortened LTL causes an increase in plasma homocysteine. A more likely explanation is that elevated homocysteine accelerates LTL attrition by increasing demand for proliferation of HSCs and a greater telomere loss per replication due to homocysteine-mediated increase in oxidative stress. Whether homocysteine enhances telomere shortening in the vascular endothelium *in vivo* is unknown. That said, homocysteine accelerates telomere attrition in cultured vascular endothelial cells and increases the expression of intracellular adhesion molecule-1 (ICAM-1) and plasminogen activator

inhibitor-1 (PAI-1), which are major factors in the development of atherosclerosis [33]. Endothelial cells retrieved from atherosclerotic lesions display increased markers of cellular senescence and telomerase inhibition in these cells leads to increased production of ICAM-1 [45]. Furthermore, the constitutive expression of human telomerase reverse transcriptase increases the regenerative capacity of endothelial progenitor cells [46]. Though, for obvious reasons, large-scale epidemiological studies cannot resort to harvesting human vascular endothelial cells, the negative correlation between LTL and plasma homocysteine suggests a similar association between telomere length in the vascular endothelium and homocysteine—a phenomenon that would be likely be driven by homocysteine-mediated increase in oxidative stress and inflammation. The modification of the association between LTL and homocysteine by CRP further supports the role of homocysteine-mediated inflammation in leukocyte telomere dynamics.

Our findings are consistent with previous observations that LTL is associated with circumstances and disorders marked by increased oxidative stress and inflammation [11–13,16,17,47,48] (reviewed elsewhere) [49]. Analysis of the placebo arm of a recent randomized controlled trial found that LTL was a potent predictor of early myocardial infarction, however, this relationship was not explained by correlation between LTL and classical CVD risk factors, although homocysteine was not considered [12]. LTL has also recently emerged as a marker for severity and presence of chronic heart failure that was primarily due to ischemic heart disease [48]. These CVD risk factors are strongly associated with increased oxidative stress and inflammation, and thus LTL may record their cumulative burden.

If homocysteine does indeed play a causal role in telomere shortening, then we would expect that this effect would be mitigated by folate. Similarly, if the mechanism whereby homocysteine decreased telomere length involved inflammation, then we would hypothesize that increased levels of systemic inflammation would potentiate this effect. Our study supports both of these postulates.

The risk attributed that homocysteine imparts on CVD appears to increase after homocysteine levels exceed 9–10 $\mu\text{mol/L}$ [3,5,6]. The mean homocysteine level in the highest tertile for homocysteine was 11.8 $\mu\text{mol/L}$. The decrease in LTL between the highest and lowest homocysteine tertiles was substantial and highly statistically significant. Thus, although homocysteine levels account for a small proportion of the variance in telomere length, our data suggests that these results may have clinical relevance.

A recent randomized controlled trial of folate supplementation in a predominantly American population did not demonstrate efficacy in the secondary prevention of stroke [50]. However, this trial may have been affected by the concomitant population-wide administration of folate to the American grain supply [51], which seems to have effectively decreased population homocysteine levels [52] and may have therefore obscured any treatment effects. As the United King-

dom does not currently fortify the grain supply with folate, our study then provides a unique opportunity to assess subjects who have not received folate supplementation. Two other recent trials have failed to demonstrate a protective effect of homocysteine on vascular events [53,54]. However, whether or not the findings from these two trials are applicable to cerebrovascular events, and especially the elderly, remains controversial [55]. Despite this, it seems apparent that vitamin therapy to lower homocysteine, at the doses used in the trials, does not clearly protect against vascular events.

Two potential limitations to this study are noteworthy. Our study sample consisted of twins, which are not necessarily independent observations and we have therefore employed robust regression methods to control for any possible non-independence of twins. In addition, our study population consistently almost entirely of women and therefore these results cannot be directly extrapolated to men.

In conclusion, our results suggest that shortened LTL is independently associated with high plasma homocysteine in a large population of healthy women and men, but the mechanism whereby these risk factors influence vascular risk remains unresolved. Our findings and previous work showing the effect of homocysteine on telomere dynamics in vascular endothelial cells, and the relationship between LTL and CVD, suggest a mechanistic link between homocysteine and CVD.

Acknowledgements

Funding Sources: Wellcome Trust (TDS, AV); Arthritis Research Campaign (TDS, AV); Canadian Institutes of Health Research (JBR); European Society for Clinical and Economic Aspects of Osteoporosis (JBR); NIH grants AG021593 and AG020132, and The Healthcare Foundation of New Jersey (AA). The authors would like to thank the subjects who participated in this study.

References

- [1] Rezvani I, Rosenblatt DS, Behrman. Methionine, Chapter 74. In: Saunders WB, editor. *Nelson Textbook of Pediatrics*. 2004. pp. 405–407.
- [2] Nygard O, Vollset SE, Refsum H, et al. Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine study. *J Am Med Assoc* 1995;274:1526–33.
- [3] Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE. Plasma homocysteine levels and mortality in patients with coronary artery disease. *N Eng J Med* 1997;337:230–7.
- [4] Homocysteine SC. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. *J Am Med Assoc* 2002;288:2015–22.
- [5] Perry IJ, Morris RW, Ebrahim SB, Shaper AG, Refsum H, Ueland PM. Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. *Lancet* 1995;346:1395–8.
- [6] Vasan RS, Beiser A, D'Agostino RB, et al. Plasma homocysteine and risk for congestive heart failure in adults without prior myocardial infarction. *J Am Med Assoc* 2003;289:1251–7.
- [7] McLean RR, Jacques PF, Selhub J, et al. Homocysteine as a predictive factor for hip fracture in older persons. *N Eng J Med* 2004;350:2042–9.
- [8] Seshadri S, Beiser A, Selhub J, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N Eng J Med* 2002;346:476–83.
- [9] Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L, Ueland PM. Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease. *Arch Neurol* 1998;55:1449–55.
- [10] Fitzpatrick AL, Kronmal RA, Gardner JP, et al. Leukocyte telomere length and cardiovascular disease in the cardiovascular health study. *Am J Epidemiol* 2007;165:14–21.
- [11] Brouillette S, Singh RK, Thompson JR, Goodall AH, Samani NJ. White cell telomere length and risk of premature myocardial infarction. *Arterioscler Thromb Vasc Biol* 2003;23:842–6.
- [12] Brouillette SW, Moore JS, McMahon AD, et al. Telomere length, risk of coronary heart disease, and statin treatment in the West of Scotland Primary Prevention Study: a nested case-control study. *Lancet* 2007;369:107–14.
- [13] Demissie S, Levy D, Benjamin EJ, et al. Insulin resistance, oxidative stress, hypertension, and leukocyte telomere length in men from the Framingham Heart Study. *Aging Cell* 2006;5:325–30.
- [14] Zhai G, Aviv A, Hunter DJ, et al. Reduction of leukocyte telomere length in radiographic hand osteoarthritis: a population-based study. *Ann Rheum Dis* 2006;65:1444–8.
- [15] Honig LS, Schupf N, Lee JH, Tang MX, Mayeux R. Shorter telomeres are associated with mortality in those with APOE epsilon4 and dementia. *Ann Neurol* 2006;60:181–7.
- [16] Valdes AM, Andrew T, Gardner JP, et al. Obesity, cigarette smoking, and telomere length in women. *Lancet* 2005;366:662–4.
- [17] Aviv A, Valdes A, Gardner JP, Swaminathan R, Kimura M, Spector TD. Menopause modifies the association of leukocyte telomere length with insulin resistance and inflammation. *J Clin Endocrinol Metab* 2006;91:635–40.
- [18] Nawrot TS, Staessen JA, Gardner JP, Aviv A. Telomere length and possible link to X chromosome. *Lancet* 2004;363:507–10.
- [19] Richards JB, Valdes AM, Gardner JP, et al. Higher serum vitamin D concentrations are associated with longer leukocyte telomere length in women. *Am J Clin Nutr* 2007;86:1420–5.
- [20] Loscalzo J. The oxidant stress of hyperhomocyst(e)inemia. *J Clin Invest* 1996;98:5–7.
- [21] Upchurch Jr, Welch GN, Fabian AJ, et al. Homocyst(e)ine decreases bioavailable nitric oxide by a mechanism involving glutathione peroxidase. *J Biol Chem* 1997;272:17012–7.
- [22] Chen JZ, Zhu JH, Wang XX, et al. Effects of homocysteine on number and activity of endothelial progenitor cells from peripheral blood. *J Mol Cell Cardiol* 2004;36:233–9.
- [23] JunHui Z, XingXiang W, JunZhu C, Jian S, FuRong Z. Reduced number and activity of circulating endothelial progenitor cells from patients with hyperhomocysteinemia. *Arch Med Res* 2006;37:484–9.
- [24] Hill JM, Zalos G, Halcox JJP, et al. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med* 2003;348:593–600.
- [25] Werner N, Kosiol S, Schiegl T, et al. Circulating endothelial progenitor cells and cardiovascular outcomes. *N Engl J Med* 2005;353:999–1007.
- [26] von Zglinicki T. Role of oxidative stress in telomere length regulation and replicative senescence. *Ann N Y Acad Sci* 2000;908:99–110.
- [27] Hall DB, Holmlin RE, Barton JK. Oxidative DNA damage through long-range electron transfer. *Nature* 1996;382:731–5.
- [28] Tchirkov A, Lansdorp PM. Role of oxidative stress in telomere shortening in cultured fibroblasts from normal individuals and patients with ataxia-telangiectasia. *Hum Mol Genet* 2003;12:227–32.
- [29] Hathcock KS, Jeffrey Chiang Y, Hodes RJ. In vivo regulation of telomerase activity and telomere length. *Immunol Rev* 2005;205:104–13.
- [30] Kurz DJ, Decary S, Hong Y, Trivier E, Akhmedov A, Erusalimsky JD. Chronic oxidative stress compromises telomere integrity and accelerates the onset of senescence in human endothelial cells. *J Cell Sci* 2004;117:2417–26.
- [31] Xu D, Neville R, Finkel T. Homocysteine accelerates endothelial cell senescence. *FEBS Lett* 2000;470:20–4.

- [32] Zhu JH, Chen JZ, Wang XX, Xie XD, Sun J, Zhang FR. Homocysteine accelerates senescence and reduces proliferation of endothelial progenitor cells. *J Mol Cell Cardiol* 2006;40:648–52.
- [33] Blackburn EH. Telomere states and cell fates. *Nature* 2000;408:53–6.
- [34] Minamino T, Kourembanas S. Mechanisms of telomerase induction during vascular smooth muscle cell proliferation. *Circ Res* 2001;89:237–43.
- [35] Ito K, Hirao A, Arai F, et al. Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. *Nat Med* 2006;12:446–51.
- [36] Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Eng J Med* 1999;340:448–54.
- [37] Andrew T, Hart DJ, Snieder H, de LM, Spector TD, MacGregor AJ. Are twins and singletons comparable? A study of disease-related and lifestyle characteristics in adult women. *Twin Res* 2001;4:464–77.
- [38] Etherington J, Harris PA, Nandra D, et al. The effect of weight-bearing exercise on bone mineral density: a study of female ex-elite athletes and the general population. *J Bone Miner Res* 1996;11:1333–8.
- [39] Ubbink JB, Vermaak WJH, Bissbort S. Rapid high-performance liquid-chromatographic assay for total homocysteine levels in human serum. *J Chromatogr -Biomed Appl* 1991;565:441–6.
- [40] Levey AS, Bosch JP, Lewis JB, et al. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 1999;130:461–70.
- [41] Benetos A, Okuda K, Lajemi M, et al. Telomere length as an indicator of biological aging—the gender effect and relation with pulse pressure and pulse wave velocity. *Hypertension* 2001;37:381–5.
- [42] Millar P. BIC: Stata module to evaluate the statistical significance of variables in a model. In: *Statistical Software Components*. Boston College Department of Economics; 2005.
- [43] Okuda K, Bardeguet A, Gardner JP, et al. Telomere length in the newborn. *Pediatr Res* 2002;52:377–81.
- [44] Akkad A, Hastings R, Konje JC, Bell SC, Thurston H, Williams B. Telomere length in small-for-gestational-age babies. *BJOG* 2006;113:318–23.
- [45] Minamino T, Miyauchi H, Yoshida T, Ishida Y, Yoshida H, Komuro I. Endothelial cell senescence in human atherosclerosis: role of telomere in endothelial dysfunction. *Circulation* 2002;105:1541–4.
- [46] Murasawa S, Llevadot J, Silver M, Isner JM, Losordo DW, Asahara T. Constitutive human telomerase reverse transcriptase expression enhances regenerative properties of endothelial progenitor cells. *Circulation* 2002;106:1133–9.
- [47] Fitzpatrick AL, Kronmal RA, Gardner JP, et al. Leukocyte telomere length and cardiovascular disease in the cardiovascular health study. *Am J Epidemiol* 2006.
- [48] van der Harst P, van der Steege G, de Boer RA, et al. Telomere length of circulating leukocytes is decreased in patients with chronic heart failure. *J Am Coll Cardiol* 2007;49:1459–64.
- [49] Minamino T, Komuro I. Vascular cell senescence: contribution to atherosclerosis. *Circ Res* 2007;100:15–26.
- [50] Toole JF, Malinow MR, Chambless LE, et al. Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: the vitamin intervention for stroke prevention (VISP) randomized controlled trial. *J Am Med Assoc* 2004;291:565–75.
- [51] Casas JP, Bautista LE, Smeeth L, Sharma P, Hingorani AD. Homocysteine and stroke: evidence on a causal link from mendelian randomisation. *Lancet* 2005;365:224–32.
- [52] Jacques PF, Selhub J, Bostom AG, Wilson PW, Rosenberg IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. *N Engl J Med* 1999;340:1449–54.
- [53] Lonn E, Yusuf S, Arnold MJ, et al. Homocysteine lowering with folic acid and B vitamins in vascular disease. *N Engl J Med* 2006;354:1567–77.
- [54] Bona KH, Njolstad I, Ueland PM, et al. Homocysteine lowering and cardiovascular events after acute myocardial infarction. *N Engl J Med* 2006;354:1578–88.
- [55] Spence JD. Homocysteine-lowering therapy: a role in stroke prevention? *Lancet neurol* 2007;6:830–8.
- [56] Aviv A, Valdes AM, Spector TD, Human telomere biology: pitfalls of moving from the laboratory to epidemiology. *Int. J. Epidemiol.*, 2006.