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Effect of vegetable consumption on the association between peripheral leucocyte telomere length and hypertension: a case–control study

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ABSTRACT

Objectives: Peripheral leucocyte telomere length has been suggested to be inversely associated with hypertension risk. Both telomere length and hypertension risk can be modified by certain dietary factors, such as fruit and vegetables. This study was to examine the potential effect of these dietary factors on the association between telomere length and hypertension risk.

Study design: A community-based case–control study.

Participants: 271 hypertensive patients and 455 normotensive controls aged 40–70 years and living in Yinzhou, Zhejiang Province, China.

Outcome measures: Peripheral leucocyte relative telomere length (RTL) was measured using quantitative real-time PCR. Dietary intake was assessed by a brief semiquantitative food frequency questionnaire. The association between RTL and hypertension risk was analysed using logistic regression, and the modulatory effect of dietary intake on RTL-related hypertension risk was analysed using likelihood ratio tests.

Results: Among controls, longer age-adjusted RTL was associated with higher vegetable intake (p=0.01). Individuals with longer age-adjusted RTL (based on median value) were 30% less likely to have hypertension (OR=0.70, 95% CI 0.52 to 0.96; p=0.03). The observed RTL-hypertension relationship appeared to be modified by vegetable intake—longer RTL was significantly associated with lower hypertension risk only in those with greater vegetable consumption (OR=0.28, 95% CI 0.14 to 0.57; p<0.001), but not in those with lower vegetable intake (P-interaction=0.008).

Conclusions: Certain dietary factors might modify telomere-related hypertension risk.

Strengths and limitations of this study

- To the best of our knowledge, this is the first population-based study to examine the potential effects of food on the association between telomere length and hypertension risk.
- The study found that longer telomere length was associated with lower risk of hypertension only in those consuming the highest levels of vegetables, suggesting a synergistically protective effect on hypertension risk.
- The study used a brief version of a Chinese food frequency questionnaire that included only 11 food items, which limited our further analysis of the relationship between telomere length and specific nutrient intake.

Telomeres are tandem repeat nucleotide sequences of TTAGGG that extend several kilobases at the end of mammalian chromosomes and play essential roles in the maintenance of chromosome stability and integrity.[1] In somatic cells, telomeres shorten with mitotic cycles due to the so-called 'end-replication problem' and also with oxidative stress. Therefore, telomere length is regarded as a marker for biological ageing and age-related diseases that reflects cell replicative history and lifetime cumulative oxidative stress from environmental exposure to cigarette smoking, poor nutrition and chronic inflammation.[1–5] Essential hypertension is an ageing-related chronic pathological condition which has been considered an important cause of morbidity and mortality around the world.[6] Animal studies found that telomerase-deficient mice with critical short telomeres developed higher blood pressure, which was associated with increased levels of circulating endothelin-1 and enhanced production of reactive oxygen species,[7] suggesting that telomere attribution may have an important role in hypertension aetiology. In humans, it was also shown that telomere length in peripheral leucocytes was inversely associated with pulse pressure,[8 9] diastolic blood pressure[10] and hypertension risk.[11–14]

It has been suggested that leucocyte telomere length could be modulated by certain

dietary factors.[15 16] Individuals following a healthy lifestyle, including a higher intake of fruit and vegetables, had a longer peripheral leucocyte telomere length.[17–19] In particular, previous studies showed that telomere length was positively associated with vegetable consumption[20 21] and dietary intake of nutrients such as dietary fibre,[22] vitamin A and β-carotene,[21 23] and vitamin C.[23] High consumption of fruit and vegetables was also related to lower risk of hypertension,[24–26] whereas interventions with diets rich in fruit and vegetables that were reported to decrease blood pressure[27 28] and the dietary approaches to stop hypertension (DASH) diet that was characterised with high intake of fruit and vegetables have been recommended as practical approaches for hypertension prevention and management by the American Heart Association.[29] Since fruit and vegetables could be associated with both hypertension risk and telomere length, we hypothesised that these dietary factors may affect the relationship between telomere length and hypertension.

In the present study, we examined the association between telomere length and hypertension risk, the association between dietary factors and telomere length, and the potential modification of certain dietary factors (ie, consumption of fruit and vegetables) on the telomere-related hypertension risk in a case–control study of hypertension.

MATERIALS AND METHODS
Participants
The community-based case–control study was conducted in Yinzhou, Zhejiang Province, China. Participants were community residents who enrolled in the New Rural Cooperative Medical Insurance and came to local Community Health Centers for biennial routine health examination between April and July 2013. Hypertensive patients were invited to participate in this study when they met the following criteria: (1) previously diagnosed with hypertension, (2) at least two medical records of systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg, (3) on antihypertensive medication and (4) aged 40–70 years. Those with conditions of diabetes mellitus, dyslipidaemia, secondary hypertension, renal disease or a history of stroke and myocardial infarction were excluded. We then selected controls matched by age and gender. The controls had to meet the following criteria: (1) never diagnosed with hypertension, (2) not receiving antihypertensive treatment and (3) systolic blood pressure <140 mm Hg and diastolic blood pressure <90 mm Hg when measured at the time of health examination. Written informed consent was obtained from all study participants.

Measurement of leucocyte relative telomere length
At the time of visiting health centres, a venous blood sample was obtained from the antecubital vein for measurement of blood lipid levels and genomic DNA extraction. Genomic DNA was extracted from blood samples using TIANamp Blood DNA Kits (Tiangen Biotech, Beijing, China) and adjusted to a final concentration of 20 ng/μL with 10 mmol/L Tris-EDTA (pH 8.0) buffer. All DNA samples were checked for integrity by electrophoresis on 1.0% agarose gel and OD260/280 ratio (1.7–1.9). Relative telomere length (RTL) was measured with a quantitative real-time PCR method that compared the ratio of the telomere repeat copy number with the copy number of the 36B4 single-copy gene in a given sample.[30] The PCR reactions were performed in an ABI Prism 7000 Sequence Detection System. The primer sequences used for the telomeres were: forward primer: 5′-CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT-3′, reverse primers: 5′-GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT-3′. The primers for the single-copy gene 36B4 were: forward primer: 5′-CAG CAA GTG GGA AGG TGT AAT CC-3′, reverse primer: 5′-CCC ATT CTA TCA TCA ACG GGT ACA A-3′. Twenty nanogram of genomic DNA was used for each amplification reaction. The thermal cycling profiles for both amplicons began with 95°C incubation for 15 min, followed by 30 cycles of 15 s at 95°C and 30 s at 60°C. The specificity of amplifications was determined by melting curve analysis. All reactions were done in 96-well PCR plates. Individual samples were measured in triplicate. A pooled DNA sample from 100 randomly selected participants was used as a reference and serially diluted (20, 6.667, 2.222, 0.741 and 0.247 ng) to generate a fresh standard curve for each plate. For each sample, the quantity of telomere repeats and the quantity of 36B4 copies were determined in comparison to the reference sample. The interbatch variability (coefficient of variation) in the study ranged from 4.9% to 12.3%, which was consistent with previous reports.[5]

Assessment of dietary intake and other potential confounders
Dietary intake was assessed via a brief semiquantitative food frequency questionnaire. All participants were asked the amount and frequency of the consumption of 11 major food groups/items during the past 12 months: vegetables, fruit, egg, meat, poultry, fish/seafood, milk, soya milk, fried food, dessert and salt. The intake of salt was self-estimated based on gram per day (see online supplementary material).

During the survey, all participants were also interviewed with a brief questionnaire regarding alcohol consumption, smoking and physical activity (see online supplementary material). Participants who smoked at least one cigarette per week and drank at least once per week were defined as current smokers and current drinkers, respectively. Since only a few people were former smokers (n=23) and former drinkers (n=31), they were counted as non-smokers or non-drinkers. Those exercising at least twice a week were defined as exercisers.

Weight, height, waist circumference and blood pressure were measured using standardised methods during the interview. Plasma concentrations of total cholesterol, triglycerides, low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol were measured on a Hitachi 7180 biochemistry automatic analyser (Hitachi High-Technologies Corporation, Minato-ku, Tokyo, Japan) as indicated in the manual.

Statistical methods

Statistical analysis was performed with SPSS V.16.0.1 (SPSS Inc, Chicago, Illinois, USA). Numeric values were presented as the mean (SD), and category values were expressed as the count (frequency) unless otherwise indicated. RTL was log-transformed to ensure the data fit normal distribution (one sample Kolmogorov-Smirnov test, p=0.20). The log-transformed RTL was further age-adjusted using a residuals method.[31] Log-transformed RTL was regressed on age, and age-adjusted RTL was computed by subtracting predicted value from log-transformed RTL. Independent t test or χ^2 test was used for comparison of parameters, such as age, gender, body mass index and lipid levels, and for comparison between hypertensive and normotensive groups. Independent t test or one-way analysis of variance was used to compare the difference in age-adjusted RTL between genders and among various intake levels of dietary factors. Logistic regression was used to relate telomere length or dietary intake to hypertension risk, after adjustment for potential confounders including age, gender, body mass index, blood total cholesterol, triglycerides, HDL cholesterol, physical activity, alcohol drinking and cigarette smoking. Telomere length was categorised to short and long based on the median value of age-adjusted RTL.

To explore whether the relationship between RTL and hypertension was modified by dietary intake, we used likelihood ratio tests to compare nested models with or without the cross product between RTL and the covariates of interest. Subgroup analyses were then conducted when we observed significant interaction. A value of p<0.05 was considered statistically significant.

RESULTS

During the recruitment period, a total of 1866 participants (1059 normotensive and 807 hypertensive patients) aged 40–70 years completed the survey and blood sample collection. After excluding 534 hypertensive patients with other conditions such as diabetes mellitus or dyslipidaemia, 277 cases were selected for final analysis. 458 controls were recruited from 709 normotensive subjects without listed chronic diseases and matched by age and gender. After further excluding 9 participants (6 cases and 3 controls) who had poor measurements of telomere length, a total of 726 individuals (271 cases and 455 controls) with complete data and acceptable DNA quality were included in the analysis (table 1).

Table 1	Characteristics of participants, according to hypertension status		
	Normotensive (n=455)	**Hypertensive (n=271)**	**p Value***
Gender			0.66
Male	231 (50.8)	133 (49.1)	
Female	224 (49.2)	138 (50.9)	
Age (years)	56.4 (7.51)	57.5 (7.97)	0.067
Regular exercise			0.61
No	309 (67.9)	189 (69.7)	
Yes	146 (32.1)	82 (30.3)	
Current alcohol consumption			0.51
No	281 (61.8)	174 (64.2)	
Yes	174 (38.2)	97 (35.8)	
Current smoking			0.014
No	303 (66.6)	204 (75.3)	
Yes	152 (33.4)	67 (24.7)	
Body mass index (kg/m^2)	22.0 (2.52)	23.2 (3.04)	<0.001
Waist circumference (cm)	78.5 (7.46)	81.5 (8.26)	<0.001
Systolic blood pressure (mm Hg)	120 (11)	148 (12.56)	<0.001
Diastolic blood pressure (mm Hg)	74.6 (7.49)	90.2 (10.7)	<0.001
Total cholesterol (mmol/L)	4.34 (0.51)	4.36 (0.51)	0.62
Triglycerides (mmol/L)	1.07 (0.33)	1.15 (0.39)	<0.01
HDL cholesterol (mmol/L)	1.38 (0.26)	1.35 (0.23)	0.074
LDL cholesterol (mmol/L)	2.62 (0.48)	2.67 (0.46)	0.24
RTL†	1.05 (0.80, 1.34)	0.98 (0.70, 1.31)	–
Age-adjusted RTL	0.069 (0.984)	−0.117 (1.015)	0.015

*Independent t test or χ^2 test.
†Data are median (IQR).
HDL, high-density lipoprotein; LDL, low-density lipoprotein; RTL, relative telomere length.

There was no significant difference in gender and age between the case and control groups (table 1). Hypertensive patients had a significantly higher prevalence of smoking, and higher waist circumference, body mass index, systolic and diastolic blood pressure, blood triglycerides and LDL cholesterol than normotensive controls. Age was inversely associated with RTL (coefficient β=−0.002, p=0.015). Further, the hypertensive group had shorter age-adjusted RTL than the normotensive group (mean age-adjusted RTL: −0.117 vs 0.069, p=0.015) (table 1). There was no significant difference in age-adjusted RTL between males and females in the case and control groups.

Since hypertension had a significant impact on telomere length, we examined the effect of dietary factors on telomere length only in the control group. In normotensive controls, higher vegetable intake was associated with longer age-adjusted RTL (p=0.01), while regular consumption of soya milk (p=0.01) and regular consumption of fried food (p=0.045) were associated with shorter telomere length. We did not observe significant associations between other dietary factors (fruit, meat, poultry, fish/seafood, milk, dessert and salt) and telomere length (table 2).

As expected, longer age-adjusted telomere length was associated with decreased risk of hypertension (OR=0.70, 95% CI 0.52 to 0.96; p=0.03), after adjustment for potential confounders (table 3). We also observed a trend between greater consumption of vegetables, but not fruit, and lower prevalence of hypertension (table 3). For other dietary factors, individuals with a greater intake of salt or a lower intake of fish/seafood had a higher likelihood of having hypertension (table 3).

We further examined whether the association between telomere length and hypertension was modified by fruit and vegetables. We observed that longer RTL was only significantly associated with lower hypertension risk in those with greater vegetable consumption (OR=0.28, 95% CI 0.14 to 0.57; p<0.001), but not in those with lower vegetable intake (P-interaction=0.008) (table 4). The significance remained even after Bonferroni correction for multiple comparisons (α=0.05/2). In contrast, the interaction between RTL and fruit was not significant (P-interaction >0.2). Similar non-significant results were also observed for other dietary factors (P-interaction >0.1 for all).

DISCUSSION

In this community-based case–control study, we found that longer telomere length in peripheral leucocytes was associated with 30% lower odds of having hypertension, and this association was more pronounced among participants with a higher intake of vegetables. To the best of our knowledge, this is the first study to show that certain dietary factors may modify the association between telomeres and hypertension risk.

Table 2 Effect of intake levels of major dietary factors on age-adjusted relative telomere length among 455 normotensive controls*

Dietary factors	n (%)	Age-adjusted RTL (SD)	p Value†
Vegetables (g/day)			
<50	72 (15.9)	−0.064 (0.94)	0.01
50–150	276 (60.8)	0.011 (0.99)	
>150	106 (23.3)	0.32 (0.98)‡	
Fruit (g/day)			
<50	141 (31.1)	0.17 (0.97)	0.35
50–150	263 (57.9)	0.031 (0.98)	
>150	50 (11.0)	0.010 (1.08)	
Meat (g/week)			
<100	240 (52.7)	0.093 (0.92)	0.58
100–200	148 (32.5)	0.083 (1.02)	
>200	67 (14.7)	−0.046 (1.11)	
Fish/seafood (g/week)			
<100	217 (47.7)	0.16 (1.00)	0.17
100–200	128 (28.1)	0.017 (0.95)	
>200	110 (24.2)	−0.044 (0.99)	
Poultry (g/week)			
<50	126 (27.7)	0.21 (0.96)	0.16
50–100	277 (60.9)	0.019 (0.96)	
>100	52 (11.4)	−0.005 (1.12)	
Milk			
None	287 (63.4)	0.13 (1.00)	0.07
Regularly	166 (36.6)	−0.04 (0.95)	
Soya milk			
None	331 (73.1)	0.15 (0.96)	0.005
Regularly	122 (26.9)	−0.15 (1.03)	
Egg (/week)			
None	104 (22.9)	−0.007 (1.02)	0.07
1–4	295 (65.0)	0.21 (0.91)	
>4	55 (12.1)	0.093 (0.92)	
Fried food			
None	178 (39.2)	0.19 (0.97)	0.045
Regularly	276 (60.8)	−0.002 (0.99)	
Dessert			
None	143 (31.5)	0.15 (0.95)	0.28
Regularly	311 (68.5)	0.038 (1.00)	
Salt (g/day)			
<6	341 (74.9)	0.08 (0.92)	0.10
6–12	100 (22.0)	0.11 (1.14)	
>12	14 (3.1)	−0.48 (1.13)	

*No significant P for trend was found for any of the analyses.
†Independent t test or one-way analysis of variance.
‡p<0.05 in comparison to vegetable intake of <50 and 50–100 g/day.

The finding that hypertensive patients had shorter peripheral telomere length than healthy controls was in agreement with previous reports.[3 11–13 32] For example, Lung et al[11] showed that the white blood cell telomere length of 73 adults with hypertension was shorter than that of 76 healthy controls in a community of southern Taiwan, and Yang et al[12] found that 388 hypertensive patients had a shorter leucocyte telomere length than 379 normotensive controls in central China. Similarly, one cohort study also showed that shorter telomere

Table 3 Adjusted ORs and 95% CIs of hypertension, according to age-adjusted relative telomere length (RTL) and dietary intake*

Variables	Normotensive n (%)	Hypertensive n (%)	OR (95% CI)	P-difference	P-trend
Age-adjusted RTL					–
Short	212 (46.6)	151 (55.7)	1.00 (reference)		
Long	243 (53.4)	120 (44.3)	0.70 (0.52 to 0.96)	0.03	
Vegetables (g/day)					0.09
<50	72 (15.9)	56 (20.7)	1.00 (reference)		
50–150	276 (60.8)	161 (59.4)	0.72 (0.48 to 1.09)	0.12	
>150	106 (23.3)	54 (19.9)	0.65 (0.40 to 1.06)	0.08	
Fruit (g/day)					0.80
<50	141 (31.1)	83 (30.6)	1.00 (reference)		
50–150	263 (57.9)	156 (57.6)	0.97 (0.69 to 1.37)	0.88	
>150	50 (11.0)	32 (11.8)	1.05 (0.61 to 1.78)	0.87	
Meat (g/week)					0.60
<100	240 (52.7)	147 (54.2)	1.00 (reference)		
100–200	148 (32.5)	88 (32.5)	0.97 (0.69 to 1.36)	0.84	
>200	67 (14.7)	36 (13.3)	0.90 (0.57 to 1.43)	0.65	
Fish/seafood (g/week)					0.035
<100	217 (47.7)	150 (55.4)	1.00 (reference)		
100–200	128 (28.1)	70 (25.8)	0.81 (0.56 to 1.16)	0.25	
>200	110 (24.2)	51 (18.8)	0.63 (0.42 to 0.95)	0.03	
Poultry (g/week)					0.83
<50	126 (27.7)	68 (25.1)	1.00 (reference)		
50–100	277 (60.9)	181 (66.8)	1.21 (0.84 to 1.72)	0.31	
>100	52 (11.4)	22 (8.1)	0.76 (0.42 to 1.38)	0.37	
Milk					–
None	287 (63.4)	177 (65.6)	1.00 (reference)		
Regularly	166 (36.6)	93 (34.4)	0.89 (0.64 to 1.23)	0.47	
Soya milk					–
None	331 (73.1)	202 (75.1)	1.00 (reference)		
Regularly	122 (26.9)	67 (24.9)	0.92 (0.65 to 1.31)	0.92	
Egg (/week)					0.56
None	104 (22.9)	62 (23)	1.00 (reference)		
1–4	295 (65.0)	168 (62.2)	0.87 (0.60 to 1.27)	0.48	
≥5	55 (12.1)	40 (14.8)	1.07 (0.63 to 1.82)	0.80	
Fried food					–
None	178 (39.2)	95 (35.2)	1.00 (reference)		
Regularly	276 (60.8)	175 (64.8)	1.14 (0.83 to 1.57)	0.42	
Dessert					–
None	143 (31.5)	79 (29.2)	1.00 (reference)		
Regularly	311 (68.5)	192 (70.8)	1.09 (0.78 to 1.53)	0.60	
Salt (g/day)					<0.001
<6	341 (74.9)	151 (55.7)	1.00 (reference)		
6–12	100 (22.0)	92 (33.9)	2.21 (1.55 to 3.14)	<0.001	
>12	14 (3.1)	28 (10.3)	4.81 (2.43 to 9.52)	<0.001	

*Adjusted for age (y), gender, body mass index (kg/m²), plasma total cholesterol, plasma high-density lipoprotein cholesterol, plasma triglycerides, exercise (2+ times per week, yes/no), current alcohol consumption (at least once a week, yes/no) and current smoking (yes/no).

length at the baseline was associated with increased risk of developing hypertension after 5 years of follow-up.[12] Shorter telomere length was associated with vascular senescence, increased oxidative stress and chronic inflammation,[1] which were all related to increased risk of hypertension.[33] Telomerase-deficient mice exhibited the phenotype of progressive telomere shortening in successive generations. These mice developed higher blood pressure than wild-type mice, which was related to higher levels of circulating endothelin-1 and enhanced production of reactive oxygen species.[7] Our results, together with previous studies, provide convincing evidence that shorter telomere length was associated with hypertension and may play an important role in the aetiology of hypertension.

In this study, we observed that greater intake of vegetables was related to longer peripheral telomere length, which is in accordance with previous reports that a healthy lifestyle including high consumption of fruit and vegetable was associated with longer telomere

Table 4 Adjusted ORs and 95% CIs of hypertension, according to longer age-adjusted relative telomere length (RTL), stratified by vegetable intake*

Vegetable intake	Age-adjusted RTL	Normotensive n (%)	Hypertensive n (%)	OR (95% CI)	P-difference	P-interaction
<50 g/day	Short	36 (50.0)	33 (58.9)	1.00 (reference)		0.008
	Long	36 (50.0)	23 (41.1)	0.68 (0.33 to 1.40)	0.330	
50–150 g/day	Short	136 (49.3)	83 (51.6)	1.00 (reference)		
	Long	140 (50.7)	78 (48.4)	0.95 (0.64 to 1.42)	0.809	
>150 g/day	Short	39 (36.8)	35 (64.8)	1.00 (reference)		
	Long	67 (63.2)	19 (35.2)	0.28 (0.14 to 0.57)	<0.001	

*Adjusted for age (y), gender, body mass index (kg/m^2), plasma total cholesterol, plasma high-density lipoprotein cholesterol, plasma triglycerides, exercise (2+ times per week, yes/no), alcohol (at least once a week, yes/no) and current smoking (yes/no).

length.[17–21] Vegetables usually contain relatively high levels of carotenoids, vitamin C and dietary fibre, the food components that were associated with the long telomere in many studies.[21–23] Unfortunately, we were not able to further analyse the effect of specific nutrients or dietary components on telomere length due to the simple dietary questionnaires used in this study.

Interestingly, we observed that long telomere length was related to lower risk of hypertension only in those consuming the highest levels of vegetables. Our results might suggest a synergistically protective effect of vegetable intake in those with a longer telomere. This finding also highlights the importance of incorporating the dietary intervention into an integrated approach to slow down the process of biological ageing and prevent against hypertension. However, this phenomenon may also be partly explained by the fact that lower levels of vegetable intake by themselves are risk factors for hypertension,[29] and the protective effect of a long telomere cannot be teased out due to limited statistical power. Consistent with this notion, Hou et al[34] showed that a short telomere was associated with a higher risk of gastric cancer only in those without risk factors for gastric cancer; and Diaz et al[35] found that healthy lifestyle behaviours modulated the association between telomere length and coronary artery calcium. In this context, more studies are warranted to elucidate the potential role of diet in the telomere-hypertension relationship.

The study had several limitations. First, telomere length was measured by the quantitative PCR method. The method usually has higher variation than that measured by southern blot (more than 10% in most references).[5] In this study, the coefficient of variation for different measurements of the same sample ranged from 10% to 20%, which was consistent with previous reports. The limitation of the method was that it required a larger sample size to find significant results, and might mask existent weak correlations (eg, the effects of other foods on the telomere-hypertension relationship) in current samples. Second, the dietary questionnaire used in the study has not been validated. However, the association between intake of salt, vegetables, and fish and hypertension risk and/or telomere length provides indirect evidence of the validity of this questionnaire. Since there were only 11 food items in the questionnaire, we were not able to calculate total energy consumption for food intake adjustment. We were also unable to further analyse the relationship between telomere length and specific nutrients or food components, which could be of importance to understand the observed associations. Further, reverse causality is of concern due to the retrospective study design. Participants might switch to a healthier dietary pattern as a result of hypertension diagnosis/symptoms. This would lead to an underestimation for the potential effect of fruit/vegetables on telomere-hypertension relationship.

In summary, the study suggests that greater vegetable intake may modify the association between telomere length and hypertension. Further studies with a validated dietary questionnaire and prospective study design are warranted to replicate our observations.

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Supplementary material: questionnaire for the study

ART 1. DEMOGRAPHIC CHRACTERISTICS

1. Name: _____
2. Telephone: _____
3. Address:_____
4. Gender: 1) male   2) female

       For female, are you menopausal?   1) yes 2) no

5. What isyour date of birth?   _____ year _____ month _____ day

6. What is your education level?

     1) No formal education

     2) Elementary school

     3) Junior high school

     4) High school/ secondary technical school

     5) Technical school/college

     6) Postgraduate degree or above

7. Are you retired?   1) Yes   2) No

8. If you are not retired, what is your primary occupation?

     1) professional/technical worker (doctor, professor, lawyer, architect, engineer, midwife, nurse, teacher, editor, photographer)

     2) administrator/executive/manager (working proprietor, government official, section chief, department or bureau director, administrative cadre, village leader)

     3) office staff (secretary, office helper)

     4) farmer, fisherman, hunter

     5) skilled or non-skill worker (foreman, group leader, craftsman, ordinary laborer, looger)

     6) service worker (housekeeper, cook, waiter, doorkeeper, hairdresser, counter salesperson, launderer, child care worker)

     7) student

     8) unemployed

     9) other (specify: _____)

PART 2. DISEASE HISTORY

Have you ever been diagnosed with the following diseases?

Name of diseases	A. Whether or not it has been diagnosed	B. When have you been diagnosed?
1. type 2 diabetes	1) yes 2) no	_____year ____ month
2. hypertension	1) yes 2) no	_____year ____ month
3. dyslipidemia	1) yes 2) no	_____year ____ month
4. coronary heart disease	1) yes 2) no	_____year ____

		month
5. stroke	1) yes 2) no	_____year _____ month
6. nephropathy (such as nephritis, kidney calculi, etc.)	1) yes 2) no	_____year _____ month
7. liver disease (such as fatty liver, hepatitis, etc.)	1) yes 2) no	_____year _____ month
8. blood system disease	1) yes 2) no	_____year _____ month
9. chronic obstructive pulmonary disease	1) yes 2) no	_____year _____ month
10. asthma	1) yes 2) no	_____year _____ month
11.other chronic disease	1) yes (name of the disease_____) 2) no	_____year _____ month

PART 3. SMOKING HABIT IN THE PAST YEAR

1. Have you ever smoked at least 100 cigarettes lifetime or smoke every day or some days now?

    1) yes (if yes, please answer the following questions)

    2) no

2. How old were you when you started to smoke? _____ years old

3. How many cigarettes did you smoke? _____ cigarettes/day

4. How long have you smoked? _____years _____months

5. Have you ever smoked but now quit smoking?

    1) yes (if yes, please answer the following question)

    2) no

6. How many years have you quitted smoking? _____years _____months ____days

PART 4. DRINKING HABIT IN THE PAST YEAR

1. Do you drink alcohol? (alcohol including liquor, beer, grape wine, rice wine and highland barley wine)?

    1) yes (if yes, please answer the following questions)

    2) no

Type of alcohol	Frequency (times/month)	Amount each time	Notes
1. liquor	_____	____ ml	Degree:□＜42 □≥42
2. beer	_____	____ml	/
3. grape wine	_____	____ ml	/
4. rice wine	_____	____ ml	/
5. fruit wine	_____	____ ml	/

2. Do you used to be a regular drinker but now quit drinking?

    1) yes (if yes, please answer the following question)

    2) no

3. How many years have you quitted drinking? _____years _____months ____days

PART 5. PHYSICAL ACTIVITY IN THE PAST YEAR

1. Which kinds of labor intensity do you belong to?
    1) Light (including retire person, office staff, watch mender, shop assistant, technician in lab, teveryers, and etc.)
    2) Moderate (including student, driver, electrician, and etc.)
    3) Heavy (including farmers, dancer, athlete, stevedore, timberjack, miner, manson, and etc.)

Regular exercise was defined as any kind of recreational or sport physical activity other than walking for work or life performed three or more days per week for at least 30 minutes.

2. Do you take regular exercise in the past year?
    1) yes (if yes, please answer the following question 2 and 3)
    2) no
3. What kind of exercise did you take? _____
4. How often did you take regular exercise? _____ times/week
5. How long did you take regular exercise each time? _____hours _____ minutes

PART 6. DIETARY HABIT IN PAST YEAR

Please describe the dietary habits during the past 12 months.
1. What is the amount of vegetables you usually eat in a day?
    1) Less than 50 gram
    2) 50～150 gram
    3) 150～300 gram
    4) more than 300 gram
2. What is the amount of fruit you usually eat in a day?
    1) Less than 50 gram
    2) 50～100 gram
    3) 100～200 gram
    4) more than 200 gram
3. What is the amount of milk you usually drink in a day?
    1) Do not drink any milk
    2) less than 200 ml
    3) 200～400 ml
    4) more than 400 ml
4. What is the amount of soymilk you usually drink in a day?
    1) Do not drink any soymilk
    2) less than 200 ml
    3) 200～400 ml
    4) more than 400 ml
5. How many eggs do you usually eat in a week?
    1) less than 1
    2) 1～4
    3) 5～7

4) more than 7

6. How many times do you eat meat of livestock or its products in a week?

    1) None

    2) 1～4 times

    3) 5～7 times

    4) more than 7 times

7. When you eat meat of livestock, what is the amount do you usually eat in each time?

    1) Less than 50 gram

    2) 50～100 gram

    3) More than 100 gram

8. How many times do you eat fish/seafood or its products in a week?

    1) None

    2) 1～4 times

    3) 5～7 times

    4) more than 7 times

9. When you eat fish/seafood or its products, what is the amount do you usually eat in each time?

    1) Less than 50 gram

    2) 50～100 gram

    3) More than 100 gram

10. How many times do you eat poultry or its products in a week?

    1) None

    2) 1～4 times

    3) 5～7 times

    4) more than 7 times

11. When you eat poultry or its products, what is the amount do you usually eat in each time?

    1) Less than 50 gram

    2) 50～100 gram

    3) More than 100 gram

12. How many times do you eat fried food in a week?

    1) none

    2) 1～4 times

    3) 5～7 times

    4) more than 7 times

13. How many times do you eat dessert in a week?

    1) none

    2) 1～4 times

    3) 5～7 times

    4) more than 7 times

14. What is the approximate amount of salt you eat in each day?

    1) less than 6 gram

    2) 6～9 gram

3) 9～12 gram

4) more than 12 gram

PART 7. BODY MEASUREMENT

1. Height: _____cm

2. Weight: _____ kg

3. Waistline: _____cm

4. Blood Pressure: Systolic _____ mmHg; diastolic _____ mmHg

5. Blood pressure:

       Total cholesterol _____mmol/L

       Triglycerides _____mmol/L

       Low density lipoprotein cholesterol _____ mmol/L

       High Density lipoprotein cholesterol _____mmol/L