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Physical activity attenuated the association of air pollutants with telomere length in rural Chinese adults

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Abbreviations

95% CI: confidence interval; Cts: cycle thresholds; CV: coefficient of variation; CVD: cardiovascular disease; FPG: fasting plasma glucose; HbA1c: glycosylated haemoglobin A1c; hsCRP: hypersensitive C-reactive protein; IFG: impaired fasting glucose; INS: insulin; IPAQ: International Physical Activity Questionnaire; IQR: interquartile range; MET: metabolic equivalent; NF- κ B: nuclear factor- κ B; NGT: normal glucose tolerance; NO₂: nitrogen dioxide; PA: physical activity; PM: particulate matter; PM₁: particulate matter with an aerodynamic diameter $\leq 1.0 \mu\text{m}$; PM_{2.5}: particulate matter with an aerodynamic diameter $\leq 2.5 \mu\text{m}$; PM₁₀: particulate matter with an aerodynamic diameter $\leq 10 \mu\text{m}$; RMB: renminbi; RT-PCR: real-time polymerase chain reaction; T2DM: type 2 diabetes mellitus; TL: telomere length. WBC: white blood cell.

Abstract

Background: Exposure to air pollutants (nitrogen dioxide (NO₂) and particulate matters (PMs)) or physical inactivity is linked to telomere length (TL) shortening. However, there is a lack of research on combined effects of either NO₂ or PMs and physical activity (PA) on TL. This study aimed to explore the joint associations of air pollutants (NO₂ or PMs) and PA with relative TL in rural Chinese adults.

Methods: This study was conducted among 2,704 participants aged 18-79 years in rural China. Concentrations of NO₂ and PMs (PM with an aerodynamics diameter $\leq 1.0 \mu\text{m}$ (PM₁), $\leq 2.5 \mu\text{m}$ (PM_{2.5}) or $\leq 10 \mu\text{m}$ (PM₁₀)) were estimated using random forest models incorporated with satellites data, meteorological data, and land use information. Relative TL of each participant was measured by a quantitative real-time polymerase chain reaction. Linear regression models were applied to examine the independent associations between PA, NO₂ or PMs and relative TL. Interaction plots were used to depict the altered associations between NO₂, PM₁, PM_{2.5}, or PM₁₀ and relative TL along with increasing PA levels.

Results: Each 1 $\mu\text{g}/\text{m}^3$ increment in NO₂, PM₁, PM_{2.5}, or PM₁₀ was associated with a 0.038 (95% confidence intervals (CI): -0.044, -0.033), 0.036 (95% CI: -0.041, -0.031), 0.052 (95% CI: -0.059, -0.045), or 0.022 (95% CI: -0.025, -0.019) decrease in relative TL among all participants; similar findings were observed among normal glucose tolerance or impaired fasting glucose (IFG) participants as well as type 2 diabetes mellitus (T2DM) patients. PA at certain levels counteracted the association of air pollutants (NO₂, PM₁, PM_{2.5}, and PM₁₀) with relative TL among IFG participants or T2DM patients.

Conclusions: Long-term exposure to NO₂ and PMs was associated with relative TL

shortening and these effects may be counteracted by PA at certain levels in IFG participants or T2DM patients.

Keywords: nitrogen dioxide; particulate matter; physical activity; relative telomere length; rural population.

1. Introduction

Telomere length (TL) has been accepted as a biomarker of senescence involved in regulating various important physiology functions such as prevention of chromosome end-to-end fusions as well as maintenance of genome stability and integrity (Blackburn, 1991; Mather et al., 2011). Telomere shortening may promote the aging process by inducing pro-inflammatory processes through activation of the transcription factor nuclear factor- κ B (NF- κ B) (Blackburn et al., 2015; Zhang et al., 2016). Telomere dysfunction is involved in the development of age-related chronic diseases such as type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD) and cancer by leading to systemic inflammatory response and inducing oxidative damage to telomeres (Blackburn et al., 2015; Lopez-Otin et al., 2013).

As economic and urbanization developing rapidly, exposure to air pollutants linked to TL has become the topic issue nowadays. A growing body of evidence suggested that exposure to particulate matter (PM) with an aerodynamic diameter $\leq 2.5 \mu\text{m}$ (PM_{2.5}) or nitrogen dioxide (NO₂) was negatively associated with TL by inducing oxidative stress or inflammatory responses (Clemente et al., 2019; Martens and Nawrot, 2016; Everson et al., 2020; Miri et al., 2019).

Physical activity (PA), as a healthy lifestyle, links to the alteration of TL. PA may induce increment in skeletal muscle secretion of interleukin-6 leading to increased adaptive response in human body (Nimmo et al., 2013). Several studies revealed that PA might associate with increased TL (Fretts et al., 2018; Shadyab et al., 2017; Tucker, 2017). For instance, one study conducted in American adults indicated that individuals reporting no regular PA were at a higher risk for TL shortening, compared to individuals

with high levels of PA (>1000 metabolic equivalent (MET)-min/week) (Tucker, 2017). However, a study performed in Northern Chinese adults suggested that there was no association of PA with relative TL (Ding et al., 2018). The inconsistent findings among previous studies may be due to the ethnic differences, sample size, and location of the studies. Aside from developed countries as well as urban cities of China, few studies have been examined the association between PA and TL in rural areas of China.

Although evidence has demonstrated the independent effect of PA or air pollutants on systemic inflammation among Taiwanese adults (Zhang et al., 2018) and PA can attenuate adverse effects of air pollutants on airway inflammation and oxidative stress among adolescents (Pasalic et al., 2016), studies on the combined effects of air pollutants and PA on TL in rural China are lacking. Considering that there exist different levels of exposure to air pollutants and lifestyles in different regions, this study aims to explore the independent and joint associations of air pollutants (NO₂ or PMs (PM with an aerodynamic diameter $\leq 1.0 \mu\text{m}$ (PM₁), PM_{2.5}, or $\leq 10 \mu\text{m}$ (PM₁₀)) and PA with relative TL in rural Chinese adults.

2 Material and methods

2.1. Study population

As shown in **Figure 1**, a total of 41,893 participants were invited to participate in the Henan Rural Cohort Study and ultimately 39,259 participants aged 18-79 years were recruited. In this study, a sub-population (n=2,775) was derived from Henan Rural Cohort Study (Li et al., 2020; Liu et al., 2019b). Briefly, 925 T2DM patients were randomly selected from the baseline of Henan Rural Cohort Study. Each T2DM patient was matched with one impaired fasting glucose (IFG) participant and one normal glucose tolerance (NGT) participant by age (± 3 years) and sex from the same population (Liu et

al., 2019a). After participants with missing data on relative TL (n=36) or the four air pollutants (n=35) were excluded, a total of 2,704 participants were finally included in the analysis.

This study was approved by Zhengzhou University Life Science Ethics Committee. Written informed consents were obtained from all participants before this study was conducted.

2.2 Data collection

A questionnaire was used to collect data on the participant's demographic characteristics, lifestyles, and personal and family histories of diseases by well-trained workers through a face-to-face interview. Averaged monthly income was classified into < 500 RMB and \geq 500 RMB groups. Education level was classified into elementary school or below, junior high school and high school or above. Marital status was classified into married/cohabitation and divorced/widowed/unmarried groups. Smoking- and drinking-status were divided into current, former as well as never groups. The levels of PA were evaluated by the International PA Questionnaire (IPAQ) (Bennie et al., 2013), which were also well described in our previous study (Tu et al., 2019). Briefly, information on the frequency and duration of different levels of PA (vigorous PA, moderate PA, and walking) over a past week was collected based on IPAQ. The total METs of each participant was assessed using the following formula: duration (hour/day) \times frequency (times/week) \times coefficient of MET for each type of activity. Compendium averaged MET coefficients were 8, 4, and 3.3 for vigorous PA, moderate PA, and walking, respectively (Ainsworth et al., 2011). PA was classified into low, moderate and high three levels, in accordance with a previous study (Tu et al., 2019).

Venous blood samples of participants were collected for measurements after an overnight (at least 8-hour) fasting period. Fasting plasma glucose (FPG) was determined by using an automatic biochemical analyzer (ROCHE Cobas C501). Glycosylated haemoglobin A1c (HbA1c) was analyzed using an ion exchange high performance liquid chromatography which can perform the automated separation and measurement of HbA1c (VARIANT II, Bio-Rad, CA, USA). T2DM Patients were defined as those who had an FPG ≥ 7.0 mmol/L, HbA1c $\geq 6.5\%$, or diagnosed T2DM by physicians and using anti-glycemic drugs in the last 2 weeks; participants with NGT was defined as FPG < 6.1 mmol/L or HbA1c $< 5.7\%$; the other participants were defined as IFG individuals (Tsuda et al., 2018).

2.3 Estimated concentrations of air pollutants

Concentrations of NO₂ and PMs (PM₁, PM_{2.5}, or PM₁₀) were predicted by a random forest model using the monitoring data, satellite remote sensing, land use and meteorology (temperature and relative humidity) data as well as other predictors. The detailed method was described in elsewhere (Chen et al., 2019; Hou et al., 2020a). Briefly, ground station daily data on PM₁ were collected from the 77 stations of the China Atmosphere Watch Network. Ground station daily data on NO₂, PM_{2.5} and PM₁₀ were collected from the 1,497 stations of China National Environmental Monitoring Center. Concentrations of ambient PMs and NO₂ were predicted by using random forest models with aerosol optical depth data or satellite-derived OMI (Daily Level-3 NO₂ Product) data as well as other predictors such as meteorological factors (relative humidity and temperature) and land use information. The models had been demonstrated a good power to predict the four air pollutants and the 10-fold cross-validation estimated R² of annual

averaged concentrations of NO₂, PM₁, PM_{2.5}, and PM₁₀ were 72%, 75%, 86%, and 81%, respectively. The 3-year averaged concentrations of PMs and NO₂ were computed by averaging the daily concentrations of each air pollutant for participants during three years before this study, according to their geocoded home address and date of investigation.

2.4 Measurement of relative telomere length

In accordance with manufacturer's protocol, whole genomic DNA of each individual was isolated from peripheral blood sample using the whole blood genomic DNA medium extraction kit III (Biotek Corporation, Beijing, China). A total of 5% of the DNA samples were randomly selected from this study population and pooled as the reference sample. The concentrations of DNA were quantified by NanoDrop 2000 Spectrophotometer (Thermo scientific, Waltham, MA, USA). The telomere repeat copy number and the single copy gene copy number were measured in triplicate using real-time polymerase chain reaction (RT-PCR) (QuantStudio™ 7 Flex, Applied Biosystems Life technologies, USA) (Cawthon, 2002). The forward and reverse telomeric primers were 5'-ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT-3' and 5'-TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA-3', respectively (Cawthon, 2009). The forward and reverse primers for β-actin were 5'-ACTCTTCCAGCCTTCCTTCC-3' and 5'-GGCAGGACTTAGCTTCCACA-3', respectively (Pieters et al., 2016). The thermal cycling conditions of RT-PCR were set at 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C for 3 seconds, annealing and extension at 60°C for 30 seconds. The melting curve analysis of each PCR reaction product was used to confirm the specificity of primers. The average of three measurements from the cycle thresholds (Cts) of telomere and β-actin were used for

analysis. The relative TL was calculated by telomere repeat copy number divided by single copy gene copy number based on $2^{-\Delta\Delta Ct}$ method with the following formula: $\Delta Ct_{\text{sample}} = \Delta Ct_{\text{telomere}} - \Delta Ct_{\beta\text{-actin}}$; $\Delta\Delta Ct = \Delta Ct_{\text{sample}} - \Delta Ct_{\text{reference}} = (Ct_{\text{telomere}} - Ct_{\beta\text{-actin}}) - (Ct_{\text{telomere}} - Ct_{\beta\text{-actin}})$, and relative TL = $2^{-\Delta\Delta Ct}$. (Hou et al., 2020b; Livak and Schmittgen, 2001). Inter-plate coefficient of variation (CV) of telomere and β -actin were 2.38% and 1.38%, respectively. Inter-day CV of telomere and β -actin were 1.49% and 0.84%, respectively.

2.5 Statistical analysis

The categorical and non-normal distributed variables of the study population were expressed as numbers (proportions) and median (interquartile range, IQR), respectively. Mann-Whitney U test or chi-square test was used to test the non-normal distributed or categorical variables by glucometabolic status. Due to the skew distribution of relative TL, thus, natural Logarithmic (log) transformation was applied to make relative TL distribution to be approximately normal. To evaluate the independent associations of exposure to NO₂, PMs and PA with relative TL by using linear regression models, three models were built: **Model 1** was adjusted for age, gender, marital status, average monthly individual income, education level, smoking and drinking status, more vegetables and fruits intake, high fat diet; **Model 2** was adjusted for the variables in the **Model 1** plus dyslipidemia, hypertension, and family histories of hypertension and T2DM; **Model 3** was adjusted for the variables in the **Model 2** plus PA or single-air pollutant (PM₁, PM_{2.5}, PM₁₀ or NO₂) for one time. Furthermore, the interaction plot was used to exhibit the interaction effects of exposure to NO₂ or PMs and PA on relative TL, after adjusted for age, gender, marital status, average monthly individual income, education level, smoking

and drinking status, more vegetables and fruits intake, high fat diet, dyslipidemia, hypertension, and family histories of hypertension and T2DM. All of the data were analyzed using R-packages of Interplot and mgcv in R software version 3.5.3. A two-tailed P -value < 0.05 was considered as statistical significance.

3. Results

3.1 Characteristics of study participants

Table 1 showed that the median (IQR) age of 2,704 participants was 61.0 (12.0) years. IFG participants or T2DM patients were likely to have higher levels of FPG, HbA1c, and insulin, compared to NGT participants. T2DM patients had higher family history disease of T2DM and lower current smokers, compared to NGT participants (all $P < 0.05$). No differences in other categorical variables were found between either IFG participants or T2DM patients and NGT groups. **Table S1** showed that the mean (SD) of the frequencies of walking, moderate PA, and vigorous PA in the total population were 6.94 ± 0.54 , 6.94 ± 0.50 , and 5.30 ± 2.34 day/week, respectively. The mean (SD) of the duration time of walking, moderate PA, and vigorous PA in the total population were 2.15 ± 1.82 , 3.43 ± 2.50 , and 1.18 ± 2.53 hour/day, respectively. Participants with IFG had lower duration time of walking than NGT participants ($P = 0.015$). T2DM patients tend to have lower duration time of walking ($P = 0.003$), duration time of moderate activity ($P < 0.001$), PA-METs ($P < 0.001$), and PA levels ($P = 0.034$) than NGT participants. **Table 2** showed that the medians of NO_2 , PM_{10} , $\text{PM}_{2.5}$, and PM_{10} of all participants were $37.27 \mu\text{g}/\text{m}^3$, $56.18 \mu\text{g}/\text{m}^3$, $71.48 \mu\text{g}/\text{m}^3$, and $128.03 \mu\text{g}/\text{m}^3$, respectively. Participants with IFG or T2DM were associated with lower exposure to the four air pollutants (NO_2 , PM_{10} , $\text{PM}_{2.5}$ and PM_{10}) as well as longer relative TL than NGT participants.

3.2 Association of air pollutants or PA with relative TL

After adjusted for age, gender, marital status, average monthly individual income, education level, smoking status, alcohol drinking habits, vegetables and fruits intake and high fat diet, **Model 1** showed that each 1 $\mu\text{g}/\text{m}^3$ increment in NO_2 , PM_{10} , $\text{PM}_{2.5}$, or PM_{10} was associated with a 0.047 (95% confidence interval (CI): -0.058, -0.036), 0.042 (95% CI: -0.052, -0.032), 0.057 (95% CI: -0.070, -0.044), or 0.026 (95% CI: -0.032, -0.020) decrease in relative TL values among NGT individuals; a 0.041 (95% CI: -0.051, -0.031), 0.040 (95% CI: -0.048, -0.031), 0.055 (95% CI: -0.068, -0.043), or 0.024 (95% CI: -0.029, -0.018) decrease in relative TL values among IFG individuals, as well as a 0.030 (95% CI: -0.040, -0.020), 0.028 (95% CI: -0.037, -0.019), 0.044 (95% CI: -0.057, -0.031), or 0.017 (95% CI: -0.023, -0.012) decrease in relative TL values among T2DM patients. The results of **Model 2** and **Model 3** were no substantial changes. No association between PA and relative TL was observed in all models (all $P > 0.05$) (**Figure 2 and Table S2**). **Table S3** showed that no associations of hypersensitive C-reactive protein (hsCRP) or white blood cell (WBC) with relative TL were observed among all participants or individuals with NGT, IFG or T2DM.

3.3 Interactive effects of air pollutants and PA on relative TL

Figure 3 and **Table S4** showed that a negative interaction effect of each of air pollutants and PA on relative TL was observed among all participants as well as IFG or T2DM patients (all $P > 0.05$). As shown in **Table S4**, each 10 hour/day increase in PA-MET value, the estimated regression coefficient of NO_2 , PM_{10} , $\text{PM}_{2.5}$, and PM_{10} associated with relative TL were a 0.0039, 0.0038, 0.0067, and 0.0026 decrease in the total population, a 0.0016, 0.0018, 0.0030, and 0.0007 increase in NGT participants, a

0.0058, 0.0058, 0.0116, and 0.0035 decrease in IFG participants, and a 0.0067, 0.0072, 0.0110, and 0.0049 decrease in T2DM patients, respectively. Moreover, the associations of NO₂, PM₁, PM_{2.5}, and PM₁₀ with relative TL were completely counteracted by PA at the cut-points of PA-MET 4.4973, 4.4973, 4.2191, and 4.4045 in IFG participants, and 3.4773, 3.3382, 3.4309, and 3.4309 in T2DM patients, respectively.

4. Discussions

Long-term exposure to high levels of ambient NO₂ and PMs was associated with relative TL shortening in all participants. Moreover, certain levels of PA counteracted relative TL shortening in response to exposure to high levels of NO₂ or PMs among IFG participants or T2DM patients, implying that PA may be an effective and costless method to delay relative TL shortening in relation to exposure to high levels of ambient air pollutants among susceptible population such as T2DM patients.

The findings of this study were in line with several previous studies. Everson et al reported that each IQR (7.0 µg/m³) increase in NO₂ was associated with a 7.3% (95% CI: -10.98%, -3.46%) decrease in TL among South African women (n=61) (Everson et al., 2020). A cross-sectional study indicated that each IQR increase in PM₁ (27.4 µg/m³), PM_{2.5} (26.0 µg/m³), or PM₁₀ (24.6 µg/m³) was associated with a 0.18 (95% CI: -0.32, -0.04), 0.23 (95% CI: -0.39, -0.08), or 0.17 (95% CI: -0.30, -0.04) decrease in relative TL among preschool children from 27 kindergartens (n=200) in Iran (Moslem et al., 2020). Results from a birth cohort study in Wuhan, China indicated that each 10 µg/m³ increase in PM_{2.5} or PM₁₀ was associated with a 3.71% or 3.24% decrease in relative TL, and the reverse association was more obvious among male infants (n=743) (Song et al., 2019). However, this study results were inconsistent with some other studies (Dioni et al., 2011; Hou et al., 2012; Walton et al., 2016; Lee et al., 2019). For instance, one study suggested

that one unit increment in NO₂, PM_{2.5}, or PM₁₀ concentrations was associated with a 0.009 (95% *CI*: 0.004, 0.015), 0.041 (95% *CI*: 0.020, 0.063), or 0.096 (95% *CI*: 0.044, 0.149) increase in relative TL values among east London schoolchildren (n=333) (Walton et al., 2016). Another study indicated that there was no association between exposure to PM_{2.5} and TL among African American youths (n=1,072) (Lee et al., 2019). The inconsistent results between this study and previous studies might be due to differences in study population, regions, sample size, levels of PMs and its components as well as the method of estimated air pollutants. For instance, a recent study showed that polycyclic aromatic hydrocarbons exposure, as one important of PM components, was associated with a relative TL reduction (Hou et al., 2020b).

Available evidences suggest that the potential mechanisms of TL shortening related to air pollutants caused by inducing an inflammatory process as well as oxidative stress. PMs and its components may enter into the lungs where it can induce excessive oxidative stress or inflammation response in the body (Pope et al., 2004). A population-based study showed increased levels of inflammation (hsCRP) and oxidative DNA damage (8-hydroxy-2-deoxyguanosine) in response to exposure to high levels of PM₁₀ or ozone among college student in Taipei (Chuang et al., 2007). Increased oxidative DNA damage may accelerate the shortening of telomeres by the telomere-p53-peroxisome proliferator-activated receptor gamma pathway related to environmental pollution (Bateson, 2016; Sahin et al., 2011). A review indicated that the antioxidants and anti-inflammatories agents might partially reduce the rate of TL shortening by attenuating the inflammation and oxidative DNA damage during ageing process (Prasad et al., 2017). Moreover, the potential mechanism of shifts in cell types may also be involved in telomere shortening in

relation to air pollutants. Exposure to air pollutants was related to induce systemic inflammation characterized by elevated levels of circulating monocytes and T-cells as well as leukocyte distribution (Atafar et al., 2019; Gao et al., 2019; Lin et al., 2016). However, although the related cell type shifts in blood were not measured in this study, associations of the systemic inflammation indicators (hsCRP and WBC) with relative TL were not observed (**Table S3**), indicated that systemic inflammation may not affect relative TL.

It is well known that regularity of physical activity may attenuate oxidative stress among healthy participants by increasing antioxidant enzyme capacities (glutathione peroxidase and catalase) and decreasing inflammatory response via reducing levels of inflammation indicators such as interleukin-1 β and hs-CRP (Mury et al., 2018). Furthermore, a study conducted in America (n=5,823) revealed that participants with higher PA values (>1000 MET-min/week) were associated with longer TL (9 years advantage of biologic senescence), compared to those who reported no regular PA (Tucker, 2017). Thus, in this study, we conferred that PA at certain levels might counteract the effect of exposure to high levels of PMs or NO₂ on relative TL shortening by alleviating inflammatory response and oxidative stress in individuals with IFG or T2DM (Mury et al., 2018; Nimmo et al., 2013). We observed a non-statistically significant result of PA and relative TL among all participants, which was consistent with a previous study (Ding et al., 2018). In addition to PA, other modifiable factors such as dietary patterns, diet component and psychosocial stress may be associated with relative TL. A systematic review showed that certain foods (processed meat, cereals and sugar-sweetened beverages) may be associated with accelerating telomere shortening, whereas

Mediterranean dietary pattern, intake fruits and vegetables were associated with longer TL (Rafie et al., 2017). Karimi and colleagues also reported that individuals with health dietary pattern including consumptions of whole grains, fish and dairy products and traditional dietary pattern such as intake of fruits and vegetables were associated with longer TL, whereas individuals with consumptions of processed meats, oils and solid fats were associated with telomere shortening among young male aged 25 to 40 years in Tehran (Karimi et al., 2018). The results from the Multi-Ethnic Study of Atherosclerosis showed that individuals with high total chronic stress were associated with 0.054 units (95% *CI*: -0.095, -0.013) telomere shortening over 10 years (Meier et al., 2019). Results from the Coronary Artery Risk Development in Young Adults Study also indicated that there was a joint effect of chronic psychosocial and financial burden on accelerating 5-year telomere shortening (Cabeza de Baca et al., 2020). However, results from the Netherlands Study of Depression in Older Persons showed that chronic psychosocial stress was not associated with TL shortening among 496 individuals aged ≥ 60 years (Schaakxs et al., 2016).

This study is the first to explore the combined effect of long-term exposure to either NO₂ or PMs (PM₁, PM_{2.5}, and PM₁₀) and PA on relative TL in rural Chinese adults. It has some limitations. First, the causal relationship between air pollutants or PA and relative TL cannot be established, because the design of this study was a cross-sectional study. Second, spatial errors of misclassification may exist, owing to estimated air pollutants of participants by using the corresponding geocoding of rural addresses. Third, although several important confounders (such as age, gender) were controlled, the other unmeasured confounding factors such as genetic factors, dietary patterns, and

psychosocial stress may also affect the results of this study.

5. Conclusions

The results of this study showed that long-term exposure to NO₂ and PMs was associated with telomere shortening and these associations may be counteracted by PA at certain levels among IFG participants or T2DM patients, implying that PA may be an effective and affordable method to reduce TL shortening in relation to exposure to high levels of air pollutants.

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Table 1 Baseline characteristics of the study participants

Variable	Total(n=2,704)	NGT(n=904)	IFG(n=914)	P-values ^a	T2DM(n=886)	P-values ^b
Age (years, median, IQR)	61.00(12.00)	61.00(12.00)	61.00(12.00)	0.897 ^c	61.00(12.00)	0.990 ^c
Gender (men, n, %)	1029(38.05)	344(38.05)	353(38.62)	0.803 ^d	332(37.47)	0.800 ^d
Education level, n (%)				0.710 ^d		0.253 ^d
Elementary school or below	1525(56.40)	520(57.52)	512(56.02)		493(55.64)	
Junior high school	945(34.95)	315(34.85)	324(35.45)		306(34.54)	
High school or above	234(8.65)	69(7.63)	78(8.53)		87(9.82)	
Average monthly income (<500 RMB, n, %)	1106(40.90)	358(39.60)	383 (41.90)	0.318 ^d	365(41.20)	0.492 ^d
Marital status (n, %)				0.832 ^d		0.702 ^d
Married/Cohabitation	2404(88.91)	803(88.83)	809(88.51)		792(89.39)	
Unmarried/Divorced/Widowed	300(11.09)	101(11.17)	105(11.49)		94(10.61)	
Smoking (n, %)				0.695 ^d		0.025 ^d
Never smokers	2023(74.82)	672(74.34)	694(75.93)		657(74.15)	
Former smokers	200(7.40)	58(6.42)	58(6.35)		84(9.48)	
Current smokers	481(17.79)	174(19.25)	162(17.72)		145(16.37)	
Alcohol consumption (n, %)				0.477 ^d		0.392 ^d
Never drinkers	2226(82.32)	744(82.30)	770(84.25)		712(80.36)	
Former drinkers	155(5.73)	48(5.31)	47(5.14)		60(6.77)	
Current drinkers	323(11.95)	112(12.39)	97(10.61)		114(12.87)	
High fat diet (Yes, n, %)	498(18.42)	166(18.36)	168(18.38)	0.992 ^d	164(18.51)	0.936 ^d
Family history of T2DM (Yes, n, %)	71(2.63)	14(1.55)	13(1.42)	0.824 ^d	44(4.97)	<0.001 ^d
PA-MET (10 hour/day, median, IQR)	1.86(0.83)	1.86(0.80)	1.86(0.83)	0.198 ^c	1.85(0.99)	<0.001 ^c
FPG (mmol/L, median, IQR)	5.44(1.99)	4.93(0.62)	5.30(0.93)	<0.001 ^c	7.92(3.16)	<0.001 ^c
HbA1c (% , median, IQR)	5.80(1.10)	5.30(0.40)	5.90(0.30)	<0.001 ^c	7.40(2.20)	<0.001 ^c
INS (pmol/L, median, IQR)	12.80(6.40)	11.91(5.90)	12.61(6.48)	<0.001 ^c	14.00(7.19)	<0.001 ^c

IQR: interquartile range; NGT: normal glucose tolerance; IFG: impaired fasting glucose; T2DM: type 2 diabetes mellitus; RMB: renminbi; PA: physical activity; MET: metabolic equivalent; FPG: fasting plasma glucose; HbA1c: glycosylated hemoglobin A1c; INS: insulin; ^a Represented the difference between IFG participants and NGT participants; ^b Represented the difference between T2DM patients and NGT participants; ^c Mann-Whitney U test was applied to compare the difference of two groups; ^d Chi-square test was applied to test the difference of two groups.

Table 2 Distributions of air pollutants and telomere length stratified by glucometabolic status

Variables	Total (n=2,704)	NGT (n=904)	IFG (n=914)	P-values ^c	T2DM(n=886)	P-values ^d
NO ₂ (µg/m ³ , median, IQR) ^a	37.27(1.86)	37.46(1.41)	37.37(1.71)	0.001	35.83(2.08)	<0.001
PM ₁ (µg/m ³ , median, IQR) ^b	56.18(2.43)	56.45(1.30)	56.18(2.00)	<0.001	54.77(1.96)	<0.001
PM _{2.5} (µg/m ³ , median, IQR) ^b	71.48(1.39)	71.58(1.21)	71.48(1.38)	<0.001	70.40(1.48)	<0.001
PM ₁₀ (µg/m ³ , median, IQR) ^b	128.03(3.22)	128.16(2.63)	128.15(3.09)	0.002	125.26(3.84)	<0.001
Relative TL (median, IQR)	1.05(0.46)	1.00(0.46)	1.06(0.44)	<0.001	1.10(0.42)	<0.001

NGT: normal glucose tolerance; IFG: impaired fasting glucose; T2DM: type 2 diabetes mellitus; IQR: interquartile range; NO₂: nitrogen dioxide; PM₁: particulate matter with an aerodynamic diameter ≤ 1.0 µm; PM_{2.5}: particulate matter with an aerodynamic diameter ≤ 2.5 µm; PM₁₀: particulate matter with an aerodynamic diameter ≤ 10 µm; TL: telomere length; ^a The concentration of NO₂ was estimated using random forest model with satellite-derived OMI (Daily Level-3 NO₂ Product) data as well as other predictors such as meteorological and land use information; ^b The concentration of PM was estimated using random forest model with aerosol optical depth data as well as other predictors such as meteorological and land use information; ^c Mann-Whitney U test was applied to compare non-normal distributed data of IFG participants and NGT participants; ^d Mann-Whitney U test was applied to compare non-normal distributed data of T2DM patients and NGT participants.

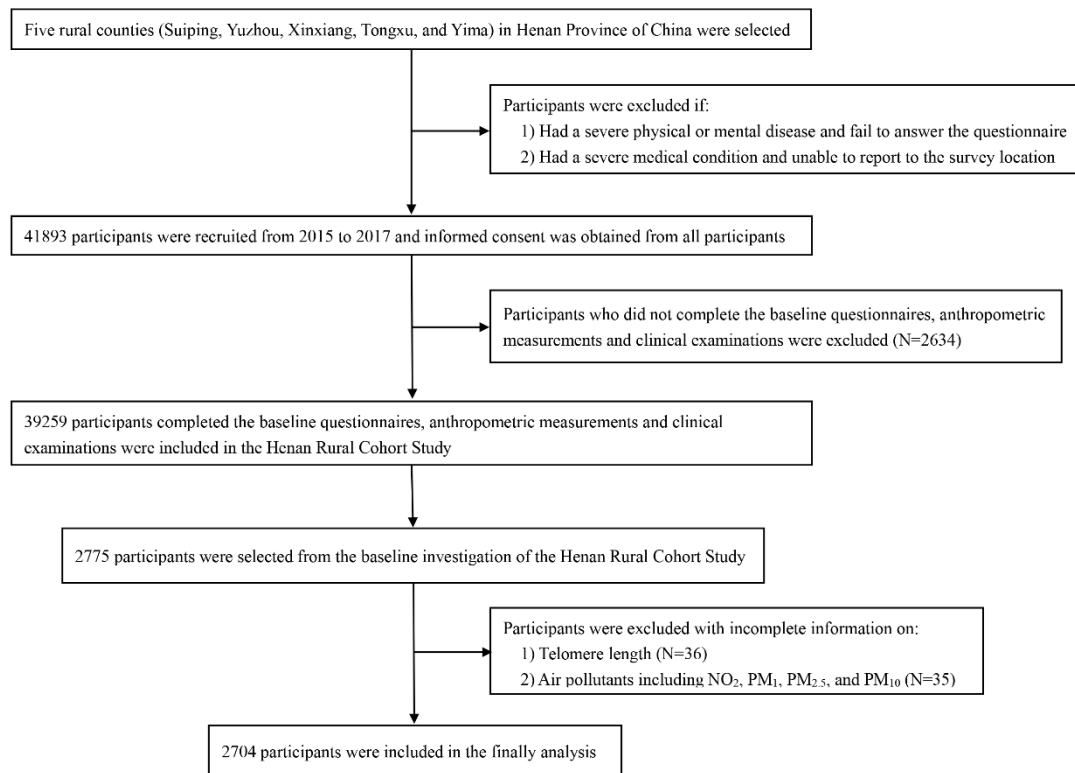


Figure 1 The flowchart of participant selection.

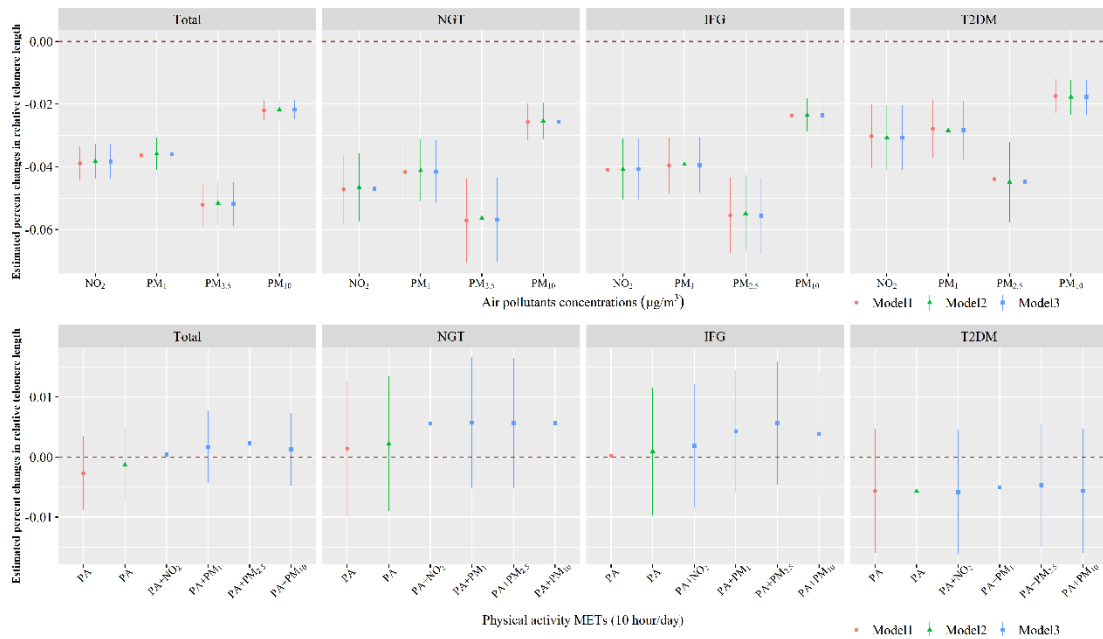


Figure 2 Associations between particulate matter, NO₂ or physical activity and relative telomere length stratified by glucometabolic status.

Model 1 adjusted for age, gender, marital status, average monthly individual income, education level, smoking and drinking status, more vegetables and fruits intake, high fat diet; Model 2 adjusted for the variables of the model 1 plus dyslipidemia, hypertension and family histories of hypertension and T2DM; Model 3 further adjusted for PA or single-air pollutant (PM₁, PM_{2.5}, PM₁₀ or NO₂) for one time. Abbreviation: NGT: normal glucose tolerance; IFG: impaired fasting glucose; T2DM: type 2 diabetes mellitus; NO₂: nitrogen dioxide; PM₁: particulate matter with an aerodynamic diameter $\leq 1.0 \mu\text{m}$; PM_{2.5}: particulate matter with an aerodynamic diameter $\leq 2.5 \mu\text{m}$; PM₁₀: particulate matter with an aerodynamic diameter $\leq 10 \mu\text{m}$; PA: physical activity; MET: metabolic equivalent; CI: confidence intervals.

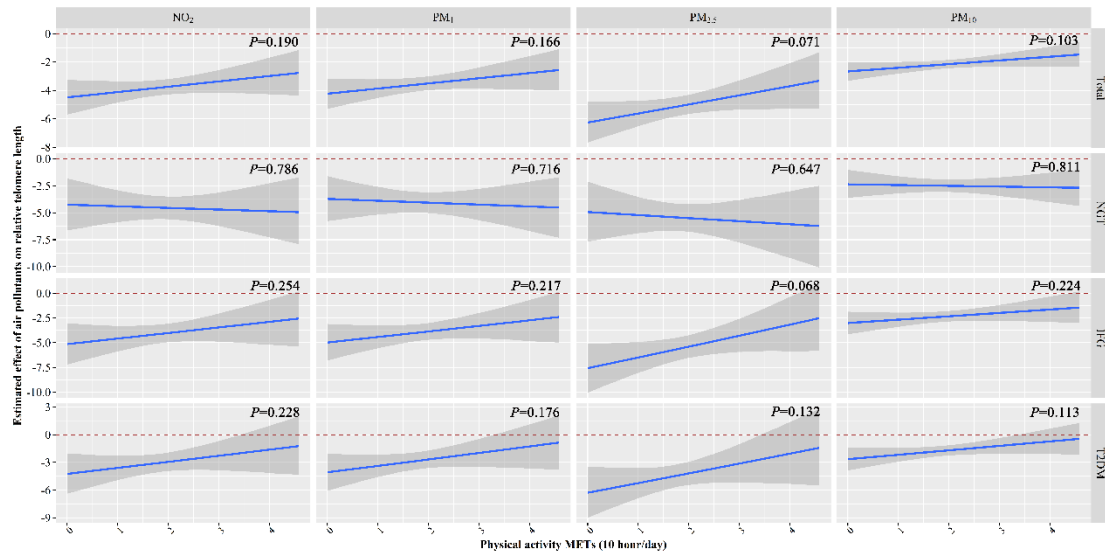


Figure 3 Interactive effects of either particulate matter or NO₂ and physical activity on relative telomere length by glucometabolic status.

The models adjusted for age, gender, marital status, average monthly individual income, education level, smoking and drinking status, more vegetables and fruits intake, high fat diet, dyslipidemia, hypertension, family history of hypertension, family history of T2DM. The X-axis represented the values of physical activity-MET. The Y-axis represented the changes of estimated percent change and 95% confidence interval in relative telomere length in response to each 1- $\mu\text{g}/\text{m}^3$ increased air pollutants with increased PA-METs. The blue lines and grey areas represented the estimated effect and 95% confidence interval.

Abbreviation: NGT: normal glucose tolerance; IFG: impaired fasting glucose; T2DM: type 2 diabetes mellitus; NO₂: nitrogen dioxide; PM₁: particulate matter with an aerodynamic diameter $\leq 1.0 \mu\text{m}$; PM_{2.5}: particulate matter with an aerodynamic diameter $\leq 2.5 \mu\text{m}$; PM₁₀: particulate matter with an aerodynamic diameter $\leq 10 \mu\text{m}$; MET: metabolic equivalent.