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Physical activity counteracted associations of exposure to mixture of air pollutants with mitochondrial DNA copy number among rural Chinese adults

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Running title: Physical activity, air pollution and mtDNA-CN

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Abstract

Background: Exposure to single air pollutant and physical activity (PA) were associated with an altered mitochondrial DNA copy number (mtDNA-CN). However, studies on the interactive effects of single or a mixture of air pollutants and PA on mtDNA-CN were limited.

Methods: A total of 2,707 Chinese adults were obtained from the Henan Rural Cohort Study. Spatiotemporal models were used to estimate particulate matter (PMs) (PM with an aerodynamic diameter ≤ 1.0 μm (PM_1), ≤ 2.5 μm ($PM_{2.5}$) or ≤ 10 μm (PM_{10})) and nitrogen dioxide (NO_2) concentrations. Relative mtDNA-CN was measured by quantitative real-time polymerase chain reaction. Linear regression and quantile g-computation models were applied to examine associations of single or mixture of air pollutants with relative mtDNA-CN. The interactive effects of single or mixture of air pollutants and PA on relative mtDNA-CN were visualized by using Interaction plots.

Results: Each 1 μg/m^3 increment in PM_1, $PM_{2.5}$, PM_{10} or NO_2 was associated with a 5.11% (95% *confidence interval*: 3.71%, 6.53%), 6.77% (4.81%, 8.76%), 3.05% (2.22%, 3.87%) or 4.99% (3.45%, 6.55%) increase in relative mtDNA-CN. Each one-quartile increment in mixture of the four air pollutants was related to a 0.053 (0.032, 0.075) increase in relative mtDNA-CN. Negative interaction effects of single or mixture of air pollutants and PA on relative mtDNA-CN were observed.

Conclusions: The positive associations of single or mixture of air pollutants with relative mtDNA-CN were counteracted by PA at certain levels, implying that PA may be a costless and effective approach to decrease negative effects of air pollution on

mtDNA-CN.

Keywords: air pollutants; physical activity; mitochondrial DNA copy number; rural population; quantile g-computation.

1. Introduction

Mitochondrion, a double-membrane organelle, is involved in a variety of biochemical reactions such as production of adenosine triphosphate (ATP), synthesis of macromolecules and regulation of apoptosis (Yee et al., 2014; Vakifahmetoglu-Norberg et al., 2017; van der Bliek et al., 2017). Mitochondrial DNA copy number (mtDNA-CN) has been suggested as a potential biomarker of aging and a sensitive target of physiological and environmental stress (Curran et al., 2007; Shen et al., 2008; Wong et al., 2009). Evidences from multiple studies indicate that mitochondrial dysfunction contributes to age-related chronic diseases such as cardiovascular diseases and type 2 diabetes mellitus (T2DM) by inducing excessive production of reactive oxygen species and abnormal cellular energy metabolism as well as modulate the expression of nuclear genes (Pello et al., 2008; Chistiakov et al., 2018; Guyatt et al., 2018; Yaribeygi et al., 2019).

Long-term exposure to ambient air pollution is linked to mtDNA-CN alteration (Hu et al., 2020; Wang et al., 2020b). For instance, exposure to particulate matter (PM) or nitrogen dioxide (NO_2) is associated with an altered mtDNA-CN via leading to excessive production of reactive oxygen species in the mitochondria and subsequently damage the structure of the mitochondria (Li et al., 2003; Bai et al., 2018; Wang et al., 2020b). Several studies show inconsistent results on mtDNA-CN alteration in relation to short- or long-term exposure to air pollution (Iodice et al., 2018; Wang et al., 2020a; Wang et al., 2020b). The differences in these studies may be due to different levels and components of air pollution, and studies' location regions and sample size. For instance, there were inconsistent results of polycyclic aromatic hydrocarbons, which were considered as one important of PM constitutes related to alteration of mtDNA-CN (Pavanello et al., 2013; Ling et al., 2017). Air pollutants-induced oxidative stress as a hierarchical event, lower oxidative stress could have protective effects by activating antioxidant defense system, while, higher oxidative stress could lead to a negative biological effect by inducing proinflammatory cytokine secretions and cytotoxic effects (Li et al., 2008).

Physical activity (PA) related to alter mtDNA-CN has been well documented (Chang et al., 2016). Regular aerobic PA may induce production of ROS and increased C-reactive protein or interleukin-6 secretion in the bodies which may increase resistance to the negative effect in response to ambient air pollution (Kelly, 2003; Nimmo et al., 2013; Fraile-Bermudez et al., 2015; Wu et al., 2017). An experimental study suggested that moderate exercise level per day could increase the mtDNA-CN of mice after 20 weeks of exercise training (Cao et al., 2012). Moreover, a study conducted in swimmers indicated that highly trained swimmers tended to have a higher mtDNA-CN compared to normal trained or non-athlete swimmers (Baykara et al., 2016), which may be attributable to increased ATP levels in blood. However, there is a lack of evidence on the combined effects of single- or mixture of air pollutants and PA on mtDNA-CN. Therefore, this study aims to: *(1)* assess associations of single- or mixture effects of air pollutants (PM with an aerodynamic diameter ≤ 1.0 μm (PM_1), ≤ 2.5 μm ($PM_{2.5}$), ≤ 10 μm (PM_{10}) and NO_2) with relative mtDNA-CN; *(2)* explore the joint effects between single- or mixture of air pollutants and PA on relative mtDAN-CN in adult population.

2 Material and methods

2.1. Study population

The participants in this study were derived from the baseline health survey of Henan Rural Cohort Study during of 2015-2017 (n=39,259) (Liu et al., 2019). Firstly, a total number of 925 T2DM patients were randomly selected from the baseline survey of this cohort study. Secondly, each T2DM patient was matched with one normal glucose tolerance (NGT) participant and impaired fasting glucose (IFG) participant by sex and age (±3 years) from the same cohort (Li et al., 2021). Participants with missing data on concentrations of air pollutants (n=35) or relative mtDNA-CN (n=33) were excluded from the study. In total, 2,707 participants were included in this study for analysis. A questionnaire was applied to collect data on each participant' general information, lifestyles as well as histories of personal and family diseases by a face-to-face interview. In accordance with the dietary guidelines for Chinese residents, participants were classified into low (< 75g/day) and high (≥ 75 g/day) fat diet intake groups by the daily intake of the live- stocks and poultry meat; participants were classified into low (< 500 g/day) and high (≥ 500 g/day) vegetable and fruit intake groups according to their daily intakes (Yang et al., 2016). Information on participants' PA including the frequency and duration of PA over a past week was collected by using the International Physical Activity Questionnaire (Bennie et al., 2013). In this study, PA metabolic equivalent (MET) values were estimated by using the following formula: duration (hour/day) × frequency (times/week) × coefficient of MET for each type of activity (vigorous PA, moderate PA, and walking were 8, 4 and 3.3, respectively) (Hou et al., 2020, Tu et al., 2019). This study was approved by Zhengzhou University Life Science Ethics Committee and written informed consents were obtained from all participants before the health survey.

2.2. Exposure assessment

Concentrations of ambient PMs (PM_1, $PM_{2.5}$, or PM_{10}) and NO_2 were predicted by using a spatiotemporal model in conjunction with remote sensing data from satellites, meteorology conditions, land use and other predictors (Chen et al., 2018a; Chen et al., 2018b; Chen et al., 2018c). Ambient PMs was estimated by using a random forest model with aerosol optical depth data (MODIS satellite) as well as other factors such as temperature and humidity information. The concentration of NO_2 was predicted with satellite-derived OMI (Daily Level-3 NO_2 Product) data plus other predictors. The estimated annual average on PM_1, $PM_{2.5}$, PM_{10} and NO_2 of 10-fold cross-validation R^2 from predicated model are 75%, 86%, 81% or 72%, respectively, which indicated a good predictive ability. *Concentrations of long-term exposure to four air pollutants were reflected by 3-year averaged concentrations before baseline of this study. They were estimated by averaging the daily concentrations of each air pollutant during the three years that incorporated with the corresponding geocoded home addresses.*

2.3. Measurement of relative mitochondrial DNA copy number

The whole genomic DNA of peripheral blood from each participant was extracted using the Whole Blood Genomic DNA Medium extraction kit III (Bioteke Corporation, Beijing, China) in accordance to the manufacturer's protocol. The concentrations of DNA were measured at least twice using NanoDrop 2000 Spectrophotometer (Thermo scientific, Waltham, MA, USA) and the difference in DNA concentration between two measurements was less than 5ng/μl. The quantitative real-time polymerase chain reaction (RT-PCR) (QuantStudio™ 7 Flex, Applied Biosystems Life technologies, USA) was used to determine mitochondrial DNA and single-gene copy number in triplicate (Cawthon, 2009). The primers of mtDNA forward and reverse were 5′-TGGCTCCTTTAACCTCTCCA-3′ and 5′-GGTTCGGTTGGTCTCTGCTA-3′, respectively (Hou et al., 2019) as well as beta actin forward and beta actin reverse were 5′-ACTCTTCCAGCCTTCCTTCC-3′ and 5′-GGCAGGACTTAGCTTCCACA-3′, respectively (Pieters et al., 2016). The relative mtDNA-CN was calculated using the average of three measurements of mtDNA divided by the average of three measurements of single copy number using the $2^{-\triangle\triangle Ct}$ method (Livak and Schmittgen, 2001; Hou et al., 2019). Inter-plate and Inter-day coefficient of variation (CV) of mtDNA-CN were 2.20% and 1.49%, the corresponding CV of beta actin were 1.38% and 0.84%, respectively.

2.4. Statistical analysis

Kolmogorov-Smirnov test was performed to test the normality of the data. Non-normal distributed and categorical variables were presented as median (interquartile range, IQR) and numbers (percent). The relative mtDNA-CN was transformed via natural logarithmic to approximate normal distribution prior to analysis. Quantile g-computation can assess the change in one outcome in response to increased one quantile of all exposures in the mixture. The contribution of each component in the mixture in the quantile g-computation did not satisfy with directional homogeneity (Niehoff et al., 2020). Linear regression models and quantile g-computation were used to explore associations of single ambient air pollutant, mixture of air pollutants or PA with relative mtDNA-CN. Three models were constructed: **Model 1** was unadjusted; **Model 2** was adjusted for gender, age, individuals' average monthly income, educational attainment, marital status, drinking and smoking status, high fat diet, more vegetables and fruits intake, hypertension, dyslipidemia as well as family histories of hypertension and T2DM diseases; **Model 3** was further adjusted for PA or single ambient air pollutant in addition to variables in the model 2. The percentage change (%) and 95% confidence interval (CI) in relative mtDNA-CN for each unit increment in air pollutants or PA was calculated by using the following formulas: $(\exp^{\beta} - 1) \times 100\%$ and $(\exp^{(\beta \pm 1.96 \times SE)} - 1) \times 100\%$, respectively. The two-tailed $P <$ 0.05 was considered significant. Furthermore, interactive effects between single- or mixture of ambient air pollutants and PA on relative mtDNA-CN were assessed by using the generalized linear regression model and visualized by using interplot method. The statistical significance of interaction was set as two tailed $P_{\text{interaction}} < 0.10$. Additionally, we conducted stratified analyses to examine the associations of air pollutants or physical activity with relative mtDNA-CN among three groups (T2DM patients, IGT population, and NGT population). All analyses were performed using R-packages of "qgcomp" and "Interplot" in R software (version 3.5.3, R foundation for Statistical Computing).

3. Results

3.1 Characteristics of study participants

Table 1 shows characteristics of 2,707 individuals. The median of age was 61.0 (IQR: 54.0, 66.0) years, PA-MET was 18.6 MET-hour/day (IQR: 14.0, 22.0 MET-hour/day), and relative mtDNA-CN was 0.9 (IQR: 0.8, 1.1). 62% of participants were women, 74.8% never smokers, 82.3% never drinkers, 56.4% educational level of ≤elementary school and 18.4% a diet high in fat in this study. **Table 2** exhibits that the range of concentrations of ambient PMs (PM_1, $PM_{2.5}$, and PM_{10}) and NO_2 were 54.1–61.2 μg/m^3, 69.7–75.6 μg/m^3, 124–134 μg/m^3 and 32.5–37.7 μg/m^3, respectively. **Figure 1** shows the correlation between each pair of the four ambient air pollutants (r ranged from 0.694-0.923).

3.2 Associations of PA, single-or mixture of air pollutant with relative mtDNA-CN

Figure 2 exhibits associations between four air pollutants (PM_1, $PM_{2.5}$, PM_{10} and NO_2) or PA and relative mtDNA-CN. In **model 1**, each 1 μg/m^3 increment in PM_1, $PM_{2.5}$, PM_{10} or NO_2 was associated with a 5.12% (95% *confidence interval* (*CI*): 3.75%, 6.51%), 6.74% (95% *CI*: 4.84%, 8.69%), 3.05% (95% *CI*: 2.24%, 3.86%) or

5.07% (95% *CI*: 3.55%, 6.60%) increase in relative mtDNA-CN. In **models 2-3**, the results were similar to the results from **model 1**. No significant associations were observed between PA and relative mtDNA-CN in all models. Stratified data analysis by glucometabolic status (**Table S1**) revealed that the associations of air pollutants and physical activity with relative mtDNA-CN in each of three groups (T2DM patients, IGT population, and NGT population) were consistent with those in all participants. **Table 3** shows results of association between mixture of air pollutants and relative mtDNA-CN using quantile g-computation approach. In **model 1**, each one-quartile increment in mixture of four ambient air pollutants was associated with a 0.055 (95% *CI*: 0.034, 0.076) increase in relative mtDNA-CN. The results were not change substantially in **models 2-3**. **Figure S1** and **Table S2** show the weight of each air pollutant estimated by quantile g-computation method. In **model 1**, the weights (%) of $PM_{2.5}$ and NO_2 in the negative direction were 54.7% and 45.3%, whereas the weights (%) of PM_1 and PM_{10} in the positive direction were 32.6% and 67.4%, respectively. There were no substantial changes in these in **models 2-3**.

3.3 Interactive effect of single or mixture of air pollutants and PA on relative mtDNA-CN

Figure 3 demonstrates interactive effects of single or mixture of air pollutants and PA on relative mtDNA-CN. There were inverse interactive effects of single or mixture of four ambient air pollutants and PA on relative mtDNA-CN (all $P_{interaction} < 0.10$), suggesting that the positive associations of exposure to single or mixture of air pollution with relative mtDNA-CN were counteracted by PA at the cut-points of PA-3.20, 3.01, 2.97, 2.74 and 3.20 for PM_1, $PM_{2.5}$, PM_{10}, NO_2 and mixture of them among all participants, respectively. **Figure S2** shows interactive effects of single or mixture of air pollutants and PA on relative mtDNA-CN stratified by glucometabolic status. The results observed in three groups of participants were consistent with the results of all participants.

4. Discussions

The findings of this study suggested that exposure to single or mixture of air pollutants were positively associated with relative mtDNA-CN and these associations were counteracted by PA at certain levels. The positive associations between air pollutants and relative mtDNA-CN in our study were similar to those in several previous studies (Hou et al., 2010; Iodice et al., 2018; Wang et al., 2020a). In Milan, Italy, a study including 199 pregnant women showed that each 10 μg/m^3 increment in PM_{10} concentrations was related to a 14% (95% *CI*: 1.08, 1.20) increased geometric mean ratio of mtDNA-CN at the first five weeks of pregnancy (Iodice et al., 2018). A cross-sectional study including 120 Chinese indicated that individuals living in a region of high air pollution tended to have a significantly higher mtDNA-CN than those in a low-pollution region (*P*=0.00097) (Wang et al., 2020a). A study conducted in Brescia, Northern Italy also revealed that either short- (PM_1: $\beta = 0.22$, 95% CI = 0.09, 0.36; PM_{10}: $\beta = 0.17$, 95% CI = 0.08, 0.27) or long-term exposure to PMs (PM_1: $\beta = 0.07$, 95% CI = -0.09, 0.24; PM_{10}: $\beta = 0.05$, 95% CI = -0.07, 0.17) was positively related to relative mtDNA-CN (Hou et al., 2010). However, some studies reported inconsistent results. One study undertaken in the United States suggested that per IQR increment in 12-month ambient PM_{10} or $PM_{2.5}$ concentrations was associated with a 0.04 ($\beta = -0.04$; 95% *CI*: -0.10, 0.02) or 0.07 ($\beta = -0.07$; 95% *CI*: -0.13, -0.01) decrease in mtDNA-CN among women nurses aged 30-50 years (Wang et al., 2020b). Another study conducted in Flanders, Belgium showed that per 10 μg/m^3 increment in traffic-derived air pollutant (NO_2) was associated with a 3.7% (95% *CI*: 2.1 to 5.3%) decrease in mtDNA-CN in elderly participants (Bai et al., 2018). The discrepancy between these studies may be due to differences in the characteristics of study population such as age, region, estimation methods of air pollutants as well as the levels and major components of ambient air pollution. Additionally, evidences demonstrated that individuals with low socio-economic status tend to be sensitive to higher levels of air pollutants such as PMs and NO_2 related adverse health effect (Havard et al., 2009; Pinault et al., 2016). Moreover, recently a study conducted in rural and urban areas of China has indicated that rural residents are more susceptible to the harmful effects of air pollution than urban residents in China (Zhao et al., 2021).

Inflammatory response and oxidative stress were proved as the two major potential mechanisms of increased mtDNA-CN in relation to exposure to high levels of air pollution. Mitochondria is sensitive to environmental factors such as PMs and NO_2 which may induce excessive reactive oxygen species production in mitochondria. A study focused on urban ambient air pollution in Taipei found that high-sensitivity C-reactive protein or 8-hydroxy-2-deoxyguanosine (the biomarkers of inflammation or oxidative stress) was increased in highly polluted regions (Chuang et al., 2007). Grevendonk et al. also indicated that mitochondrial 8-hydroxy-2'-deoxyguanosine (8-OHdG), as a promising biomarker of oxidative DNA damage, was related to high levels of air pollutants (Grevendonk et al., 2016). Moreover, increment in 8-OHdG values was positively associated with increased mtDNA-CN (Zheng et al., 2019). Population-based research indicated that repeatedly exposed to high levels of air pollutants may induce mitochondrial oxidative damage by activation of nuclear factor erythroid 2-p45 related factor 2 or nuclear factor kappa-B signaling pathways (Iodice et al., 2018) and lead to an increase in mtDNA-CN to compensate for the oxidative damage.

Moderate PA could increase the number of normal mitochondria and reduce damage to mitochondrial (Cao et al., 2012; Chang et al., 2016). Regularity exercise or PA may reduce mitochondrial aging and inhibit oxidative damage by stimulating the secretion of anti-inflammatory molecules and increasing mitochondrial oxidative capacities (Mury et al., 2018). It is well known that oxidative damage is an important mechanism of mtDNA-CN alteration in response to air pollution. Epidemiological studies demonstrated that increased PA was negatively associated with inflammatory response in human body (Hou et al., 2010; Iodice et al., 2018). Therefore, we inferred it plausible that negative interactive effects of single or mixture of the four ambient air pollutants and PA on relative mtDNA-CN were observed among all participants. The potential mechanism of these effects could be inflammatory response and oxidative stress induced by PA and air pollutants. Our findings have implied that regularity PA may be an affordable and effective way to reduce the harmful effects of air pollution on mtDNA-CN.

To our best knowledge, the current study is the first to investigate the mixed effects of exposure to air pollutants simultaneously and joint effects of exposure to air pollutants and PA on relative mtDNA-CN in rural Chinese adults. There are some limitations in the study. First, spatial errors of misclassification may not be avoided. To minimize its effect, we used the geocoding rural address to estimate air pollutants. Second, although many important related risk factors were adjusted in this study, some other confounders (such as genetic factors) were not measured for adjustment, that might have residual effects on the association between ambient air pollution and relative mtDNA-CN. Third, the participants of this study limited the generalizability of the findings to the whole Chinese population. Finally, causal inferences of this study were not established, due to its cross-sectional study design.

5. Conclusions

Exposure to single or mixture of air pollutants was positively associated with relative mtDNA-CN and these associations were counteracted by PA at certain levels. This study suggested that mtDNA-CN may be considered as a potential biomarker of age-related disease in relation to exposure to mixture of air pollution. Regularity PA could be accepted as a costless and effective way to reduce adverse effects of air pollution on mtDNA-CN.

Abbreviations

ATP: adenosine triphosphate; CI: confidence interval; CV: coefficient of variation; IFG: impaired fasting glucose; IQR: interquartile range; MET: metabolic equivalent; mtDNA-CN: mitochondrial DNA copy number; NGT: normal glucose tolerance; NO_2: nitrogen dioxide; PA: physical activity; PMs: particulate matters; PM_1: particulate matter with an aerodynamics diameter ≤ 1.0 μm; $PM_{2.5}$: particulate matter with an aerodynamics diameter ≤ 2.5 μm; PM_{10}: particulate matter with an aerodynamics diameter ≤ 10 μm; RT-PCR: real-time polymerase chain reaction; T2DM: type 2 diabetes mellitus; 8-OHdG: 8-hydroxy-2'-deoxyguanosine.

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Table 1

Descriptive characteristic of the study participants

Characteristic	Median (Q25, Q75)	Number (proportion)
Age (years)	61.0 (54.0, 66.0)	
Gender (men)		1039 (38.0%)
Education level		
Elementary school or below		1527 (56.4%)
Junior high school		946 (35.0%)
High school or above		234 (8.6%)
Average monthly income (<500		1106 (40.9%)
Marital status		
Married/Cohabitation		2407 (88.9%)
Unmarried/Divorced/Widowed		300 (11.1%)
Smoking		
Current smokers		480 (17.7%)
Former smokers		201 (7.4%)
Never smokers		2026 (74.8%)
Alcohol consumption		
Current drinkers		322 (11.9%)
Former drinkers		156 (5.8%)
Never drinkers		2229 (82.3%)
High fat diet (Yes)		497 (18.4%)
Vegetables and fruits intake (High)		1707 (63.1%)
Family history of T2DM (Yes)		72 (2.7%)
Physical activity (MET-10 hour/day)	1.86 (1.4, 2.2)	
Relative mtDNA-CN	0.9 (0.8,1.1)	

RMB: renminbi; T2DM: type 2 diabetes mellitus; MET: metabolic equivalent; mtDNA-CN: mitochondrial DNA copy number.

Table 2
Distributions of air pollutants among study population

Variables	Mean	Min	Q_{25}	Q_{50}	Q_{75}	Max	IQR
PM_1 (μg/m^3)	55.7	54.1	54.3	56.2	56.8	61.2	2.43
$PM_{2.5}$ (μg/m^3)	71.0	69.7	70.2	71.5	71.6	75.6	1.39
PM_{10} (μg/m^3)	127	124	125	128	128	134	3.22
NO_2 (μg/m^3)	36.6	32.5	35.6	37.3	37.5	37.7	1.87

Min: minimum; Max: maximum; IQR: interquartile range; PM_1: particulate matter with an aerodynamic diameter ≤ 1.0 μm; $PM_{2.5}$: particulate matter with an aerodynamic diameter ≤ 2.5 μm; PM_{10}: particulate matter with an aerodynamic diameter ≤ 10 μm; NO_2: nitrogen dioxide.

Table 3

Quantile g-computation estimates for the change in relative mtDNA-CN for one quartile increase in mixture of air pollutants

Model	Estimate	Lower *CI*	Upper *CI*
Model 1	0.055	0.034	0.076
Model 2	0.054	0.033	0.075
Model 3	0.053	0.032	0.075

mtDNA-CN: mitochondrial DNA copy number; CI: confidence interval; Model 1 was unadjusted. Model 2 was adjusted for gender, age, individuals' average monthly income, educational attainment, marital status, drinking and smoking status, high fat diet, more vegetables and fruits intake, hypertension, dyslipidemia as well as family histories of hypertension and T2DM diseases; Model 3 was further adjusted for PA based on model 2.

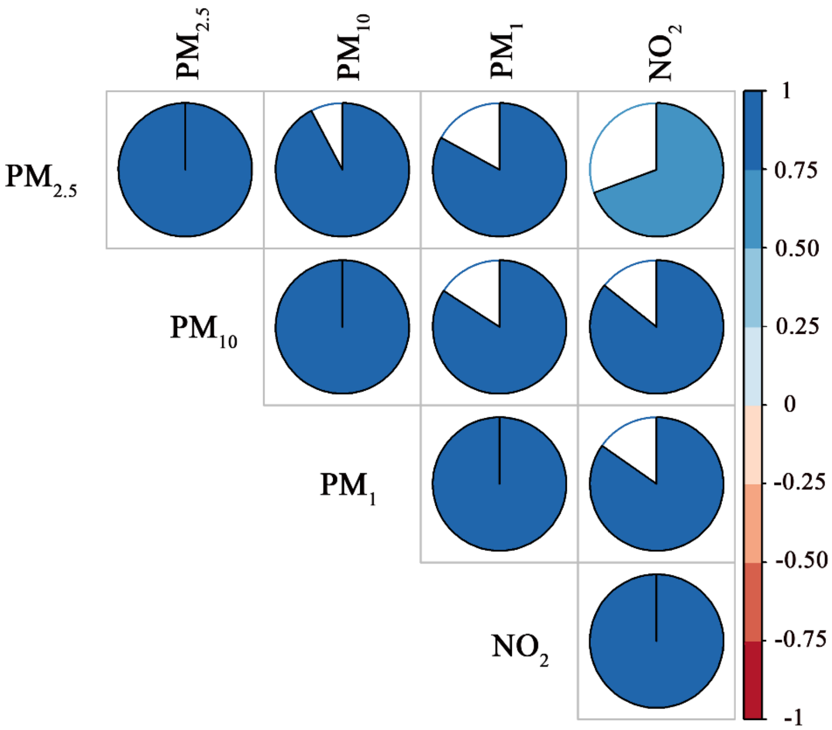

Figure 1 Pairwise correlations of ambient air pollutants.

Abbreviation: NO_2: nitrogen dioxide; PM_1: particulate matter with an aerodynamic diameter ≤ 1.0 µm; $PM_{2.5}$: particulate matter with an aerodynamic diameter ≤ 2.5 µm; PM_{10}: particulate matter with an aerodynamic diameter ≤ 10 µm.

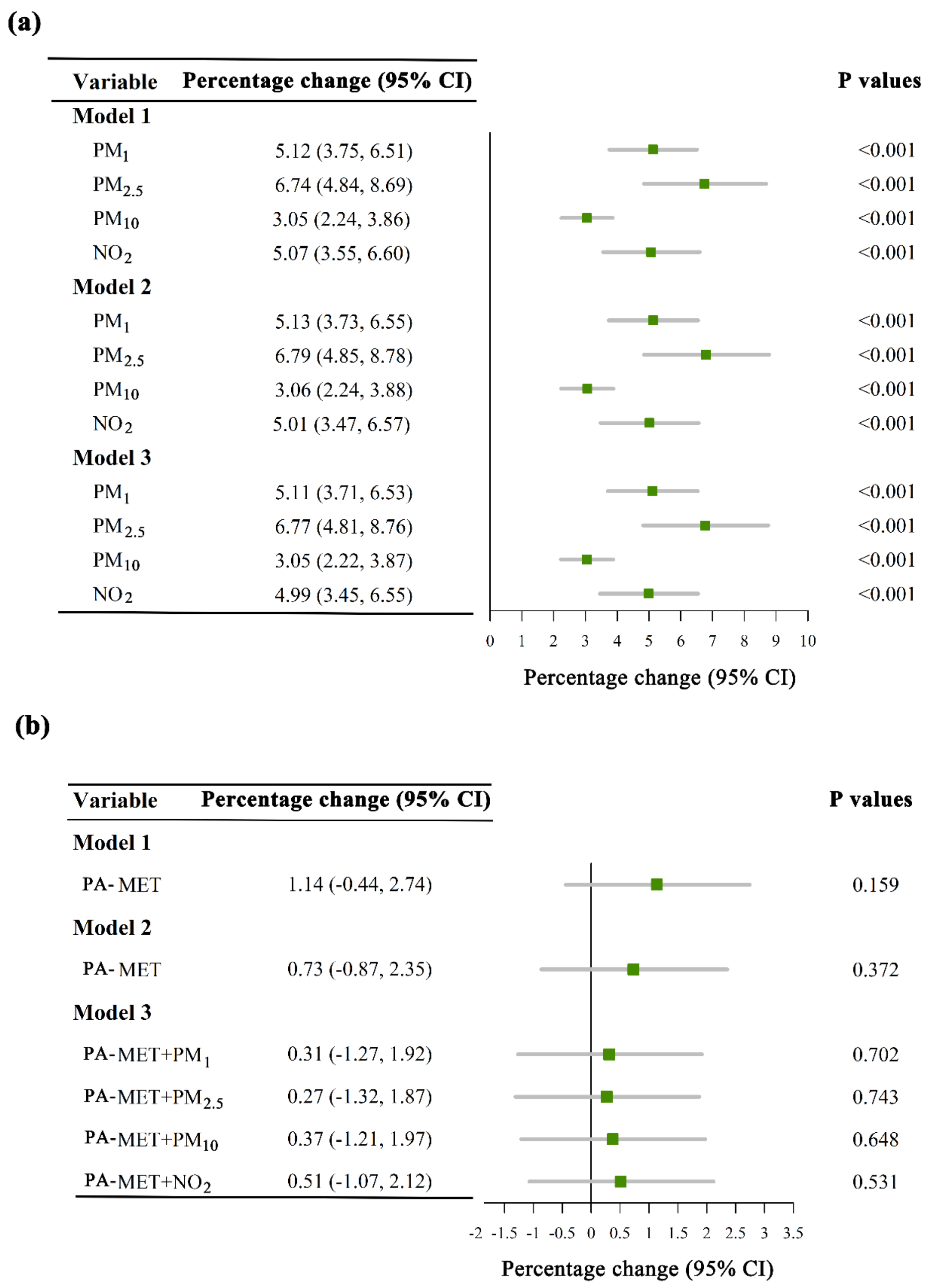

Figure 2 Associations of ambient air pollutants (a) or physical activity (b) with relative mtDNA-CN.

Model 1 was unadjusted; Model 2 was adjusted for gender, age, individuals' average monthly income, educational attainment, marital status, drinking and smoking status, *high fat diet, more vegetables and fruits intake, hypertension, dyslipidemia as well as family histories of hypertension and T2DM diseases; Model 3 was further adjusted for physical activity or ambient air pollutants.*

Abbreviation: mtDNA-CN: mitochondrial DNA copy number; NO_2: nitrogen dioxide; PM_1: particulate matter with an aerodynamic diameter ≤ 1.0 μm; $PM_{2.5}$: particulate matter with an aerodynamic diameter ≤ 2.5 μm; PM_{10}: particulate matter with an aerodynamic diameter ≤ 10 μm; MET: metabolic equivalent; CI: confidence intervals.

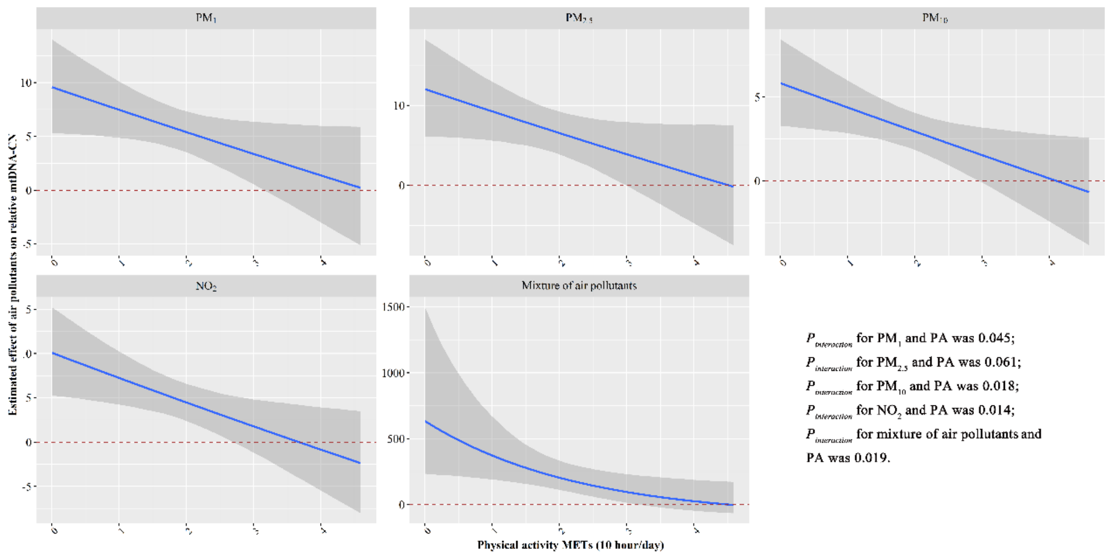

Figure 3 Interactive effects of single or mixture of air pollutants and PA on relative mtDNA-CN.

The models was adjusted for gender, age, individuals' average monthly income, educational attainment, marital status, drinking and smoking status, high fat diet, more vegetables and fruits intake, hypertension, dyslipidemia as well as family histories of hypertension and T2DM diseases.

Abbreviation: PA: physical activity; mtDNA-CN: mitochondrial DNA copy number; NO_2: nitrogen dioxide; PM_1: particulate matter with an aerodynamic diameter ≤ 1.0 μm; $PM_{2.5}$: particulate matter with an aerodynamic diameter ≤ 2.5 μm; PM_{10}: particulate matter with an aerodynamic diameter ≤ 10 μm; MET: metabolic equivalent.