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Factors Associated with Age-Related Changes in Non-Smoking Urban Men and Women in China Determined by Low-Dose Computed Tomography Imaging

Authors' Contribution:

Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF 1 **Shujing Li**
BCDEF 1 **Yaguang Li**
BCD 1 **Meibao Kong**
BCD 1 **Chenguang Zhang**
CDE 2 **Yulan Geng**
BCD 1 **Mengyue Sun**
BC 1 **Li He**
BEF 1 **Shengnan Li**
ABCDEF 3 **Huaijun Liu**

1 Department of Radiology, The First Hospital of Hebei Medical University, Shijiazhuang, Hebei, PR China
2 Department of Laboratory, The First Hospital of Hebei Medical University, Shijiazhuang, Hebei, PR China
3 Department of Medical Imaging, The Second Hospital of Hebei Medical University, Shijiazhuang, Hebei, PR China

Corresponding Author: Huaijun Liu, e-mail: huaijunliu2@126.com

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Background: Respiratory function usually worsens in the elderly with aging. This study aimed to retrospectively investigate tracheal changes caused by "normal aging" through use of low-dose CT (LDCT) in non-smoking asymptomatic urban residents and the related factors influencing tracheal changes.


Material/Methods: A total of 733 Chinese subjects who underwent LDCT were recruited. The trachea shape, width, and calcification degree of the tracheal wall were measured and compared between males and females and among different age groups. The effects of age, sex, trachea morphology, BMI, BP, GLU, TC, TG, HDL, and LDL on the width and calcification of tracheal wall were analyzed by multiple linear regression.

Results: Significant sex differences in trachea shape were found, as type II and type I were found mainly in the males and females, respectively. The values of anterior-posterior inner diameter (AP), left-right inner diameter (LR), width, and calcification score of tracheae in the males were higher than that in the females. In both males and females, trachea AP, wall width, and calcification scores increased with age, but this trend was not observed in tracheal LR. Age, sex, and trachea shape had significant effects on the width and calcification scores of tracheal walls, and trachea calcification was one of the factors influencing tracheal wall width.

Conclusions: Tracheal aging can be evaluated by measuring trachea shape, thickness, and the degree of calcification of the tracheal wall by LDCT, while sex and age should be taken into consideration comprehensively for judging normal trachea aging. In addition, obesity may aggravate trachea aging.

Keywords: **Aging • Body Mass Index • Calcification, Physiologic • Multidetector Computed Tomography • Trachea**

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Background

The problem of an aging population is becoming serious all around the world. According to the World Population Prospects 2019 Highlights[1], it is expected that the trend of increasing life expectancy will continue, and the proportion of the elderly population will proceed to increase. Particularly, the population over 65 years old will be the fastest growing aging group [1,2]. In 2019, 1/11 of the world's population was older than 65 years, which is expected to increase to 1/6 in 2050, and even 1/4 in Europe and North America [1,2]. Thus, in-depth understanding of the changes in every organ system during physiological aging will no doubt benefit clinical decision-making to improve public health in the elderly.

Aging, defined as an inevitable, time-dependent functional decline that affects most living organisms, causes a progressive impairment of the integrity of physiological structures, impairs organ function, and subsequently increases susceptibility to a wide range of deadly diseases [3,4]. The respiratory system is constantly sampling diverse, potentially injurious, substances that have a great impact on the whole body. Compared with the younger population, the elderly population has increased risk due to the cumulative exposure time and exposure to environmental toxins [5]. However, among the elderly, their respiratory immune system function and respiratory muscle function decline along with changes in the shape and structure of tracheae and lungs, resulting in compromised pulmonary functions [6-8]. In addition, cough reflex and mucociliary function usually decline in the elderly with aging, and the susceptibility to and severity of age-related infection increase [9]. It has been shown that aging can increase the incidence of chronic obstructive pulmonary disease [10], idiopathic pulmonary fibrosis [11], diabetes, and malignant pulmonary tumors [12]. Therefore, how to distinguish "healthy" aging from "disease" aging in the respiratory system is vital, as the establishment of a "normal" appearance of an organ system in the elderly can avoid over-diagnosis and over-treatment of diseases.

The trachea and bronchus are the respiratory channels of the human body [13]. Computed tomography (CT) is the best non-invasive method for evaluating tracheobronchial lesions. Compared to the routine-dose CT, low-dose CT (LDCT) provides a 70.76% dose reduction, and facilitates better airway quantification [14]. Most studies on aging of the respiratory system have focused on changes in lung structure and function, while there have been few investigations of tracheal aging, and large-scale systematic studies on tracheal calcification are particularly rare. For example, Sakai et al investigated age-related changes in the trachea in 83 healthy male adults, and found that aging resulted in increased tracheal area and a distortion of the roundness [15]. Kim et al performed a retrospective analysis of CT data from 119 Korean non-smokers and 45 ever-smokers with normal lung

function, and identified significant differences in quantitative CT parameters, including bronchial wall thickness of inner perimeter of a 10-mm-diameter airway (Pi10) according to sex, age, and smoking history [16]. However, no study on tracheal aging in a large-scale Chinese population has been reported so far. In addition, biological tissue engineering research on tracheal scaffolds showed age-related tracheal changes, and suggested that some histological alterations in tracheal cartilage and connective tissue may be associated with age-related tracheosclerosis [17]. Therefore, an intensive study on the factors influencing tracheal aging evaluated by the non-invasive CT method is necessary to better inform subsequent clinical applications.

As we know, the probabilities of abnormal blood pressure, disordered lipid profile, and dysregulated glucose homeostasis, and obesity increase with age in normal males and females [3,4,12]. Since both the LDCT data and these indicators are accessible in subjects after routine physical examinations, we conducted an intensive, retrospective study on tracheal aging and the influence of these routine indicators on tracheal aging in a cohort of non-smoking asymptomatic urban Chinese. This study aimed to distinguish the manifestations of tracheal changes caused by "normal aging", which could provide an objective imaging basis for targeted slowing of tracheal aging and reducing its negative impact on human health.

Material and Methods

Study Population

This retrospective study included subjects who underwent a routine physical examination with pulmonary LDCT in the Department of Radiology of the First Hospital of Hebei Medical University (Shijiazhuang, China) from January 2017 to December 2019. The inclusion criteria were as follows: (1) 18-80 years of age; (2) no known history of pulmonary disease; (3) no chest symptoms or signs; (4) no systemic diseases with potential impact on pulmonary health; (5) no smoking history; (6) urban residents (continuous urban residence for more than 15 years [18] and no less than 6 months per year). The exclusion criteria were as follows: (1) with respiratory symptoms and history of inhalation of irritants; (2) with pulmonary and pleura diseases, such as COPD, emphysema, or lung infection; (3) history of chest trauma, operation (including tracheal intubation), or pulmonary tumor and radiotherapy; (4) with organic heart disease; (5) images with motion and metal artifacts; (6) with abnormal pulmonary function. A total of 733 subjects (424 males with a mean age of 50.75 ± 15.98 years; and 309 females with a mean age of 51.26 ± 16.75 years) were enrolled. According to age, all eligible subjects were categorized into 6 groups (Table 1). This single-center retrospective study received approval from the Ethics Committee of the First Hospital of Hebei Medical University

Table 1. Baseline data of 733 non-smoking asymptomatic urban subjects.

| G | Sex | BMI | | | BP | | GLU | | TC | | TG | | HDL-C | | | LDL-C | |
|----|----------|-----|----|----|----|----|-----|----|----|----|----|----|-------|---|----|-------|----|
| | | N | O | Ob | N | H | N | H | N | H | N | H | L | N | H | | |
| G1 | M (n=42) | 21 | 12 | 9 | 37 | 5 | 41 | 1 | 38 | 4 | 34 | 8 | 37 | 0 | 5 | 38 | 4 |
| | F (n=43) | 37 | 4 | 2 | 40 | 3 | 43 | 0 | 42 | 1 | 40 | 3 | 41 | 2 | 0 | 42 | 1 |
| G2 | M (n=77) | 31 | 27 | 19 | 64 | 13 | 72 | 5 | 52 | 25 | 45 | 32 | 68 | 0 | 9 | 54 | 23 |
| | F (n=48) | 40 | 8 | 0 | 48 | 0 | 48 | 0 | 40 | 8 | 45 | 3 | 46 | 2 | 0 | 43 | 5 |
| G3 | M (n=81) | 33 | 36 | 12 | 62 | 19 | 70 | 11 | 56 | 25 | 48 | 33 | 69 | 1 | 11 | 47 | 34 |
| | F (n=50) | 36 | 11 | 3 | 46 | 4 | 50 | 0 | 34 | 16 | 43 | 7 | 45 | 5 | 0 | 37 | 13 |
| G4 | M (n=78) | 28 | 29 | 21 | 42 | 36 | 53 | 25 | 50 | 28 | 41 | 37 | 74 | 1 | 3 | 49 | 29 |
| | F (n=51) | 33 | 14 | 4 | 40 | 11 | 46 | 5 | 21 | 30 | 39 | 12 | 45 | 5 | 1 | 26 | 25 |
| G5 | M (n=74) | 28 | 33 | 13 | 42 | 32 | 46 | 28 | 58 | 16 | 54 | 20 | 62 | 1 | 11 | 51 | 23 |
| | F (n=59) | 29 | 20 | 10 | 44 | 15 | 51 | 8 | 35 | 24 | 39 | 20 | 56 | 2 | 1 | 32 | 27 |
| G6 | M (n=72) | 33 | 28 | 11 | 35 | 37 | 49 | 23 | 52 | 20 | 59 | 13 | 64 | 3 | 5 | 50 | 22 |
| | F (n=58) | 24 | 25 | 9 | 30 | 28 | 32 | 26 | 28 | 30 | 38 | 20 | 52 | 3 | 3 | 30 | 28 |

G – group; M – Male; F – Female; BMI – body mass index; BP – blood pressure; GLU – blood glucose; TC – total cholesterol; TG – triglyceride; HDL-C – high density lipoprotein cholesterol; LDL-C – low density lipoprotein cholesterol. N – normal; O – overweight; Ob – obesity; H – higher; L – lower. G1, <30 y; G2, 30-39 y; G3, 40-49 y; G4, 50-59 y; G5, 60-69 y; G6, 70-79 y.

(No. 20190909), and the requirement for informed consent was waived due to the retrospective nature of this study.

CT Examination

For pulmonary imaging, we used a Neusoft 64-Slice spiral CT (NeuViz64 In, Neusoft, Shenyang, China) and a Toshiba 320-Slice spiral CT (Aquilion ONE, Toshiba, Otawara, Japan). All subjects underwent volumetric CT without contrast in the craniocaudal direction while they were placed in the supine position with 2 arms raised above head. Each subject was instructed to hold their breath at full inspiration, and the scanning range ranged from lung tip to the costophrenic angle. The scanning parameters of the former were as follows: voltage, 120 kV; current, 60 mA; matrix, 512×512; acquisition thickness, 5 mm; acquisition interval, 5 mm; reconstruction thickness, 1.0 mm; reconstruction interval, 1.0 mm; and F50 reconstruction kernel. The scanning parameters of the Toshiba 320-Slice spiral CT were as follows: voltage, 120 kV; Sure EXP 3D, quality (Auto mAs); matrix, 512×512; acquisition thickness, 5 mm; acquisition interval, 5 mm; collimation width, 0.5×80 mm; reconstruction thickness, 1.0 mm; reconstruction interval, 1.0 mm; and FC52 reconstruction kernel.

CT Measurements and Observations Analysis

The selected CT sections included thoracic entrance, aortic arch, and superior plane of tracheal carina, which represented the

upper, middle, and lower segment of the trachea, respectively. The CT observations included the following parameters: (1) the width of tracheal wall (W), for which the left-right diameters of the trachea were measured; (2) the trachea shape (S), which was classified into 3 types according to trachea index (Ti, Ti is a radiological parameter calculated as the ratio of left-right inner diameter (LR) to anterior-posterior inner diameter (AP) in the trachea [19]) as follows: type I, round, $Ti=0.95-1.05$; type II, vertical ellipse, $Ti<0.95$; type III, transverse ellipse, $Ti>1.05$; (3) trachea calcification (Ca) and distribution, in which length measurement was achieved by use of the Python program. Specifically, the region of interest (ROI) was acquired through interactive operation analysis, while the lumen and wall of the trachea in the ROI was extracted using the watershed algorithm [20], and the tracheal circumference was obtained. Subsequently, the pixels in the annular area of the trachea wall were taken to cover the calcified area. Otsu's thresholding method [21] was used to segment the extracted ring region to distinguish the highlighted calcified components. The area of calcified region was obtained by calculating the number of highlighted pixels. Finally, the length of the calcified area needed to be further extracted by skeleton line extraction method [22]. The length of calcified area was obtained by counting the number of pixels contained in the line segment.

The semi-quantitative Ca score was calculated according to the percentage of calcified length of tracheal wall in the tracheal

circumference. The scoring system of trachea calcification was defined as follows: 0, no calcification; 1, 1-24% calcification; 2, 25-50% calcification; 3, 51-74% calcification; 4, $\geq 75\%$ calcification. The calcification statuses of the anterior, left, right, and posterior walls of each trachea were measured. To ensure the consistency of the measurements, 120 patients (20 for each group) were premeasured by 3 radiologists, and the intraclass correlation coefficient (ICC) was used for statistical analysis. The ICC values of the 3 radiologists for W, AP, LR, and Ca of the trachea were 0.815, 0.923, 0.883, and 0.885, respectively. In case of disagreement, consensus must be reached after consultation among all the participating radiologists.

Hypertension, Body Mass Index, and Laboratory Analysis

Hypertension was diagnosed as systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg [23]. According to body mass index (BMI), the subject was deemed as normal (N, BMI < 25 kg/m²), overweight (O, BMI ≥ 25 -29.9 kg/m²), or obese (Ob, BMI ≥ 30 kg/m²) [24]. Fasting serum concentrations of glucose (GLU), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were determined using the Beckman Coulter AU5821 automatic biochemistry analyzer (Beckman Coulter, CA, USA). Subjects with GLU ≥ 6.1 mmol/L were considered as hyperglycemic [25], while the subjects with 1 or more of the following blood parameters were considered as hyperlipidemic: TC ≥ 240 mg/dL, TG ≥ 200 mm/dL, HDL-C < 40 mm/dL, or LDL-C ≥ 160 mm/dL [26,27].

Statistical Analysis

Statistical analyses were performed using SPSS version 24.0 (SPSS, Inc., Chicago, IL, USA). Measurements are expressed as mean \pm standard deviation. The 2 independent samples *t* test or one-way analysis of variance (ANOVA) were used with normal distribution and homogeneous variance to evaluate the difference between groups; otherwise, the Mann-Whitney U test or Kruskal-Wallis H test were used. The chi-square test was used to compare data counts and ratios. Multiple regression analysis was performed to identify the factors associated with trachea wall width and calcification score. *P* values < 0.05 were considered statistically significant.

Results

The Baseline data of 733 Non-smoking Asymptomatic Urban Subjects

A total of 733 qualified subjects were enrolled in this study, and their baseline data on BMI, blood pressure, and regular blood testing parameters, including glucose, total cholesterol,

triglyceride, and high-/low-density lipoprotein cholesterol, are summarized in **Table 1**. Based on age, these subjects were divided into 6 age groups: Group 1, less than 30 years old; Group 2, 30-39 years old; Group 3, 40-49 years old; Group 4, 50-59 years old; Group 5, 60-69 years old; and Group 6, 70-79 years old. The males appeared to have a higher percentage of overweight or obesity than the females in all age groups. Along with the increase of age, both men and women showed rising percentages of subjects with hypertension (high blood pressure (BP)), hyperglycemia (high GLU) and hyperlipidemia (high TC/TG/LDL-C, and/or low HDL-C) to various extents.

Effects of Sex on the Shape, Inner Diameter of Trachea and Tracheal Wall Width, and Calcification in Different Age Groups

Significant differences in trachea shape were found between the males and the females. Male tracheas were mainly type II in shape (71.462%), which was followed by type I (26.179%), and the least common was type III (2.358%). However, the shape of female tracheas was predominately type I (59.547%), followed by type II (30.097%), and type III was the least common (10.356%) (**Figure 1**). The Ti of the males was 0.830 ± 0.137 , and that of the females was 0.962 ± 0.123 ($F = 182.450$, $P < 0.05$) (**Figure 2**). The females had a significantly higher Ti than the males in all age groups (**Figure 3**, **Table 2**). Nevertheless, the AP, LR, and W values, and Ca scores of male tracheae were higher than those of female tracheae ($U = 10262.500$, $P < 0.001$; $U = 33496.000$, $P < 0.001$; $F = 107.599$, $P < 0.001$; $F = 43.485$, $P < 0.001$). We found that the distribution of tracheal calcification was similar between the males and the females ($\chi^2 = 11.246$, $P = 0.264$). Specifically, in all the males and the females, the trachea AP values were 20.729 ± 2.740 mm and 15.928 ± 2.138 mm; the LR values were 16.988 ± 2.360 mm and 15.155 ± 1.605 mm; the width values were 1.985 ± 0.356 mm and 1.724 ± 0.389 mm; and the calcification scores were 7.101 ± 2.287 and 5.968 ± 2.314 , respectively (**Tables 2, 3**). In addition, similar differences in these parameters between the males and the females were observed in all age groups (**Tables 2, 3**).

Effects of Age on the Shape, Inner Diameter of Trachea And Tracheal Wall Width and Calcification

We found that the shape of tracheae changed with increasing age; especially, the ratio of type II increased, but this trend was slightly later in females than in males. For the males over 50 years old, the proportion of type II trachea shape increased gradually with age, while such a trend was only found for the females before 60 years old (**Figure 4A**). It is also observed that, in both the males and the females, the AP, W values of tracheae increased with age (**Figure 4B, 4D**, **Tables 3, 4**). However, such a trend in tracheal LR was not identified (**Figure 4C**). Further analysis demonstrated that no significant difference was found

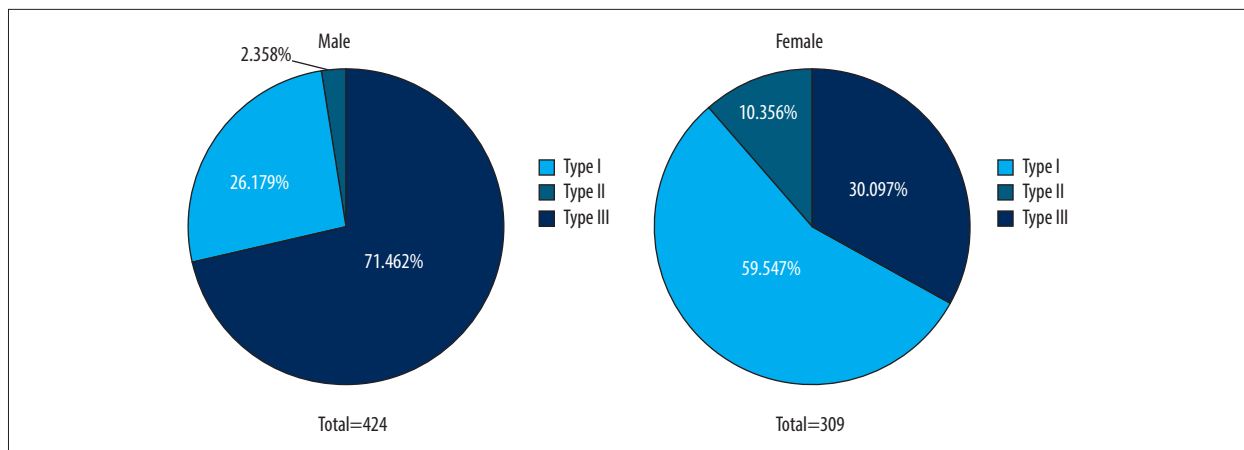


Figure 1. Trachea shape distribution in the males and the females of the study population.

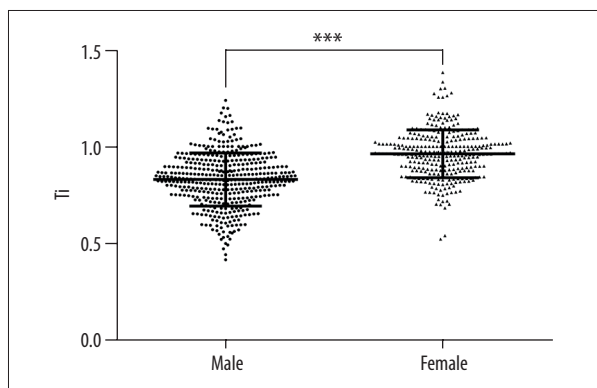


Figure 2. Comparison of Ti (trachea index) values between the males and the females. n=424 for the male group, n=309 for the female group; *** $P < 0.01$.

between the group under 30 years old and the group 30-39 years old in male trachea AP, Ti ($Z=1.768$, $P=1.000$; $Z=1.985$, $P=0.707$, respectively), and in female trachea W, AP, and Ti ($Z=0.252$, $P=1.000$; $Z=2.458$, $P=0.209$; $Z=2.045$, $P=0.614$, respectively) (Table 4). As a result, statistical analysis was instead carried out between groups above and below 40 years old. We found that the values of trachea AP (21.230 ± 2.729 vs 19.442 ± 2.324 , 16.180 ± 2.215 vs 15.322 ± 1.817) and W (2.121 ± 0.294 vs 1.635 ± 0.245 , 1.860 ± 0.252 vs 1.399 ± 0.149) were significantly higher in the males and the females ≥ 40 years old, compared with those below 40 years old (Figure 5A, 5B). On the contrary, the Ti value was lower for the group ≥ 40 years old, as compared with the group whose ages were below 40 years old (0.808 ± 0.138 vs 0.886 ± 0.115 , 0.953 ± 0.130 vs 0.986 ± 0.104 ; all $P < 0.05$; Figure 5C). However, no statistically significant differences were observed in tracheal LR between these 2 groups ($P > 0.05$; Figure 5D).

Tracheal wall calcification appeared more likely to occur on the anterior wall and both side walls of the tracheae, while the posterior wall was less likely to have calcification (Figure 6).

Older age was found to be positively correlated with the score and distribution of tracheal wall calcification. Tracheal calcification increased along with age in both the males and the females ($H=256.941$, $P < 0.001$; $H=235.640$, $P < 0.001$). The changes in posterior wall calcification among different age groups in males and females were statistically significant ($\chi^2=54.987$, $P < 0.001$; $\chi^2=65.429$, $P < 0.001$), particularly for the middle section of tracheal walls ($H=83.293$, $P < 0.001$; $H=61.185$, $P < 0.001$; Figure 6). There were significant differences in tracheal calcification scores ($U=33939.000$, $P < 0.001$; $U=19276.000$, $P < 0.001$) and calcification distributions ($\chi^2=65.429$, $P < 0.001$; $\chi^2=5.751$, $P=0.016$) in men and women between the group under 40 years old and the group ≥ 40 years old (Table 3).

Analysis of Factors Influencing Tracheal Wall Width and Calcification

Multiple linear regression (stepwise method) was used to predict tracheal wall width using the factors age, sex, tracheal wall calcification score, trachea shape, BMI, BP, and HDL-C. We found that the resulting regression model with predictors including age, sex, tracheal wall calcification score, trachea shape, BP, BMI, and HDL-C was statistically significant ($F=106.112$, $P < 0.001$, Adjusted $R^2=0.589$). Age ($t=13.061$, $P < 0.05$), sex ($t=-9.636$, $P < 0.05$), trachea calcification score ($t=3.017$, $P=0.003$), trachea shape (type II, $t=2.192$, $P=0.029$), BMI ($t=2.191$, $P=0.029$), and HDL-C ($t=-2.456$, $P=0.014$) were important factors for tracheal wall width prediction, while BP ($t=1.949$, $P=0.052$) was not (Table 5).

A similar methodology was applied to tracheal wall calcification score prediction, where age, sex, trachea shape, BMI, BP, TG, and HDL-C were considered as potential predictive variables. The final model was statistically significant ($F=153.403$, $P < 0.001$, adjusted $R^2=0.680$), which included age, sex, trachea shape, TG, BP, BMI, and HDL-C as predictors. Among those factors, we found that age ($t=33.285$, $P < 0.05$), sex ($t=-7.998$,

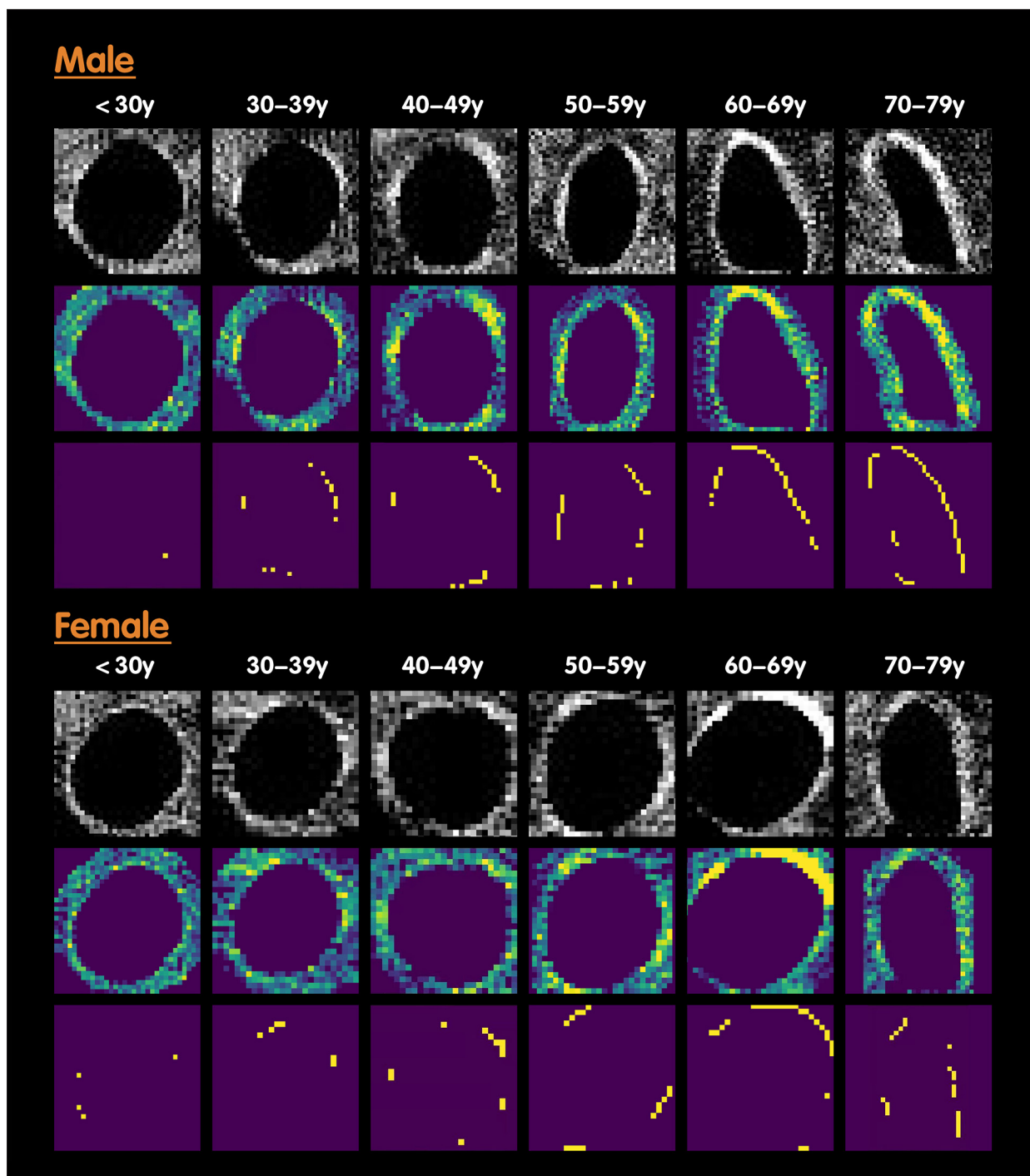


Figure 3. Representative CT images of tracheae from the males and the females in different age groups. The first column, the original image; the second column, the pseudo-color images; the third column, the extracted yellow lines that represent calcification areas.

Table 2. Relationship between sex and trachea inner diameter, wall width in different age groups.

| Group | Sex | W (mm) | AP (mm) | LR (mm) | Ti |
|-------|-------|-------------|--------------|--------------|-------------|
| G1 | M | 1.507±0.180 | 18.867±1.611 | 17.162±1.551 | 0.915±0.100 |
| | F | 1.393±0.140 | 14.735±1.575 | 14.795±1.289 | 1.010±0.088 |
| | Value | U=580.500 | F=143.027 | U=200.500 | F=21.596 |
| | P | 0.004 | <0.001 | <0.001 | <0.001 |
| G2 | M | 1.705±0.249 | 19.756±2.589 | 16.986±1.872 | 0.870±0.120 |
| | F | 1.404±0.157 | 15.848±1.872 | 15.137±1.391 | 0.964±0.113 |
| | Value | U=615.000 | F=82.378 | U=733.500 | F=19.063 |
| | P | <0.001 | <0.001 | <0.001 | <0.001 |
| G3 | M | 1.914±0.239 | 20.728±2.460 | 17.174±1.994 | 0.836±0.110 |
| | F | 1.658±0.230 | 15.656±2.243 | 15.692±1.753 | 1.015±0.130 |
| | Value | U=911.000 | F=140.409 | U=1475.000 | F=71.176 |
| | P | <0.001 | <0.001 | <0.001 | <0.001 |
| G4 | M | 2.121±0.278 | 21.078±2.174 | 16.346±2.133 | 0.781±0.106 |
| | F | 1.849±0.170 | 16.202±1.745 | 15.437±1.175 | 0.959±0.084 |
| | Value | U=780.000 | F=180.376 | U=1202.500 | F=102.514 |
| | P | <0.001 | <0.001 | 0.013 | <0.001 |
| G5 | M | 2.209±0.273 | 21.554±3.142 | 17.001±2.892 | 0.801±0.162 |
| | F | 1.898±0.260 | 15.956±2.091 | 14.973±1.775 | 0.949±0.134 |
| | Value | U=896.500 | U=292.000 | U=1159.000 | F=31.907 |
| | P | <0.001 | <0.001 | <0.001 | <0.001 |
| G6 | M | 2.268±0.257 | 21.628±3.034 | 17.364±3.060 | 0.815±0.166 |
| | F | 2.003±0.206 | 16.841±2.547 | 14.909±1.877 | 0.899±0.137 |
| | Value | U=890.000 | F=92.043 | U=1002.500 | F=9.491 |
| | P | <0.001 | <0.001 | <0.001 | 0.003 |
| G1-G6 | M | 1.985±0.356 | 20.729±2.740 | 16.988±2.360 | 0.830±0.137 |
| | F | 1.724±0.389 | 15.928±2.138 | 15.155±1.605 | 0.962±0.123 |
| | Value | F=107.599 | U=10262.500 | U=33496.000 | F=182.450 |
| | P | <0.001 | <0.001 | <0.001 | <0.001 |

G1, <30 y; G2, 30-39 y; G3, 40-49 y; G4, 50-59 y; G5, 60-69 y; G6, 70-79 y. W – width; AP – anterior-posterior inner diameter; LR – left-right inner diameter; Ti – trachea index.

$P < 0.05$), trachea shape (type II, $t = 3.371$, $P = 0.001$), TG ($t = 2.512$, $P = 0.012$), and BP ($t = 1.977$, $P = 0.048$) were factors influencing the prediction of tracheal wall calcification scores, while BMI and HDL-C ($P > 0.05$) were not (Table 5).

Discussion

The purpose of this study was to investigate the “normal aging”-induced changes of trachea in non-smoking asymptomatic urban residents through evaluating LDCT image data and other available clinical information. In this study, the effects

of age, sex, trachea morphology, BMI, BP, GLU, TC, TG, HDL-C, and LDL-C on the width and calcification of tracheal wall were analyzed. We found that the shape of tracheae changed increased with age; especially, the ratio of type II increased. In both males and females, the AP and W values of tracheae and tracheal calcification increased with age. Tracheal wall calcification appeared more likely to occur on the anterior wall and both side walls of the tracheae, while the posterior wall was less likely to have calcification. However, this trend was slightly different between females and males. The values of AP, LR, W, and Ca scores of the male tracheae were higher than that of the female tracheae. In addition, in both males and females,

Table 3. Analyses of tracheal calcification score and distribution in different sex groups, tracheal segments, and age groups.

| Variable | | Calcification score | Calcification distribution | |
|----------|---------|----------------------------|----------------------------|---------------------------|
| Sex | Segment | | PW with Ca | PW without Ca |
| M | | 7.101±2.287 | 193 (15.20%) | 1079 (84.80%) |
| F | | 5.968±2.314 | 157 (16.90%) | 770 (83.10%) |
| Value | | F=43.485 | $\chi^2=11.246$ | |
| P | | <0.001 | 0.264 | |
| M | Upper | 2.380±0.933 ^a | 24 (5.70%) ^a | 400 (94.30%) ^a |
| | Middle | 2.651±0.845 ^b | 121 (28.50%) ^b | 303 (71.50%) ^b |
| | Lower | 2.071±0.808 ^c | 48 (11.30%) ^c | 376 (88.70%) ^c |
| Value | | H=83.293 | $\chi^2=93.540$ | |
| P | | <0.001 | <0.001 | |
| F | Upper | 1.821±0.864 ^a | 27 (8.70%) ^a | 282 (91.30%) ^a |
| | Middle | 2.328±0.900 ^b | 91 (29.40%) ^b | 218 (70.60%) ^b |
| | Lower | 1.838±0.831 ^a | 39 (12.60%) ^a | 270 (87.40%) ^a |
| Value | | H=61.185 | $\chi^2=53.247$ | |
| P | | <0.001 | <0.001 | |
| M | 1 | 3.880±1.173 ^a | 12 (9.50%) ^a | 114 (90.50%) ^a |
| | 2 | 5.000±1.328 ^{a,b} | 31 (13.40%) ^a | 200 (86.60%) ^a |
| | 3 | 6.840±1.545 ^c | 27 (11.10%) ^a | 216 (88.90%) ^a |
| | 4 | 7.720±1.494 ^d | 22 (9.40%) ^a | 212 (90.60%) ^a |
| | 5 | 8.510±1.590 ^{d,e} | 34 (15.30%) ^a | 188 (84.70%) ^a |
| | 6 | 9.400±1.381 ^{e,f} | 67 (31.00%) ^b | 149 (69.00%) ^b |
| Value | | H=256.941 | $\chi^2=54.987$ | |
| P | | <0.001 | <0.001 | |
| F | 1 | 2.880±1.258 ^a | 19 (14.70%) ^a | 110 (85.30%) ^a |
| | 2 | 3.650±1.062 ^{a,b} | 19 (13.20%) ^a | 125 (86.80%) ^a |
| | 3 | 5.620±1.086 ^c | 20 (13.30%) ^a | 130 (86.70%) ^a |
| | 4 | 6.530±1.239 ^{c,d} | 14 (9.20%) ^a | 139 (90.80%) ^a |
| | 5 | 7.170±1.177 ^{d,e} | 20 (11.30%) ^a | 157 (88.70%) ^a |
| | 6 | 8.760±1.247 ^f | 65 (37.40%) ^b | 109 (62.60%) ^b |
| Value | | H=235.640 | $\chi^2=65.429$ | |
| P | | <0.001 | <0.001 | |
| M | <40y | 4.605±1.379 | 70 (11.70%) | 530 (88.30%) |
| | ≥40y | 8.075±1.775 | 123 (18.30%) | 549 (81.70%) |
| Value | | U=33939.000 | $\chi^2=10.848$ | |
| P | | <0.001 | 0.001 | |
| F | <40y | 3.286±1.214 | 58 (13.70%) | 365 (86.30%) |
| | ≥40y | 7.087±1.648 | 99 (19.60%) | 405 (80.40%) |
| Value | | U=19276.000 | $\chi^2=5.751$ | |
| P | | <0.001 | 0.016 | |

PW with Ca – posterior wall with calcification; PW without Ca – posterior wall without calcification; a-f – there is no statistical difference between groups with the same letter in the subscript.

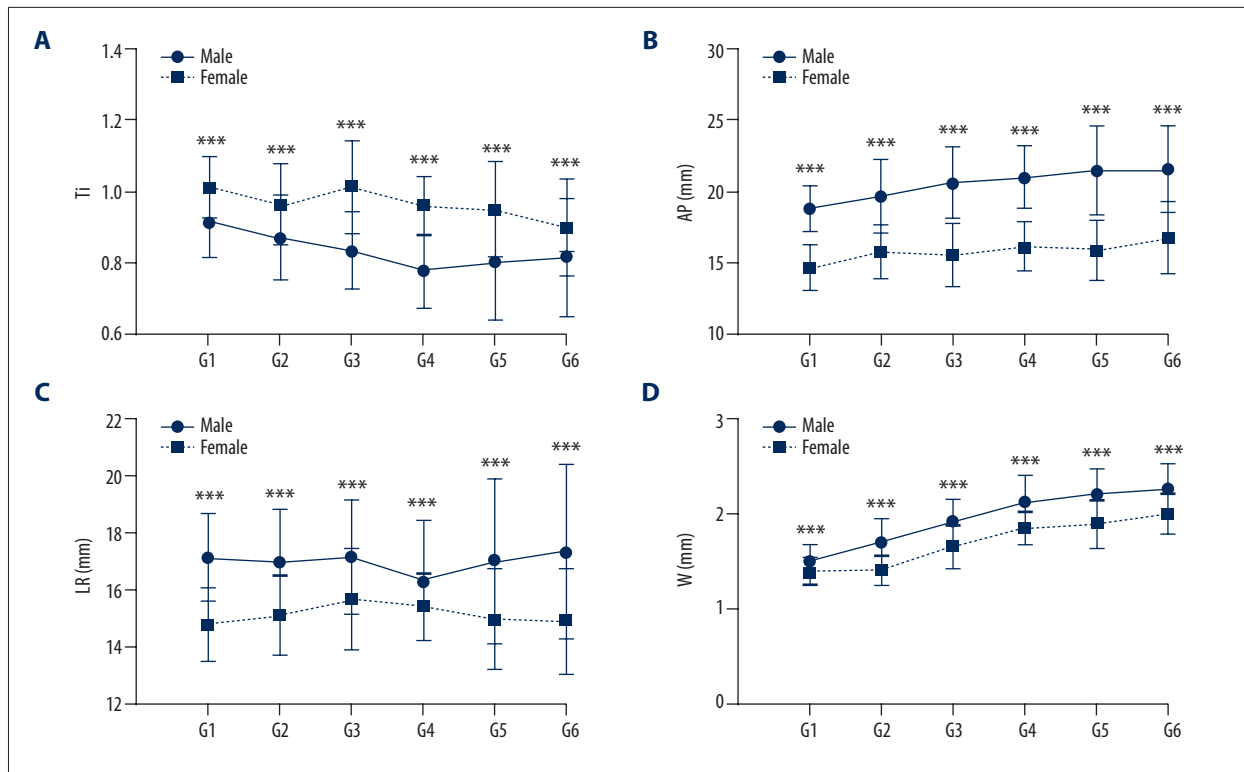


Figure 4. The distributions of trachea-associated parameters in the males and the females from the indicated age groups. (A-D) Distributions of Ti (A), tracheal AP (B), tracheal LR (C), and tracheal wall width (D) with age. Group [G] 1, less than 30 years old; Group 2, 30-39 years old; Group 3, 40-49 years old; Group 4, 50-59 years old; Group 5, 60-69 years old; and Group 6, 70-79 years old.

the values of trachea AP and W, and Ca scores increased, but such trends were not identified in tracheal LR. Further analysis of different age groups showed that males and females there was no significant difference between the group below 30 years and the group 30-39 years old in trachea shape, AP, and Ca, and similar results for female trachea W were noticed. Therefore, we conducted comparative analyses between the subjects below and above 40 years old. Compared with the below 40 years old group, the ≥ 40 years old group had significantly increased trachea AP and W values and Ca score (especially for the calcification of the posterior tracheal wall), and decreased Ti value. However, there was no significant difference in tracheal LR. Thus, we concluded that the age of 40 years may be a threshold age for tracheal aging. Furthermore, multiple linear regression analyses revealed that age, sex, and trachea shape had significant effects on the W and Ca of the tracheal wall, and Ca was one of the factors influencing tracheal wall width.

The reasons for the sex and age differences in trachea may be related to the anatomy and pathophysiology of trachea. The trachea is about 10-12 cm in length, and is connected with cricothyroid cartilage through the cricothyroid ligament from the lower part of the cricoid cartilage [28-30]. The tracheal scaffold

is constructed by 16 to 20 "C"-shaped transparent cartilage rings. The adjacent cartilage rings are connected by annular ligaments and end at the level of the tracheal carina, which are divided into the left and right main bronchus [28-30]. The anterior and lateral wall of the trachea account for about 2/3 of the trachea, which is composed of tracheal cartilage, connective tissue, elastic fibers, glands, arterioles, lymphatic vessels, nerves, and epithelial tissues [28-30]. The posterior wall accounts for about 1/3 of the trachea and is composed of smooth muscle, connective tissue, elastic fiber, and other tissues, which are connected with esophageal muscle by loose connective tissue [28-30]. Safshekan et al studied the cartilage, smooth muscle, and connective tissue of trachea in patients with brain death [31]. They found that the elderly trachea became hard as a whole, and the histology revealed mainly cartilage and connective tissue. They concluded that the histological changes of cartilage and connective tissue were the basis of tracheal hardening [31]. Kamel et al measured the trachea tissues of 60 live volunteers (20-88 years, 40 males and 20 females) and 10 donated cadavers (60-101 years, 7 males and 3 females) [32]. They found that the maximum trachea AP, LR, and volume of the males were significantly larger than those of the females ($P \leq 0.01$) and there were significant sex differences in trachea morphology [32]. Sošnik et al found that

Table 4. Paired group comparisons of trachea shape, inner diameter, and wall width.

| Sex | Group | W | | AP | | Ti | | |
|----------|-------------------|-----------------|------------------|------------------|------------------|------------------|------------------|------------------|
| | | Value | P | Value | P | Value | P | |
| M | | F=84.520 | <0.001 | H=49.718 | <0.001 | H=43.178 | <0.001 | |
| | Paired comparison | | | | | | | |
| | | G1-G2 | LSD-t=-4.088 | <0.001 | Z=-1.768 | 1.000 | Z=1.985 | 0.707 |
| | | G1-G3 | LSD-t=-8.463 | <0.001 | Z=-3.792 | 0.002 | Z=3.497 | 0.007 |
| | | G1-G4 | LSD-t=-12.688 | <0.001 | Z=-4.769 | <0.001 | Z=5.646 | <0.001 |
| | | G1-G5 | LSD-t=-14.393 | <0.001 | Z=-5.183 | <0.001 | Z=4.741 | <0.001 |
| | | G1-G6 | LSD-t=-15.516 | <0.001 | Z=-5.427 | <0.001 | Z=3.777 | 0.002 |
| | | G2-G3 | LSD-t=-5.183 | <0.001 | Z=-2.400 | 0.246 | Z=1.785 | 1.000 |
| | | G2-G4 | LSD-t=-10.235 | <0.001 | Z=-3.571 | 0.005 | Z=4.356 | <0.001 |
| | | G2-G5 | LSD-t=-12.264 | <0.001 | Z=-4.068 | 0.001 | Z=3.288 | 0.015 |
| | | G2-G6 | LSD-t=-13.593 | <0.001 | Z=-4.359 | <0.001 | Z=2.151 | 0.472 |
| | | G3-G4 | LSD-t=-5.164 | <0.001 | Z=-1.209 | 1.000 | Z=2.621 | 0.132 |
| | | G3-G5 | LSD-t=-7.285 | <0.001 | Z=-1.743 | 1.000 | Z=1.562 | 1.000 |
| | | G3-G6 | LSD-t=-8.664 | <0.001 | Z=-2.054 | 0.599 | Z=0.423 | 1.000 |
| | | G4-G5 | LSD-t=-2.170 | 0.031 | Z=-0.545 | 1.000 | Z=-1.014 | 1.000 |
| | | G4-G6 | LSD-t=-3.574 | <0.001 | Z=-0.863 | 1.000 | Z=-2.124 | 0.505 |
| | | G5-G6 | LSD-t=-1.401 | 0.162 | Z=-0.317 | 1.000 | Z=-1.103 | 1.000 |
| | <40y vs ≥40y | | F=256.515 | <0.001 | U=25460.000 | <0.001 | F=29.168 | <0.001 |
| F | | | H=181.024 | <0.001 | H=26.163 | <0.001 | H=32.081 | <0.001 |
| | Paired comparison | | | | | | | |
| | | G1-G2 | Z=-0.252 | 1.000 | Z=-2.458 | 0.209 | Z=2.045 | 0.614 |
| | | G1-G3 | Z=-4.179 | <0.001 | Z=-2.591 | 0.144 | Z=0.327 | 1.000 |
| | | G1-G4 | Z=-7.334 | <0.001 | Z=-3.054 | 0.034 | Z=2.220 | 0.396 |
| | | G1-G5 | Z=-8.234 | <0.001 | Z=-3.623 | 0.004 | Z=2.836 | 0.068 |
| | | G1-G6 | Z=-9.934 | <0.001 | Z=-4.938 | <0.001 | Z=4.783 | <0.001 |
| | | G2-G3 | Z=-4.039 | 0.001 | Z=0.162 | 1.000 | Z=-1.788 | 1.000 |
| | | G2-G4 | Z=-7.288 | <0.001 | Z=-0.526 | 1.000 | Z=0.151 | 1.000 |
| | | G2-G5 | Z=-8.221 | <0.001 | Z=-1.200 | 1.000 | Z=0.717 | 1.000 |
| | | G2-G6 | Z=-9.974 | <0.001 | Z=-2.500 | 0.186 | Z=2.733 | 0.094 |
| | | G3-G4 | Z=-3.263 | 0.017 | Z=-0.352 | 1.000 | Z=1.968 | 0.737 |
| | | G3-G5 | Z=-4.067 | 0.001 | Z=-1.025 | 1.000 | Z=2.605 | 0.138 |
| | | G3-G6 | Z=-5.856 | <0.001 | Z=-2.305 | 0.318 | Z=4.635 | <0.001 |
| | | G4-G5 | Z=-0.693 | 1.000 | Z=0.721 | 1.000 | Z=0.571 | 1.000 |
| | | G4-G6 | Z=-2.504 | 0.184 | Z=-2.063 | 0.587 | Z=2.620 | 0.132 |
| | | G5-G6 | Z=-1.883 | 0.895 | Z=-1.269 | 1.000 | Z=2.130 | 0.497 |
| | <40y vs ≥40y | | U=18509.500 | <0.001 | F=10.666 | 0.001 | F=4.502 | 0.035 |

LSD-t – t value in least significant difference t test; F – F value in one-way ANOVA; H – H value in Kruskal-Wallis test; Z – Z value in Bonferroni-Dunn test.

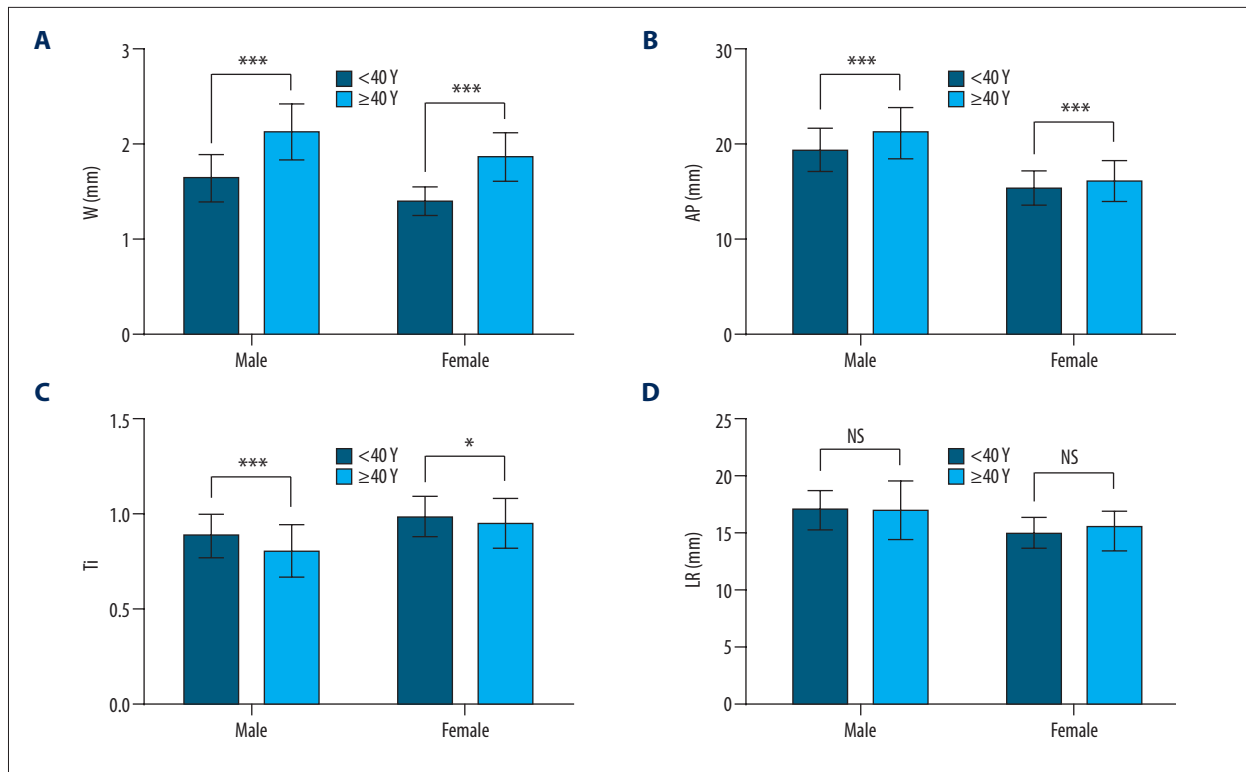


Figure 5. Comparison of trachea-associated parameters in the males and the females between the <40 years old group and the ≥40 years old group. (A-D) Comparison of tracheal wall width (A), tracheal AP (B), tracheal shape (C), tracheal LR (D) between the <40 years old and ≥40 years old groups. n=119 males and 91 females for the <40 years old group; n=305 males and 218 females for the ≥40 years old group; * P<0.05. *** P<0.01; NS – not significant.

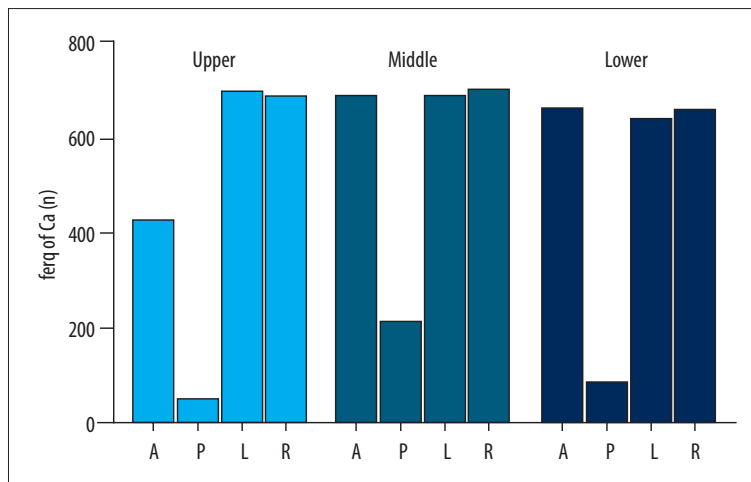


Figure 6. The distributions of frequencies of tracheal wall calcification in upper, middle, and lower sections of all tracheae. The frequencies of tracheal calcification in the indicated sides of tracheal walls were summarized. A – anterior of trachea; P – posterior of trachea; L – left side of trachea; R – right side of trachea.

there were significant sex differences in the dynamic changes of human trachea morphology with age, and these changes in the females were smaller than those in the males [33].

All the above studies are consistent with our results, and the differences can be explained from the aspects of anatomy and pathophysiology. For example, Wansleben et al performed histological studies on the mucociliary pseudostratified epithelium

of mouse trachea and main stem bronchi, and confirmed that there were age-related gland-like structures (ARGLS) in the submucosa, especially in the intercartilage regions and carina [34]. In addition, in comparison to young mice, old mice displayed low-grade chronic inflammation and had increased numbers of immune cells in the tracheae [34]. Sakai et al investigated age-related changes in trachea area and the shape of trachea in 83 normal male volunteers [15]. They found that the trachea

Table 5. Multiple regression analysis of trachea wall width and trachea calcification.

| Variable | B | Standard error | Bate | t | P |
|------------------------------|--------|----------------|--------|--------|--------|
| Tracheal wall width | | | | | |
| Constant | 1.342 | 0.031 | | 42.708 | <0.001 |
| Age | 0.116 | 0.009 | 0.529 | 13.061 | <0.001 |
| Sex (M)* | | | | | |
| Sex (F) | -0.196 | 0.02 | -0.269 | -9.636 | <0.001 |
| Ca | 0.019 | 0.006 | 0.126 | 3.017 | 0.003 |
| BP | 0.041 | 0.021 | 0.051 | 1.949 | 0.052 |
| BMI (N)* | | | | | |
| BMI (O) | 0.043 | 0.02 | 0.057 | 2.191 | 0.029 |
| BMI (Ob) | 0.11 | 0.026 | 0.11 | 4.158 | <0.001 |
| HDL (N)* | | | | | |
| HDL (H) | -0.117 | 0.047 | -0.059 | -2.456 | 0.014 |
| HDL (L) | 0.022 | 0.034 | 0.015 | 0.642 | 0.521 |
| Shape (type I) * | | | | | |
| Shape (type II) | 0.043 | 0.02 | 0.06 | 2.192 | 0.029 |
| Shape (type III) | -0.023 | 0.038 | -0.015 | -0.602 | 0.547 |
| Trachea calcification | | | | | |
| Constant | 2.722 | 0.153 | | 17.751 | <0.001 |
| Age | 1.086 | 0.033 | 0.753 | 33.285 | <0.001 |
| Sex (M)* | | | | | |
| Sex (F) | -0.912 | 0.114 | -0.191 | -7.998 | <0.001 |
| Ca | 0.285 | 0.114 | 0.054 | 2.512 | 0.012 |
| BP | 0.242 | 0.122 | 0.046 | 1.977 | 0.048 |
| BMI (N)* | | | | | |
| BMI (O) | -0.019 | 0.116 | -0.004 | -0.161 | 0.872 |
| BMI (Ob) | -0.065 | 0.155 | -0.01 | -0.416 | 0.678 |
| HDL (N)* | | | | | |
| HDL (H) | -0.378 | 0.278 | -0.029 | -1.364 | 0.173 |
| HDL (L) | -0.285 | 0.2 | -0.03 | -1.423 | 0.155 |
| Shape (type I) * | | | | | |
| Shape (type II) | 0.386 | 0.115 | 0.081 | 3.371 | 0.001 |
| Shape (type III) | -0.087 | 0.223 | -0.009 | -0.392 | 0.695 |

* Control.

area increased with age ($r=0.37$, $P=0.0006$), and the roundness of tracheal shape was distorted ($r=-0.32$; $P=0.00364$), while Ti was not associated with age ($r=-0.20$; $P=0.0697$) [15]. However, in the present study, we found that a significant difference between age and Ti. The possible reason for this difference might be due to the sample size, and the larger sample size in this study could allow a higher statistical power. In addition, we found that there was statistical significance between AP and age, but not between LR and age. This might be due to the following reasons. First, the posterior wall of trachea is a membranous structure without cartilage support, while the anterior and bilateral walls are mainly composed of cartilage and connective tissue. Second, in terms of the cartilage and connective tissue hardening with age, the elasticity of trachea decreased. As mentioned above, the anterior and lateral walls of the trachea are mainly composed of cartilage and connective tissue. According to the results of the ultrastructural anatomy of the trachea of 10 donated cadavers by Kamel et al [32], there are extensive and obvious elastic tissue nets in the trachea, and the elastic fibers are mainly longitudinal, while the tracheal membrane wall is composed of tracheal membrane fibers. The smooth muscle layer in the deep layer of elastic membrane and scattered longitudinal muscle bundles are mostly embedded in the fibrous elastic membrane at the distal end of the trachea [35]. Therefore, the diameter changes of trachea occur mainly in AP and only minor changes are observed in LR.

We found that there were sex differences in the wall width and calcification score of tracheae. The trachea width and degree of calcification increased with age, and tracheal calcification was one of the factors influencing wall width. The reasons may be as follows. First, osseocalcineous metaplasia (OCM) is a contributing factor. Sošnik et al carried out a series of studies about OCM, and they found that OCM was associated with age and sex. Specifically, the incidence of OCM in the males was higher than that in the females [36]. Before the age of 50 years, the incidence of OCM in the males was 9 times higher than that in the females. However, after the age of 50 years, the incidence of OCM was only twice that in the females [33]. In addition, the average thickness of trachea cartilage in the males was larger than in the females, and there was a positive correlation between the thickness of trachea cartilage and age. Moreover, the trachea cartilage with OCM was significantly thicker than that without OCM [37]. Second, calcification

and deposition of trachea cartilage occur differently in males and females. The proportions of calcium deposition (13.2% vs 9.7%) and cartilage ossification (20.5% vs 3.6%) in the males were higher than that in the females, while 8.6% of the males and 3.9% of the females had calcification and OCM simultaneously [38]. Cartilage ossification, calcium deposition, and OCM can result in tracheal wall thickening and visible calcification.

The current investigation has several limitations. First, was a single-center study. Second, although the volunteers were strictly screened, there may still have been selection bias. Third, we used semi-quantitation in this study, which may be subject to measurement errors. Fourth, the proposed influencing factors are relatively few. Multi-center studies with a large-scale and multi-racial population are needed to further consolidate our current findings.

Conclusions

LDCT can be used as a non-invasive examination method to evaluate tracheal aging. By accurately measuring and evaluating the shape of the trachea, width, and the degree of calcification of the tracheal wall, the characterization of trachea aging and determination of the degree of aging can be achieved. For the judgment of tracheal aging, sex and age should be taken into consideration comprehensively. The shape of trachea from males and females at different ages is diverse. The width of the tracheal wall of males is thicker than that of females in the same age group. In addition, tracheal wall calcification in males appears slightly earlier than in females, and the extent of calcification in males is greater than in females. The increase in BMI or HDL-C values has slight effects on tracheal wall width and calcification, and we deduced that obesity may aggravate tracheal aging.

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Conflict of Interest

None.

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