# Serum α-Klotho level, lung function, airflow obstruction and inflammatory markers in US adults

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**Higher serum α-Klotho, a pleiotropic anti-ageing protein, is associated with higher lung function measures, lower odds of airflow obstruction and lower levels of inflammatory biomarkers in adults with and without airflow obstruction** https://bit.ly/3EP7jEq

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## Abstract

*Background* α-Klotho is a pleiotropic protein that may have anti-oxidative and anti-inflammatory properties in the lung, but its role in airflow obstruction or lung function is largely unknown.

*Methods* This was a cross-sectional study of 6046 adults aged 40–79 years in the US National Health and Nutrition Examination Survey (NHANES) 2007–2012. We used multivariable logistic or linear regression to examine the relation between serum α-Klotho level and airflow obstruction, defined as forced expiratory volume in 1 s ($FEV_1$) <80% of predicted and $FEV_1$/forced vital capacity (FVC) ratio <0.70; $FEV_1$, FVC and $FEV_1$/FVC as percentage of predicted; and inflammatory markers in blood (white blood cell count, eosinophils, neutrophils and C-reactive protein (CRP)).

*Results* α-Klotho levels in the second to fourth quartiles (Q2–Q4) were associated with significantly decreased odds of airflow obstruction (adjusted OR for Q2–Q4 *versus* lowest quartile (Q1) 0.54 (95% CI 0.35–0.81)) in never-smokers and ex-smokers with <10 pack-years of smoking, but not in current smokers or ex-smokers with ⩾10 pack-years of smoking. In all participants, each unit increment in $log_{10}$-transformed α-Klotho level was significantly associated with 5.0% higher $FEV_1$ % pred and 3.7% higher FVC % pred. Higher α-Klotho was also associated with lower eosinophils, neutrophils and CRP in participants both with and without airflow obstruction.

*Conclusions* Higher serum α-Klotho is associated with lower inflammatory markers and higher lung function in adults with and without airflow obstruction, and with decreased odds of airflow obstruction in never-smokers and ex-smokers with <10 pack-years of smoking. Further studies are warranted to replicate our findings and evaluate underlying mechanisms.

## Introduction

α-Klotho is a pleiotropic protein mainly derived from the kidney that is expressed predominantly in kidney tubular epithelium, and to a lesser extent in the parathyroid gland and epithelial cells of the choroid plexus [1]. α-Klotho may be involved in several ageing-related physiological functions. In a murine model, defective expression of the α-Klotho gene causes a complex ageing-like phenotype, including arteriosclerosis, skin atrophy, osteoporosis, emphysema, infertility and reduced lifespan [2]. Emerging evidence suggests that α-Klotho acts as a paracrine and endocrine hormonal factor with ageing suppressing effects in multiple organs in humans. α-Klotho forms a complex with fibroblast growth factor (FGF) receptors involved in ageing processes; regulates oxidative stress, growth factor signalling and ion homeostasis; and suppresses inflammation-mediated tissue damage [3–5]. While it remains unclear whether there are α-Klotho receptors in the lung, α-Klotho is a coreceptor of FGF23 and enhances FGF23 signalling, which has been shown to be relevant to lung health [6, 7].

Normal lung ageing is associated with physiological changes that result in decreased lung function, lessened regeneration and repair, altered airway remodelling, weakened innate and adaptive immune responses, and increased susceptibility to respiratory diseases [8–10]. Aside from its anti-ageing properties, extracellular α-Klotho circulates in a soluble form that has anti-oxidative, anti-inflammatory and anti-fibrotic properties in the lung [11, 12]. Reduced α-Klotho expression in airway epithelial cells from patients with COPD is associated with increased oxidative stress, inflammation and apoptosis in the lung [13]. On the contrary, overexpression or supplementation of α-Klotho may reduce inflammation by decreasing interleukin (IL)-8 secretion and counteracting transforming growth factor (TGF)-β signalling in the bronchial epithelium of patients with cystic fibrosis [14].

Whether circulating α-Klotho is associated with lung function or airflow obstruction in a general adult population is largely unknown. Given a plausible role of α-Klotho on mediating ageing and inflammatory processes in the lung, we hypothesised that higher serum α-Klotho levels would be associated with higher lung function measures, lower odds of airflow obstruction, and lower levels of atopic and inflammatory markers. We tested these hypotheses in adults aged 40–79 years who participated in the US National Health and Nutrition Examination Survey (NHANES).

## Methods

### *Study design and study population*

NHANES is a cross-sectional nationwide survey designed to assess the health and nutrition of the civilian non-institutionalised US population. NHANES adopts a stratified multistage probability design to select a representative sample of about 5000 persons each year. As part of the study design, racial and ethnic minorities (non-Hispanic Blacks, Hispanics and Asians), low-income persons (⩽130% of the federal poverty level) and adults aged ⩾80 years are oversampled to increase the statistical power for data analysis in these groups. The NHANES protocol includes health interviews, examination components and laboratory tests administered by highly trained personnel. The unweighted response rates ranged from 71% to 84% for the interviewed sample and from 68% to 80% for the examined sample for NHANES 1999–2014. Serum α-Klotho and lung function measures were available in participants 40–79 years old from the NHANES 2007–2012 study cycles. The flowchart for selection of participants for the current analysis is shown in figure 1. Of the 8360 participants aged 40–79 years in NHANES 2007–2012 who had measures of serum α-Klotho, 6046 had spirometry measures of good quality and relevant covariates and were thus included in the current analysis.

NHANES is approved by the Institutional Review Board of the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC). Informed consent is obtained from all study participants. Further details of the methods, protocols and definitions used in NHANES can be found at https://www.cdc.gov/nchs/nhanes.htm.

### *Measurement of serum soluble α-Klotho*

Serum α-Klotho was measured in NHANES following a standardised protocol. Pristine serum samples were processed, stored and shipped to the CDC for analysis. All samples were stored at −80°C until analyses, which were performed by trained technicians. α-Klotho was analysed with commercially available ELISA kits (Immuno-Biological Laboratories, Fujioka-Shi, Japan). Sample measurements were done in duplicate with two quality controls (low and high concentration of α-Klotho), with the average of the two duplicates used to calculate the final value. A detailed description of quality assurance and quality control protocols used for the analyses is provided in the NHANES Laboratory Procedures Manual [15].

### *Outcome and covariate measurements*

Based on the NHANES protocol, lung function measures and blood sampling were collected at the mobile examination centre on the study visit day. Eligible NHANES participants performed spirometry following American Thoracic Society/European Respiratory Society recommendations [16]. The best forced expiratory volume in 1 s ($FEV_1$) and forced vital capacity (FVC) were selected for analysis. Percentage predicted $FEV_1$, FVC and $FEV_1$/FVC were calculated using Global Lung Initiative 2012 equations that account for age, sex, ethnic group and height [17]. Airflow obstruction was defined as $FEV_1$ <80% of predicted and $FEV_1$/FVC ratio <0.70 [18]. Per NHANES protocols, participants were not eligible for spirometry if they had current chest pain or a physical problem with forceful expiration; were taking supplemental oxygen; had recent surgery of the eye, chest or abdomen; had had a heart attack, stroke or tuberculosis exposure; had recently coughed up blood; or had a personal history of a detached retina or a collapsed lung.

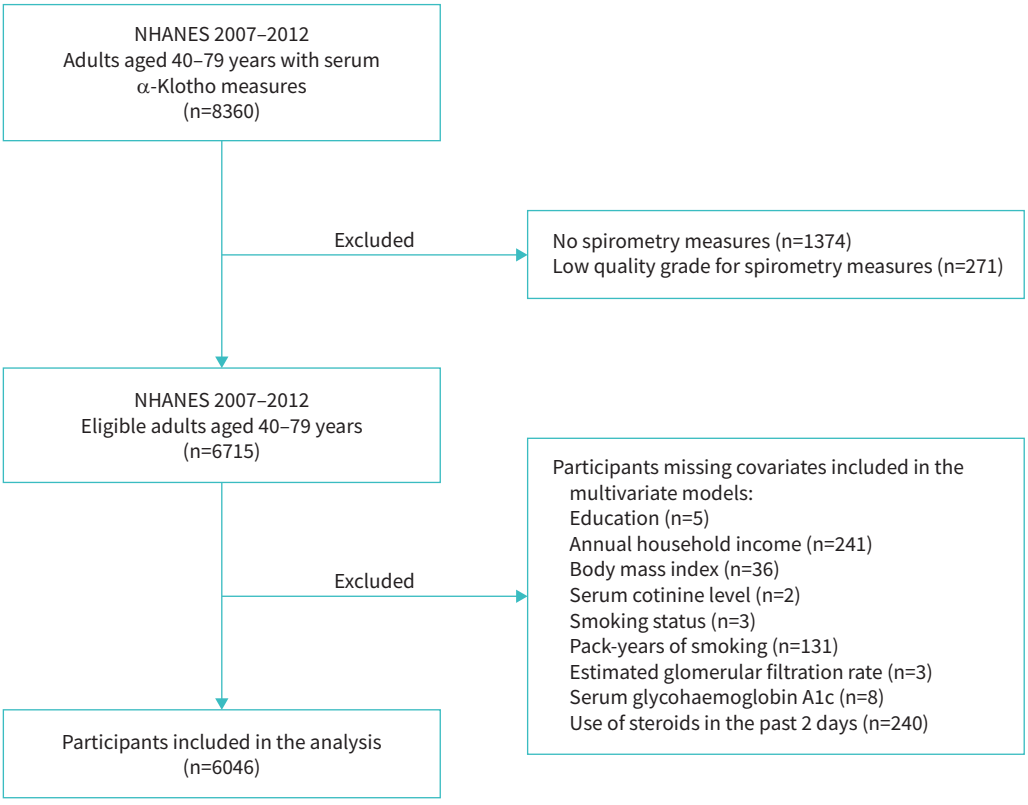


FIGURE 1 Flowchart for selection of US National Health and Nutrition Examination Survey (NHANES) participants included in the study.

White blood cell (WBC), eosinophil and neutrophil counts were measured by the Beckman Coulter method of counting and sizing, in combination with an automatic diluting and mixing device for sample processing, and a single beam photometer for haemoglobinometry. Serum C-reactive protein (CRP) was available in NHANES 2007–2008 and 2009–2010, and was quantified by latex-enhanced nephelometry. Estimated glomerular filtration rate (eGFR) and serum glycohaemoglobin were included in the models because prior studies have shown that kidney function and diabetes mellitus are associated with serum α-Klotho levels [19–21], and glucose dysregulation has been associated with worse lung function [22, 23]. eGFR was calculated using the 2021 Chronic Kidney Disease Epidemiology Collaboration creatinine equation that includes factors for age and sex but not racial or ethnic group [24]. Serum glycohaemoglobin measurements were performed on the Tosoh G7 high-performance liquid chromatography glycohaemoglobin A1c analyser (Tosoh Medics, San Francisco, CA, USA).

### *Statistical analysis*

Sampling weights, stratification and clusters provided in the NHANES dataset were incorporated into the analysis to obtain proper effect estimates and their standard errors that account for the complex NHANES survey design. Two-sided Wald Chi-squared tests and t-tests were used for bivariate analyses. Because of a skewed distribution, serum α-Klotho was analysed as quartiles and as $\log_{10}$-transformed variable. Logistic regression was used for the multivariable analysis of serum α-Klotho and airflow obstruction, which was adjusted for known or potential confounders including age, gender, race/ethnicity (non-Hispanic White, non-Hispanic Black, Mexican American/Other Hispanic and Others), education (less than high school, high school or General Educational Development or Associate of Art, and college and above), annual household income (<USD 20 000 *versus* ⩾USD 20 000 per year), body mass index (BMI ($kg \cdot m^{-2}$)), smoking status (never-smoker, ex-smoker and current smoker), pack-years of cigarette smoking, serum cotinine level (as an objective proxy for short-term exposure to smoking or second-hand smoke), eGFR, serum glycohaemoglobin and use of oral or inhaled steroids in the past 2 days. As in previous studies using NHANES data, current asthma was defined by an affirmative answer to both of the following questions: "Has a doctor or other health professional ever told you that you have asthma?" and "Do you still have asthma?". Self-reported diagnosis of emphysema or chronic bronchitis was defined by an affirmative answer

to either of the following questions: "Has a doctor or other health professional ever told that you had emphysema?" and "Has a doctor or other health professional ever told that you had chronic bronchitis?".

Linear regression was used for the multivariable analysis of $\log_{10}$-transformed serum α-Klotho level and lung function measures or inflammatory markers (WBC count, eosinophils, neutrophils and CRP), which was first conducted in all participants and then separately in participants with and without airflow obstruction. Models for lung function were adjusted for education, annual household income, BMI, serum cotinine level, smoking status, pack-years of cigarette smoking, eGFR, serum glycohaemoglobin, use of oral or inhaled steroids in the past 2 days and (in the analyses of all participants) airflow obstruction. Models for inflammatory markers were additionally adjusted for age, sex and race/ethnicity.

As part of the multivariable analysis of lung function, we tested for an interaction between gender or smoking status and α-Klotho levels on lung function measures, given known effects of sex hormones or smoking on both α-Klotho levels and lung function [25–27]. In addition, we performed a sensitivity analysis with airflow obstruction defined by $FEV_1$/FVC below the lower limit of normal (LLN) [28].

All statistical analyses were conducted using the SAS SURVEY procedure and SAS version 9.4 software (SAS Institute, Cary, NC, USA).

## Results

Table 1 shows the main characteristics of study participants by airflow obstruction. Compared with adults without airflow obstruction (n=5529), those with airflow obstruction (n=517) were more likely to be older, non-Hispanic White and current smokers; to have annual household income <USD 20 000 and to lack a college degree; to self-report being diagnosed with emphysema or chronic bronchitis, to have current asthma and to have used oral or inhaled steroids in the previous 2 days; to have higher serum cotinine levels, pack-years of cigarette smoking, serum glycohaemoglobin, WBC counts, eosinophil counts, neutrophil counts and CRP; and to have lower BMI and eGFR, and lower lung function measures ($FEV_1$, FVC and $FEV_1$/FVC). Serum α-Klotho level was significantly lower in subjects with airflow obstruction.

The results of the multivariable analysis of serum α-Klotho levels and airflow obstruction are shown in table 2. In all participants, subjects whose serum α-Klotho levels were in the second quartile (Q2) had 35% lower odds of airflow obstruction than those whose serum α-Klotho levels were in the first (lowest) quartile (Q1) (OR 0.65 (95% CI 0.43–0.98)). Based on this quartile analysis (quartiles with adjusted OR <1.0), we then dichotomised serum α-Klotho level as high (second to fourth quartile (Q2–Q4)) or low (first quartile (Q1)). In this analysis, adults with higher serum α-Klotho levels had 30% decreased odds of airflow obstruction compared with those with lower serum α-Klotho levels (OR 0.70 (95% CI 0.50–0.98)). To account for potential effects of smoking, we stratified the multivariable analysis of airflow obstruction by smoking status. While the analysis in never-smokers and ex-smokers with <10 pack-years of cigarette smoking yielded similar results to that in all participants, there was no significant association between serum α-Klotho and airflow obstruction in the analysis conducted in current smokers or ex-smokers with ⩾10 pack-years of cigarette smoking. Further, similar results were obtained in a sensitivity analysis with additional adjustment for current asthma and a self-reported diagnosis of emphysema or chronic bronchitis (supplementary table S1).

The results of the multivariable analysis of serum α-Klotho and lung function measures are shown in table 3. In the analysis of all participants, each unit increment in $\log_{10}$-transformed serum α-Klotho level was significantly associated with 5.0% (95% CI 2.7–7.3%) higher $FEV_1$ % pred and 3.7% (95% CI 1.0–6.4%) higher FVC % pred. After stratification by airflow obstruction status, higher serum α-Klotho levels were associated with increased $FEV_1$ % pred and FVC % pred in adults without airflow obstruction, and with increased $FEV_1$ % pred in adults with airflow obstruction. We then conducted sensitivity analyses using lung function measures as raw values and as z-scores, obtaining similar results (supplementary table S2).

Because previous studies have reported that aerobic training may increase α-Klotho levels [29], we conducted a sensitivity analysis of lung function measures after additional adjustment for moderate intensity aerobic physical activity (defined as at least 150 min per week of moderate intensity aerobic activity or 75 min per week of vigorous aerobic activity, or a combination of both [30]), obtaining similar results (supplementary table S3). We did not find a significant interaction between serum α-Klotho and either gender or smoking on lung function measures ($p>0.15$ in all instances).

**TABLE 1** Characteristics of adult participants in the US National Health and Nutrition Examination Survey (NHANES) 2007–2012, by status of airflow obstruction (n=6046)

| | No airflow obstruction (n=5529) | With airflow obstruction (n=517) |
|---|---|---|
| **Age (years)** | 54.7±0.2 | 59.3±0.5* |
| **Female** | 2798 (51.8) | 211 (44.8) |
| **Race/ethnicity** | | |
| Mexican American/Other Hispanic | 1449 (10.3) | 44 (2.5)* |
| Non-Hispanic White | 2595 (75.6) | 347 (86.7) |
| Non-Hispanic Black | 1107 (8.8) | 105 (7.7) |
| Other | 378 (5.4) | 21 (3.2) |
| **Have health insurance coverage** | 4435 (86.0) | 442 (88.3) |
| **Annual household income <USD 20 000** | 1056 (11.0) | 141 (16.0)* |
| **Education** | | |
| Less than high school | 1411 (15.5) | 165 (21.5)* |
| High school/GED/AA | 2758 (51.4) | 273 (58.1) |
| College and above | 1360 (33.1) | 79 (20.4) |
| **BMI ($kg \cdot m^{-2}$)** | 29.4±0.1 | 28.6±0.4* |
| **Serum cotinine level ($ng \cdot mL^{-1}$)** | 53.7±2.8 | 125.3±9.6* |
| **Smoking status** | | |
| Never-smoker | 2977 (55.2) | 106 (20.4)* |
| Ex-smoker | 1546 (27.7) | 194 (38.0) |
| Current smoker | 1006 (17.1) | 217 (41.6) |
| **Pack-years of cigarette smoking** | 9.2±0.5 | 27.9±2.0* |
| **⩾150 min per week of moderate intensity aerobic physical activity** | 3293 (64.3) | 278 (59.3) |
| **eGFR ($mL \cdot min^{-1} \cdot 1.73\ m^{-2}$)** | 90.7±0.3 | 85.9±1.4* |
| **Serum glycohaemoglobin (%)** | 5.7±0.02 | 5.9±0.05* |
| **Diagnosis of emphysema or chronic bronchitis** | 53 (0.9) | 56 (10.5)* |
| **Current asthma** | 337 (6.2) | 102 (20.9)* |
| **Use of oral or inhaled steroids in the past 2 days** | 202 (4.0) | 99 (20.8)* |
| **$FEV_1$ % pred** | 98.9±0.3 | 66.6±0.7* |
| **FVC % pred** | 102.0±0.3 | 87.3±0.7* |
| **$FEV_1$/FVC % pred** | 96.7±0.2 | 76.2±0.5* |
| **White blood cell count ($\times 10^3\ \mu L^{-1}$)** | 7.0±0.01 | 7.5±0.1* |
| **Peripheral neutrophils ($\times 10^3\ \mu L^{-1}$)** | 4.2±0.03 | 4.6±0.09* |
| **Peripheral eosinophils ($\mu L^{-1}$)** | 194±3.0 | 237±8.6* |
| **CRP ($mg \cdot dL^{-1}$)#** | 0.38±0.02 | 0.57±0.08* |
| **Serum α-Klotho ($pg \cdot mL^{-1}$, $log_{10}$ transformed)** | 2.91±0.01 | 2.89±0.01* |

Data are presented as mean±SE or n (%) for binary variables; denominators may be different due to missingness. GED: General Educational Development; AA: Associate of Art; BMI: body mass index; eGFR: estimated glomerular filtration rate; $FEV_1$: forced expiratory volume in 1 s; FVC: forced vital capacity; CRP: C-reactive protein. #: CRP was only available in NHANES 2007–2008 and 2009–2010. *: p<0.05 for comparison between with or without airflow obstruction (defined as $FEV_1$ <80% of predicted and $FEV_1$/FVC ratio <0.70).

We conducted an additional sensitivity analysis defining airflow obstruction as $FEV_1$/FVC below the LLN. This analysis yielded similar results for α-Klotho level and lung function measures (supplementary table S4), and similar but less significant findings for airflow obstruction (supplementary table S5).

Table 4 shows the results of the multivariable analysis of serum α-Klotho level and inflammatory biomarkers. In all participants, each $log_{10}$ unit increment in serum α-Klotho was associated with lower eosinophil and neutrophil counts (β −36 (95% CI −70– −2.0) for eosinophils and β −435 (95% CI −823– −47) for neutrophils). Similar but weaker associations were observed in an analysis stratified by airflow obstruction, likely due to reduced statistical power due to small sample size. Further, each $log_{10}$ unit increment in serum α-Klotho was associated with lower CRP in adults with and without airflow obstruction (β −0.22 (95% CI −0.41– −0.03) for those without airflow obstruction and β −0.50 (95% CI −1.00– −0.01) for those with airflow obstruction). There was no significant association between serum α-Klotho and WBC count.

**TABLE 2** Multivariable analysis of serum α-Klotho level and airflow obstruction, by smoking status

| Serum α-Klotho level | All participants (n=6046) | Never- or ex-smokers with <10 pack-years (n=3931) | Current or ex-smokers with ⩾10 pack-years (n=2115) |
|---|---|---|---|
| **Q1** | 1.0 (reference) | 1.0 (reference) | 1.0 (reference) |
| **Q2** | 0.65 (0.43–0.98)* | 0.52 (0.33–0.81)* | 0.72 (0.42–1.23) |
| **Q3** | 0.76 (0.51–1.13) | 0.59 (0.31–1.11) | 0.84 (0.51–1.39) |
| **Q4** | 0.70 (0.46–1.06) | 0.50 (0.30–0.83)* | 0.86 (0.53–1.37) |
| **Q2–4 *versus* Q1** | 0.70 (0.50–0.98)* | 0.54 (0.35–0.81)* | 0.80 (0.53–1.19) |

Data are presented as odds ratio (95% CI). Q: quartile. All models were adjusted for age, gender, race/ethnicity, education, annual household income, body mass index, smoking status (in all participants), pack-years of cigarette smoking, serum cotinine level, estimated glomerular filtration rate, serum glycohaemoglobin and use of oral or inhaled steroids in the past 2 days. *: p<0.05.

**TABLE 3** Multivariable analysis of serum α-Klotho level ($log_{10}$ transformed) and lung function, by status of airflow obstruction

| | All participants (n=6046) | No airflow obstruction (n=5529) | With airflow obstruction (n=517) |
|---|---|---|---|
| **$FEV_1$ % pred** | 5.00 (2.67–7.34)* | 4.64 (2.04–7.24)* | 7.35 (0.40–14.31)* |
| **FVC % pred** | 3.69 (0.99–6.39)* | 3.41 (0.34–6.49)* | 4.62 (−5.80–15.03) |
| **$FEV_1$/FVC % pred** | 1.55 (−0.02–3.12)** | 1.38 (−0.33–3.08) | 4.31 (−2.07–10.69) |

Data are presented as β coefficient (95% CI) for the % pred of each lung function index. $FEV_1$: forced expiratory volume in 1 s; FVC: forced vital capacity. All models were adjusted for education, annual household income, body mass index, smoking status, pack-years of cigarette smoking, serum cotinine level, estimated glomerular filtration rate, serum glycohaemoglobin, use of oral or inhaled steroids in the past 2 days and airflow obstruction (in all participants). *: p<0.05; **: p=0.05.

**TABLE 4** Multivariable analysis of serum α-Klotho level ($log_{10}$ transformed) and inflammatory markers, by status of airflow obstruction

| | All participants | No airflow obstruction | With airflow obstruction |
|---|---|---|---|
| **White blood cell count** | −475 (−978–27) | −439 (−933–55) | −717 (−2166–732) |
| **Eosinophils** | −36 (−70– −2.0)* | −37 (−73– −1.2)* | −19 (−116–79) |
| **Neutrophils** | −435 (−823– −47)* | −358 (−743–27)** | −1010 (−2161–139)** |
| **CRP[#]** | −0.24 (−0.41– −0.07)* | −0.22 (−0.41– −0.03)* | −0.50 (−1.00– −0.01)* |

Data are presented as β coefficient (95% CI). CRP: C-reactive protein. All models were adjusted for age, gender, race/ethnicity, education, annual household income, body mass index, smoking status, pack-years of cigarette smoking, serum cotinine level, estimated glomerular filtration rate, serum glycohaemoglobin and use of oral or inhaled steroids in the past 2 days. Models for all participants were additionally adjusted for airflow obstruction. [#]: CRP was only available in NHANES 2007–2008 and 2009–2010. *: p<0.05; **: p<0.10.

## Discussion

In a cohort of US adults, higher serum α-Klotho levels were associated with lower odds of airflow obstruction, and with higher lung function measures in adults with and without airflow obstruction. Further, serum α-Klotho levels were associated with lower neutrophil and eosinophil counts in peripheral blood, and with lower serum CRP. To the best of our knowledge, this is the first study of serum α-Klotho, airflow obstruction and lung function in a large population-based adult cohort.

We focused on objective measures of lung function and airflow obstruction because COPD (comprising emphysema and chronic bronchitis) and asthma can be underdiagnosed and are dependent on access to and quality of healthcare [31, 32]. Airflow obstruction can be caused by airway inflammation due to exposure to cigarette smoking or occupational exposure to chemical agents and fumes [18]. α-Klotho has been shown to play a role in the regulation of autophagy and cellular senescence in smokers [33], and here we

report an inverse association between serum α-Klotho and airflow obstruction only in adults who were never-smokers or ex-smokers with <10 pack-years of cigarette smoking. Further, the strength of this association was similar for α-Klotho levels between the second and fourth quartiles, suggesting a threshold effect. The mechanisms underlying this association warrant further investigation.

Very few epidemiological studies have reported an association between α-Klotho, lung function or inflammatory biomarkers. In a study of participants with interstitial lung abnormalities in imaging studies, lower serum α-Klotho was associated with lower $FEV_1$, FVC and diffusing capacity of the lung for carbon monoxide [34]. In a separate study of patients with established cardiovascular disease and preserved renal function, lower α-Klotho was linked to a pro-inflammatory status defined by higher tumour necrosis factor-α/IL-10 ratio and higher CRP levels [35]. Serum α-Klotho levels have also been inversely associated with inflammatory biomarkers such as WBC count, CRP and uric acid [36]. Thus, our findings for serum α-Klotho and lower eosinophils and neutrophils are intriguing, but must be interpreted cautiously pending confirmation studies.

In contrast to the low number of epidemiological and clinical studies on α-Klotho and lung disease, several experimental studies have shown that α-Klotho may influence lung health. α-Klotho overexpression in adult mice is associated with decreased mortality, less oxidative damage in the lungs, enhanced recovery from acute hyperoxia and lung cytoprotective effects in a cigarette smoke injury model [37]. α-Klotho knockout mice develop COPD and show increased IL-6 and Klotho-mediated FGF receptor (FGFR4) expression in their lungs, while overexpression of α-Klotho leads to reduced airway inflammation in murine models [38]. *In vitro* and *ex vivo*, α-Klotho overexpression or supplementation has been shown to regulate mucociliary clearance by increasing airway surface liquid volume, improving large conductance calcium-activated potassium channel activity and downregulating IL-8 [39]. In two murine models, α-Klotho attenuated pulmonary fibrosis by suppressing TGF-β-induced fibroblast activation and extracellular matrix production, as well as by downregulating vascular endothelial growth factor and TGF-β1/Smad3 signalling [40, 41]. Furthermore, α-Klotho supplementation in neonatal hyperoxia-exposed pre-term rat pups preserves lung alveolar and vascular structure, decreases pulmonary hypertension and oxidative stress, and reduces pulmonary vascular remodelling [42]. Taken together, these findings suggest that α-Klotho could be a potential therapeutic target in airway diseases.

Our study has several strengths, including the use of data from a large and racially/ethnically diverse cohort of adults designed to represent the US population, as well as the ability to adjust for potential confounders such as cigarette smoking (measured by questionnaires and serum cotinine), kidney function (eGFR) and serum glucose regulation (glycohaemoglobin). We also acknowledge several study limitations. First, we cannot determine a temporal relationship between α-Klotho level, airflow obstruction or lung function in this cross-sectional study. Second, we lack data on other chronic diseases or genetic variants that may influence α-Klotho expression and circulating levels. Third, unmeasured confounders such as exposure to outdoor air pollution or use of medication such as long-acting β-agonists or long-acting muscarinic antagonists should be considered in future studies. Lastly, serum α-Klotho levels may not reflect long-term status, which is best assessed by repeated measures within an individual over time.

In summary, we show that among adults who participated in a nationwide study in the USA, higher serum α-Klotho levels were associated with lower odds of airflow obstruction and positively associated with lung function in participants with and without airflow obstruction. Moreover, serum α-Klotho level was associated with lower eosinophil counts, neutrophil counts and serum CRP in all participants. Our cross-sectional findings should be confirmed in future longitudinal studies of serum α-Klotho and airway inflammation and lung function in adults with and without airflow obstruction.

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Author contributions: Y-Y. Han, J.C. Celedón and E. Forno participated in study design, study implementation and data analysis. Y-Y. Han wrote the initial draft of the manuscript. J.C. Celedón and E. Forno participated in the review of the manuscript. All authors approved the final version of the manuscript prior to submission. Y-Y. Han had full access to all the data and takes responsibility for the integrity and accuracy of the analysis.

Conflict of interest: J.C. Celedón has received research materials from Merck (inhaled steroids) to provide medications free of cost to participants in NIH-funded studies, unrelated to the current work. Y-Y. Han and E. Forno have nothing to disclose.

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