Research Paper

Low serum DHEA-S is associated with impaired lung function in women

Giancarlo Pesce[a,*], Kai Triebner[b,c], Diana A. van der Plaat[d], Dominique Courbon[e],
Steinar Hustad[b,c], Torben Sigsgaard[f], Dennis Nowak[g], Joachim Heinrich[g,h], Josep M. Anto[i,j,k,l],
Sandra Dorado-Arenas[m], Jesús Martinez-Moratalla[n], Jose A. Gullon-Blanco[o],
José L. Sanchez-Ramos[p], Chantal Raherison[q], Isabelle Pin[r], Pascal Demoly[a],
Thorarinn Gislason[s,t], Kjell Torén[u], Bertil Forsberg[v], Eva Lindberg[w], Elisabeth Zemp[x,y],
Rain Jogi[z], Nicole Probst-Hensch[x,y], Shyamali C. Dharmage[aa], Debbie Jarvis[ab],
Judith Garcia-Aymerich[i,j,k], Alessandro Marcon[ac], Francisco Gómez-Real[b,ad,#],
Bénédicte Leynaert[ae,af,#,**]

[a] Sorbonne Université and INSERM UMR-S 1136, Epidemiology of Allergic and Respiratory Diseases (EPAR), Pierre Louis Institute of Epidemiology and Public Health (IPLESP), Saint-Antoine Medical School, F-75012, Paris, France
[b] Department of Clinical Science, University of Bergen, Bergen, Norway
[c] Core Facility for Metabolomics, University of Bergen, Bergen, Norway
[d] Population Health and Occupational Disease, National Heart and Lung Institute (NHLI), Imperial College, London, United Kingdom
[e] INSERM UMR 1152, Pathophysiology and Epidemiology of Respiratory Diseases, Paris, France. University Paris Diderot Paris 7, UMR 1152, F-75890, Paris, France
[f] Institute of Public Health, Aarhus University, Aarhus, Denmark
[g] Institute and Clinic for Occupational, Social and Environmental Medicine, University Hospital, LMU Munich, Comprehensive Pneumology Centre Munich, German Centre for Lung Research (DZL), Munich, Germany
[h] Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Epidemiology I, Neuherberg, Germany
[i] ISGlobal, Barcelona, Spain
[j] Universitat Pompeu Fabra (UPF), Barcelona, Spain
[k] CIBER Epidemiología y Salud Pública (CIBERESP), Spain
[l] IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain
[m] Pulmonology Department, Galdakao Hospital, Bizkaia, Spain
[n] Servicio de Neumología del Hospital General Universitario de Albacete. Albacete, Spain
[o] Pneumology Deptarment, Universitary Hospital San Agustín, Avilés, Spain
[p] Department of Nursing, University of Huelva, Huelva, Spain
[q] Université de Bordeaux, Inserm, Bordeaux Population Health Research Center, Team EPICENE, UMR 1219, Bordeaux, France
[r] Pédiatrie CHU Grenoble Alpes; Inserm Unité E2R2H; Université Grenoble Alpes, Grenoble, France
[s] Department of Sleep, Landspitali, The National University Hospital of Iceland, Reykjavík (Iceland)
[t] University of Iceland, Faculty of Medicine, Reykavík, Iceland
[u] Occupational and environmental medicine, School of Public Health, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
[v] Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden
[w] Department of Medical Sciences: Respiratory, Allergy and Sleep research, Uppsala University, Uppsala, Sweden
[x] Department Epidemiology and Public Health, Swiss Tropical and Public Health Institute, Basel, Switzerland
[y] University of Basel, Basel, Switzerland
[z] Lung Clinic, Tartu University Hospital, Tartu, Estonia
[aa] Allergy and Lung Health Unit, School of Population and Global Health, The University of Melbourne, Melbourne, Australia
[ab] National Heart and Lung Institute, Imperial College, London UK
[ac] Unit of Epidemiology and Medical Statistics, Department of Diagnostics and Public Health, University of Verona, Verona, Italy
[ad] Department of Obstetrics and Gynaecology, Haukeland University Hospital, Bergen, Norway
[ae] Inserm UMR-S 1168, VIMA, Villejuif, France
[af] UMR-S 1168, UVSQ, Université Versailles St-Quentin-en-Yvelines, St-Quentin-en-Yvelines, France

* Corresponding authors. Giancarlo Pesce. Sorbonne Université and Inserm UMR-S 1136, Pierre Louis Institute of Epidemiology and Public Health (IPLESP), Saint-Antoine Medical School, 27, rue Chaligny 75012 Paris, France. Phone: +39 34 58 13 42 19.
** Corresponding authors. Bénédicte Leynaert, Inserm UMR-S 1168, VIMA: Aging and chronic diseases. Epidemiological and public health approaches, 16, avenue Paul Vaillant Couturier, 94807 Villejuif, France. Phone: +33 (0)1 45 59 51 96.
E-mail addresses: giancarlo.pesce@inserm.fr (G. Pesce), benedicte.leynaert@inserm.fr (B. Leynaert).
# these authors equally contributed to this work.

ARTICLE INFO

ABSTRACT

Background: Emerging evidence suggests that androgens and estrogens have a role in respiratory health, but it is largely unknown whether levels of these hormones can affect lung function in adults from the general population. This study investigated whether serum dehydroepiandrosterone sulfate (DHEA-S), a key precursor of both androgens and estrogens in peripheral tissues, was related to lung function in adult women participating in the European Community Respiratory Health Survey (ECRHS).

Methods: Lung function and serum DHEA-S concentrations were measured in $n = 2,045$ and $n = 1,725$ women in 1999−2002 and in 2010−2013, respectively. Cross-sectional associations of DHEA-S levels (expressed as age-adjusted z-score) with spirometric outcomes were investigated, adjusting for smoking habits, body mass index, menopausal status, and use of corticosteroids. Longitudinal associations of DHEA-S levels in 1999−2002 with incidence of restrictive pattern and airflow limitation in 2010−2013 were also assessed.

Findings: Women with low DHEA-S (z-score$<$-1) had lower FEV1 (% of predicted, adjusted difference: -2.2; 95%CI: -3.5 to -0.9) and FVC (-1.7; 95%CI: -2.9 to -0.5) and were at a greater risk of having airflow limitation and restrictive pattern on spirometry than women with higher DHEA-S levels. In longitudinal analyses, low DHEA-S at baseline was associated with a greater incidence of airflow limitation after an 11-years follow-up (incidence rate ratio, 3.43; 95%CI: 1.91 to 6.14).

Interpretation: Low DHEA-S levels in women were associated with impaired lung function and a greater risk of developing airflow limitation later in adult life. Our findings provide new evidence supporting a role of DHEA-S in respiratory health.

Funding: EU H2020, grant agreement no.633212

1. INTRODUCTION

Sex hormones have biological effects in many non-reproductive organs. The presence of sex steroid receptors in the lungs and in the airways suggests that androgens and estrogens might play a role in the regulation of the respiratory function [1]. However, despite supporting findings from experimental studies, there is little evidence from clinical or epidemiological studies. In women, hormonal balance might be especially important, as changes of the hormonal status such as those occurring with menopause, have been associated with lung function decline [2,3]. In peripheral tissues, androgens and estrogens are produced intracellularly from dehydroepiandrosterone (DHEA) [4]. DHEA is a steroid, which is mainly produced in the *zona reticularis* of the adrenal glands in response to adrenocorticotropic hormone (ACTH). DHEA is mainly secreted as a sulfate ester (DHEA-S), which is the most abundant sex steroid in human serum. Its concentration peaks around the age of 20 and then steadily decreases with age [4].

There is evidence that DHEA-S can exert immunomodulatory and anti-inflammatory functions [5−7]. DHEA-S naturally antagonizes some of the immunosuppressive and catabolic effects of glucocorticoids, and its decline with age is thought to be one of the causes of immune-senescence [8−10].

Low DHEA-S levels were associated with all-cause mortality in several population-based studies [11], and were also observed in patients with cardiovascular and inflammatory diseases [12−19]. No population-based study has investigated the association of DHEA-S with lung function decline, which is a significant predictor of mortality in long term follow-up studies [20]. As low DHEA-S has been associated with early death, we hypothesized that DHEA-S concentrations might be associated with lung function impairment, which may be mediating this association.

Serum DHEA-S concentrations were measured in women participating in the European Community Respiratory Health Study (ECRHS) as part of a program aimed at investigating the associations between hormonal factors and lung function decline in women [2,21], In this manuscript, we investigated if DHEA-S levels were associated with lung function and lung function decline in 10 years in women from the general population, independently from age, smoking history, and other potential confounders.

Research in context

Evidence before this study

We searched Pubmed for articles using the terms ("lung function" OR "pulmonary function" OR "airflow obstruction") AND ("DHEA-S" OR "DHEA" OR "Sex steroids" OR "Sex hormones"), with no date restrictions and not limited to English-language publications. In population-based surveys, recent mendelian randomization studies indicate that changes in hormonal status (e.g. with menarche or menopause) might affect lung function. Another recent study suggested that exogenous sex steroids may reduce the lung aging in menopausal women. Low DHEA-S concentrations have been associated with inflammatory lung diseases, and greater mortality in COPD patients. DHEA-S had been associated with improved lung function in male adolescents with asthma, and nebulized DHEA-S was reported to improve asthma control. In experimental animal models, DHEA-S was found to prevent bronchospasm and relax airway smooth muscles. One cross-sectional study in men, found significant correlations but no association between DHEAs and FEV1 and FVC in multivariate analysis. We found no studies that investigated the associations between DHEA-S levels and lung function decline in women from the general population.

Added value of this study

We showed that low DHEA-S levels are associated with poor lung function, reduced FVC and FEV1/FVC ratio in a population-based sample of about 3000 women from 19 centres and 9 European countries. In longitudinal analyses, low DHEA-S levels at baseline were associated with 3-fold greater risk of developing airflow limitation (FEV1/FVC$<$lower limit of normal) after an 11-years follow-up.

Implications of all the available evidence

Our findings provide new evidence supporting a beneficial effect of DHEA-S in women's respiratory health. Further studies are needed to assess these relationships in men and to evaluate if restoring DHEA-S to normal levels might improve respiratory function and decrease the risk of airflow limitation.

2. METHODS

2.1. Study design and population

The ECRHS (www.ecrhs.org) is an international population-based cohort study of adults born in 1945−1970 [22]. Participants underwent clinical examinations, including spirometry and standardized interviewer-led questionnaires in 1991−1993 (ECRHS-1), 1999−2002 (ECRHS-2) and 2010−2013 (ECRHS-3). At ECRHS-2 and ECRHS-3, blood samples were collected in women from centers taking part in the hormonal measurement protocol. All the women with spirometry and DHEA-S measurement in at least one of these surveys were considered for the present analysis. Overall, 2969 women from 19 centers in 9 European countries were included in this study [Table E1]. Ethical approval was obtained for each center from the appropriate ethics committee, and written consent was obtained from each participant. This manuscript adheres to the STROBE guidelines.

2.2. Respiratory outcomes

Forced vital capacity (FVC) and forced expiration volume in 1 s (FEV1) were measured according to the American Thoracic Society criteria for repeatability. FEV1, FVC, and FEV1/FVC ratio percentage of predicted and lower limit of normal (LLN) were calculated using the Global Lung Initiative equations [23]. Airflow limitation was defined as FEV1/FVC <LLN; restrictive pattern on spirometry was defined as FVC<LLN in subjects without airflow limitation [23].

2.3. DHEA-S measurements

Blood samples were drawn and stored following standardized procedures. DHEA-S serum concentrations were determined centrally using an immuno-competitive assay (Elecsys® DHEA-S electrochemiluminescence immunoassay, Roche Diagnostics, Germany) at ECHRS-2, while a high-throughput LC-MS/MS system (Aquity UPLC coupled with a Xevo TQ-S mass spectrometer, Waters Corp., Milford, MA, USA) was used at ECRHS-3.

To account for the dependency of DHEA-S on age and as the laboratory assessments differed between the two examinations, we derived age-adjusted DHEA-S z-scores separately for ECRHS-2 and ECRHS-3, using mixed-effects linear regression models on log-transformed DHEA-S concentrations with age included as independent variable and study center as a random effect. The resulting z-score represents how far (in units of the population standard deviation, SD) a measured concentration is from the mean of a same-age population. Participants with a z-score <−1 (representing 14.3% of the sample) were considered as having "low" DHEAs levels.

2.4. Statistical analysis

To investigate the cross-sectional associations of DHEA-S with subjects' characteristics and respiratory outcomes, data from ECRHS-2 and ECRHS-3 were pooled together, and we fitted multilevel models with subject ID (as a level-2 unit, to account for repeated measures) nested in the center (level-3). Associations were modeled using mixed-effects linear or Poisson with robust error variance regressions, with DHEA-S z-score included either as a continuous variable or as a categorical variable. Body mass index, smoking history, education as proxy of socioeconomic status, physical activity, and menopausal status were considered for adjustment. Age was included as precision variable because of its strong association with spirometry outcomes. Stratified analyses were performed for median age, to investigate if a different association was observed in older women (when DHEA-S is lower), and for menopausal status, as the effects of DHEA-S might change with the hormonal context.

Longitudinal association of DHEA-S z-score at baseline (ECRHS-2) and lung function decline between ECRHS 2 and 3 was assessed using linear models. In addition, incidence of new subjects with spirometric restriction pattern and airflow limitation was evaluated at follow-up, and mixed-effects Poisson regression models were used to derive incident rate ratios (IRR) in function of baseline DHEA-S z-score.

Sensitivity analyses for the associations of DHEA-S with spirometric outcomes were performed after exclusion from the models of: 1) corticosteroids users, as corticosteroids are known to affect the adrenal function and DHEA-S secretion; 2) women with asthma, as DHEA-S has been associated in literature with asthma occurrence; 3) current smokers, to avoid potential residual confounding by smoking which can significantly alter both lung function and DHEA-S secretion; 4) women sampled in the ECHRS symptomatic sample, to ascertain that the results were not driven by unspecified respiratory conditions.

In all models, women with missing data on a covariate were removed from the models. The statistical analyses were performed using STATA v14.2 (StataCorp, College Station, TX, USA).

Additional details are provided in the online data supplement. [Text E1]

3. RESULTS

3.1. Sample characteristics and associations with DHEA-S

A total of 3770 observations with valid lung function and DHEA-S measurements ($n = 2045$ at ECRHS-2 and $n = 1725$ at ECRHS-3) were considered for the cross-sectional analyses; 801 women had DHEA-S measured at both ECRHS-2 and ECRHS-3 (repeated measures). Among the 2045 women with DHEA-S and lung function at ECRHS-2, 1216 were followed-up with spirometry and clinical interviews at ECRHS-3 (average follow-up time: 11.0 ± 1.1 years) and were included in the longitudinal analyses.[Table E1]

The distribution of the covariates by survey and their associations with DHEA-S levels are shown in Table 1. Compared to lifetime non-smokers, current smokers had significantly higher DHEA-S concentrations, while ex-heavy smokers had lower DHEA-S concentrations. DHEA-S concentrations were also higher in overweight women (Table 2). We observed no association of age-adjusted DHEA-S z-score with menopausal status, as well as with passive smoke, school education, and physical activity. Both inhaled and oral corticosteroid use in the past year were significantly associated with lower DHEA-S levels ($p<0.001$).

3.2. Cross-sectional associations of DHEA-S levels with lung function indices

The cross-sectional associations of DHEA-S with pre-bronchodilator FEV1, FVC and FEV1/FVC ratio suggest that the association with FEV1 and FVC might not be linear [Fig. 1]. In particular, women with low DHEA-S (z-score<−1) had significantly lower FEV1 and FVC compared to women with normal levels of DHEA-S for their age (z-score between −0.5 and +0.5), while women with DHEA-S z-score above 1 did not have significantly different FEV1 or FVC. Accordingly, it was found that the proportion of women with low DHEA-S was significantly higher among women with a restrictive pattern (21.8%) than among women with normal lung function (13.5%). This difference was found also among never-smokers or ex-smokers with a cumulative history of less than 15 pack-years tobacco smoke, in whom the percentage of women with low DHEA-S reached 24.5% in women with a restrictive spirometric pattern (vs. 13.8% in women with normal lung function).

For modeling both linear and non-linear associations, the associations of lung function outcomes with DHEA-S were estimated with z-scores included either as a continuous or dichotomous variable

Table 1

Participants' characteristics by survey. Distributions are expressed as number (percentage), median [range] or mean ± standard deviation.

	ECRHS-2 $n = 2045$	ECRHS-3 $n = 1725$
Age, years	43.4 [28.0,56.8]	53.7 [39.7,66.9]
Smoking habits		
Non smokers	928 (45.4%)	774 (44.9%)
Past smokers with <15py	387 (18.9%)	472 (27.4%)
Past smokers with ≥15py	119 (5.8%)	155 (9.0%)
Current smokers with <15py	249 (12.2%)	102 (5.9%)
Current smokers with ≥15py	272 (13.3%)	132 (7.7%)
Smokers with unknown py	86 (4.2%)	89 (5.2%)
missing	4 (0.2%)	1 (0.1%)
Exposure to passive smoke in the last 12 months		
No	1275 (62.4%)	1376 (79.8%)
Yes	759 (37.1%)	337 (19.5%)
missing	11 (0.5%)	12 (0.7%)
Body mass index,		
<25 kg/m^2	1194 (58.4%)	736 42.7%)
[25−30) kg/m^2	591 (28.9%)	578 (33.5%)
≥30 kg/m^2	258 (12.6%)	400 (23.2%)
missing	2 (0.1%)	11 (0.6%)
Age completed full time education		
16years or younger	357 (17.5%)	252 (14.6%)
17−20 years	653 (31.9%)	614 (35.6%)
21years or older	1030 (50.4%)	818 (47.4%)
missing	5 (0.2%)	41 (2.4%)
Vigorous physical activity		
No	1207 (59.0%)	979 (56.8%)
Yes	808 (39.5%)	742 (43.0%)
missing	30 (1.5%)	4 (0.2%)
Menstrual Periods		
Regular	1199 (58.6%)	464 (26.9%)
Never been regular	120 (5.9%)	72 (4.2%)
Recently irregular	220 (10.8%)	139 (8.1%)
Have stopped (>6 months)	464 (22.7%)	840 (48.7%)
missing	42 (2.0%)	210 (12.2%)
Use of corticosteroids in the last 12 months		
No	1824 (89.2%)	1538 (89.2%)
Inhaled corticosteroids only	150 (7.3%)	68 (3.9%)
Oral corticosteroids	38 (1.6%)	20 (1.2%)
missing	33 (1.6%)	99 (5.7%)
FEV1, (mL)	2959 ± 505	2574 ± 500
FEV1,% of predicted	98.7 ± 13.5	94.4 ± 14.9
FVC, (mL)	3675 ± 588	3392 ± 593
FVC,% of predicted	99.8 ± 12.6	99.0 ± 13.5
FEV1/FVC ratio (%)	80.6 ± 6.6	75.9 ± 6.5
FEV1/FVC ratio,% of predicted	98.5 ± 7.8	94.7 ± 7.9
Airflow limitation (FEV1/FVC<LLN)	129 (6.3%)	174 (10.1%)
Restrictive pattern on spirometry (FVC<LLN and FEV1/FVC≥LLN)	81 (4.2%)	62 (4.0%)

(Table 3). After adjusting for potential confounders, women with low DHEA-S had significantly lower FEV1 and FVC (−2.0 and −1.7% of predicted, respectively) than women with higher DHEA-S. For FEV1/FVC ratio, a linear increase of one unit in DHEA-S z-score was associated with an increase of +0.3 in FEV1/FVC% of predicted.

Although different methods were used at ECRHS 2 and ECRHS 3 to measure DHEA-S, the associations of z-score DHEA-S with FEV1 and FVC were similar at the two surveys (Table E3). No significant interaction was found between DHEA-S and age or between DHEA-S and menopausal status (Table E4).

When analyzing spirometric outcomes as binary variables, we found that women with low DHEA-S had a 62% greater risk of having restrictive pattern (RR: 1.62, 95%CI: 1.11−2.38; $p = 0.013$) and 35% greater risk of having pre-bronchodilator airflow limitation (RR: 1.35, 95%CI: 1.08−1.69; $p = 0.008$) than women with higher DHEA-S [Fig. 2]. When we repeated the cross-sectional analysis using only data at ECRHS-3, where post-bronchodilator measures were available, we found that low DHEA-S was also associated with a 66%

significantly greater risk of post-bronchodilator airflow limitation (Table E3).

3.3. Longitudinal associations of DHEA-S levels with lung function decline and incidence of spirometric restriction and airflow limitation

The characteristics of women who did and did not participate in both surveys and were included in the longitudinal analyses are shown in the online supplement.[Table E5] A faster decline in pre-bronchodilator FEV1/FVC ratio (−0.13% of predicted/year, $p = 0.003$) was observed in women with low DHEA-S compared to those who had higher DHEA-S levels at baseline.[Table E6] DHEA-S at baseline was not associated with FVC decline.

After excluding women with prevalent airflow limitation at ECRHS-2, women with low DHEA-S at baseline had a 3-fold greater risk to develop pre-bronchodilator airflow limitation at ECRHS-3 (IRR = 3.43, $p<0.001$) (Table 4). This association showed a significant interaction with age (p for interaction=0.013), and low DHEA-S was prospectively associated with a greater risk of developing airflow limitation in women older than the median age (i.e.age≥43 years at baseline, IRR=9.59; 95%CI:4.03−22.8), but not in younger women (IRR=1.32;95%CI:0.48−3.68). Low DHEA-S at baseline was also associated with a higher risk of post-bronchodilator airflow limitation at ECRHS-3 (IRR = 2.81, $p = 0.040$) (Table 4). No association between DHEA-S at baseline and having restrictive pattern at follow-up was observed, but the number of incident cases was very low.

3.4. Sensitivity analyses

We found similar results when data from ECRHS 2 and ECRHS 3 were analyzed separately for cross-sectional associations (Table E4). The cross-sectional associations of DHEA-S with lung function outcomes were less strong, but still generally statistically significant, when women who used corticosteroids or had asthma were excluded from the analyses (Table 3, Fig. 2, Table E7). The sensitivity analyses performed considering only women sampled in the ECRHS "random sample" to exclude potential bias related to other respiratory conditions, as well as in "non-smokers only" to avoid potential residual confounding by smoking, confirmed the main results (Table 3, Fig. 2, Table E7). The longitudinal association between low DHEA-S and higher risk of developing airflow limitation remained significant in the sensitivity analyses (Table E8).

4. DISCUSSION

To the best of our knowledge, this is the first epidemiological study investigating the association between DHEA-S levels and lung function in adult women from the general population, and the first longitudinal study investigating the associations of DHEA-S with lung function decline. We found that women with low DHEA-S levels were significantly more likely to have airflow limitation and restrictive pattern on spirometry than women with higher DHEA-S levels. Moreover, in longitudinal analyses, having low DHEA-S was associated with a greater risk of developing airflow limitation after 11-years of follow-up.

These associations were independent from potential confounders, such as smoking habits and use of corticosteroids, which were found to affect the concentration of DHEA-S. In fact, DHEA-S levels were severely reduced in women treated with oral corticosteroids, but also in women who used only inhaled corticosteroids, reflecting that even inhaled corticosteroids may have systemic effects and cause adrenal suppression. Nicotine has also been reported to affect the HPA axis [24]. In our study, current smokers had higher DHEA-S concentrations than non-smokers, confirming previous reports [25].

Women who had DHEA-S concentrations lower than one standard deviation from the average for their age had significantly lower FEV1

Table 2
Associations of women's characteristics with DHEA-S z-score. *adjusted for age plus all the other covariates included in the table.

	Unadjusted Beta (95%CI)	p-value	Adjusted* beta (95%CI)	p-value
Smoking habits				
Non smokers	reference	–	reference	–
Past smokers with <15py	−0.00 (−0.08,0.08)	.921	0.00 (−0.08,0.09)	.942
Past smokers with ≥15py	−0.04 (−0.16,0.09)	.575	−0.13 (−0.26,0.00)	.052
Current smokers with <15py	**0.18 (0.07,0.29)**	**.001**	**0.18 (0.06,0.29)**	**.003**
Current smokers with ≥15py	**0.23 (0.13,0.33)**	**<0.001**	**0.21 (0.10,0.33)**	**<0.001**
Smokers with unknown py	0.00 (−0.14,0.15)	.958	−0.02 (−0.18,0.14)	.810
Exposure to passive smoke in the last 12 months				
No	Reference	–	Reference	–
Yes	**0.09 (0.02,0.16)**	**.008**	0.04 (−0.06,0.12)	.355
Body mass index,				
<25 kg/m²	Reference	–	Reference	–
[25−30) kg/m²	**0.09 (0.02,0.16)**	**.014**	**0.08 (0.01,0.16)**	**.023**
≥30 kg/m²	0.03 (−0.06,0.12)	.488	0.05 (−0.04,0.14)	.282
Age completed full time education				
16 years or younger	−0.03 (−0.13,0.07)	.554	−0.07 (−0.17,0.04)	.213
17−20 years	Reference	–	Reference	–
21 years or older	−0.01 (−0.08,0.06)	.705	−0.02 (−0.09,0.05)	.569
Vigorous physical activity				
No	Reference	–	Reference	–
Yes	−0.05 (−0.11,0.02)	.160	−0.05 (−0.11,0.02)	.173
Menstrual Periods				
Regular	reference	–	reference	–
Never been regular	−0.05 (−0.19,0.09)	.447	−0.06 (−0.20,0.08)	.386
Recently irregular	**0.11 (0.01,0.09)**	**.038**	0.07 (−0.04,0.18)	.204
Have stopped (>6 months)	−0.02 (−0.09,0.05)	.523	−0.06 (−0.14,0.03)	.192
Use of corticosteroids in the last 12 months				
No	reference	–	reference	–
Inhaled corticosteroids only	**−0.41 (−0.54,−0.28)**	**<0.001**	**−0.41 (−0.54,−0.27)**	**<0.001**
Oral corticosteroids	**−0.82 (−1.05,−0.59)**	**<0.001**	**−0.77 (−1.01,−0.52)**	**<0.001**

and FVC than women with higher DHEA-S, resulting in a greater risk of both airflow limitation and restrictive pattern on spirometry. There are no other studies in women to be compared to ours. We only found similar investigation in two cohorts of community-dwelling men: the Tromsø and the Busselton studies [26,27]. In these cross-sectional studies, including respectively 2197 Norwegian and 1768 Australian adult men, high serum levels of testosterone and dihydro-testosterone, but not estrogens, were significantly associated with better lung function [26,27]. In the Tromsø study, DHEA-S concentrations were also measured, but no significant linear association was found between FEV1 and FVC and DHEA-S after adjustments [26].

However, our findings suggest that the associations between DHEA-S and these lung function outcomes might not be linear. Moreover, as the Tromsø cohort was composed exclusively of men, the difference with our findings might be due to the lower concentrations of DHEA-S in women as compared to men, to different distribution of unmeasured confounders, or indicate that DHEA-S has a different impact on respiratory function in men and women.

Considering that DHEA-S is almost the exclusive source of androgens in peripheral tissues in women [4], these data together suggests that DHEA-S, directly or indirectly through its androgenic metabolites, might contribute in sustaining the respiratory function. Since
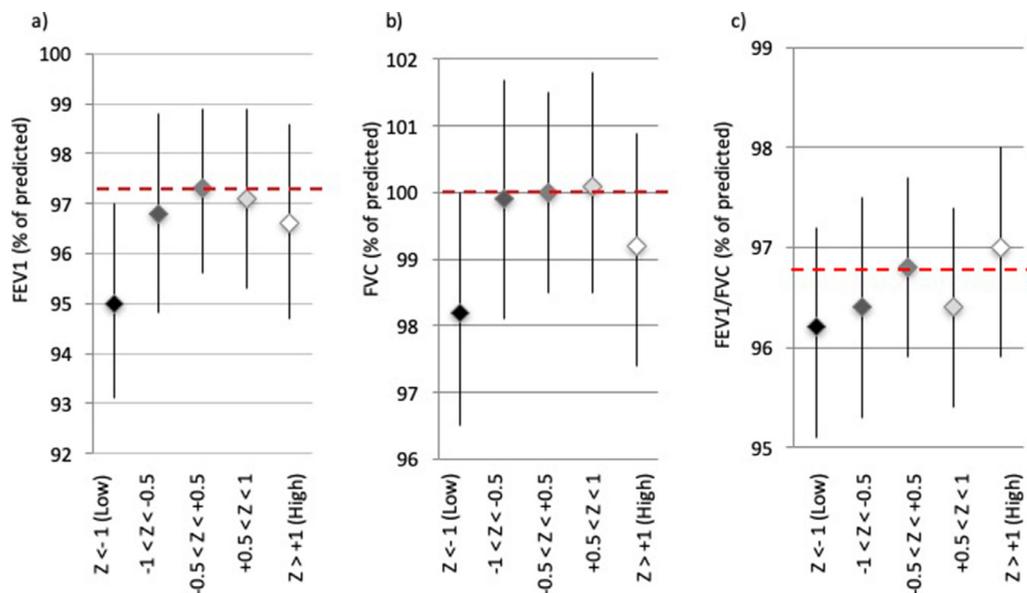

Fig. 1. Women's spirometric measures by DHEAS z-score group. FEV1 (a), FVC (b), and FEV1/FVC ratio (c) are expressed as percentage of predicted. P-values for the pairwise comparisons between different DHEA-S levels are shown in Table E2 in the Online Supplement.

Table 3
Association of DHEA-S z-scores with FEV1, FVC, and FEV1/FVC. Results from main model and sensitivity analyses.

	Obs.	DHEA-S z-score (continuous) Beta (95% CI)	p-value	Low* DHEA-S vs.higher Beta (95% CI)	p-value
FEV1 (% of predicted)					
Unadjusted	3516	0.52 (0.35,1.00)	**.036**	−2.18 (−3.52,−0.85)	**.001**
Main model[†]	3516	**0.65 (0.18,1.12)**	**.007**	**−2.02 (−3.33,−0.71)**	**.002**
w/o CS users[†1]	3245	**0.49 (0.00,0.97)**	**.049**	**−1.65 (−3.01,−0.28)**	**.018**
w/o asthma[†2]	3089	0.33 (−0.15,0.82)	.180	**−1.52 (−2.90,−0.15)**	**.030**
w/o current smokers[†3]	2633	**0.92 (0.38,1.46)**	**.001**	**−2.35 (−3.82,−0.87)**	**.002**
w/o symptomatic sample[†4]	2895	**0.59 (0.06,1.12)**	**.029**	**−1.99 (−3.44,−0.54)**	**.007**
FVC (% of predicted)					
Unadjusted	3460	0.27 (−0.16,0.70)	.219	**−1.70 (−2.90,−0.49)**	**.006**
Main model[†]	3460	0.34 (−0.08,0.77)	.114	**−1.64 (−2.83,−0.46)**	**.006**
w/o CS users[†1]	3193	0.20 (−0.25,0.64)	.386	**−1.40 (−2.65,−0.15)**	**.028**
w/o asthma[†2]	3037	0.06 (−0.40,0.52)	.800	**−1.33 (−2.62,−0.04)**	**.043**
w/o current smokers[†3]	2586	**0.72 (0.23,1.21)**	**.004**	**−2.15 (−3.49,−0.80)**	**.002**
w/o symptomatic sample[†4]	2848	0.29 (−0.20,0.78)	.241	**−1.77 (−3.11,−0.43)**	**.010**
FEV1/FVC ratio (% of predicted)					
Unadjusted	3412	**0.35 (0.08,0.62)**	**.010**	−0.60 (−1.36,0.15)	.118
Main model[†]	3412	**0.31 (0.04,0.58)**	**.024**	−0.55 (−1.28,0.19)	.147
w/o CS users[†1]	3151	**0.29 (0.02,0.56)**	**.033**	−0.28 (−1.03,0.48)	.469
w/o asthma[†2]	2995	**0.28 (0.02,0.55)**	**.038**	−0.26 (−1.01,0.50)	.505
w/o current smokers[†3]	2552	*0.27 (−0.04,0.57)*	*.086*	−0.35 (−1.18,0.48)	.409
w/o symptomatic sample[†4]	2812	*0.33 (0.04,0.62)*	*.061*	−0.30 (−1.09,0.49)	.456

FEV1: Forced expiratory volume in the 1st second, FVC: Forced vital capacity, CS: corticosteroids.
*low DHEAS is defined as having Z-score < −1.
[†] adjusted for age, BMI, smoking history, passive smoke exposure, physical activity, schooling and periods.
Sensitivity analyses were performed in (1) women who were not using corticosteroids; (2) women who did not report asthma; (3) after excluding women recruited as part of the symptomatic sample; (4) non-smokers only.
Statistically significant associations ($p < 0.05$) are shown in bold; borderline associations ($0.05 \leq p < 0.10$) are in italics.

androgens have known anabolic effects, one might speculate that DHEA-S deficiency contributes to respiratory muscle weakness [9], with a simultaneous restrictive lung function impairment. In our study, low DHEA-S was associated with restrictive pattern on spirometry only in the cross-sectional analysis, while it was not associated with FVC decline in the longitudinal analysis. If the association was causal, this might point toward a short-term, maybe reversible, effect

of DHEA-S deficiency on pulmonary vital capacity. Noteworthy, among never-smokers or light smokers, one out of four women with a restrictive pattern was found to have low DHEA-S levels.

As regards airflow limitation, we found significant relationships in the cross-sectional analyses and in the longitudinal analysis. Low DHEA-S at baseline was prospectively associated with faster FEV1/FVC ratio decline and with about 3-fold greater risk of developing
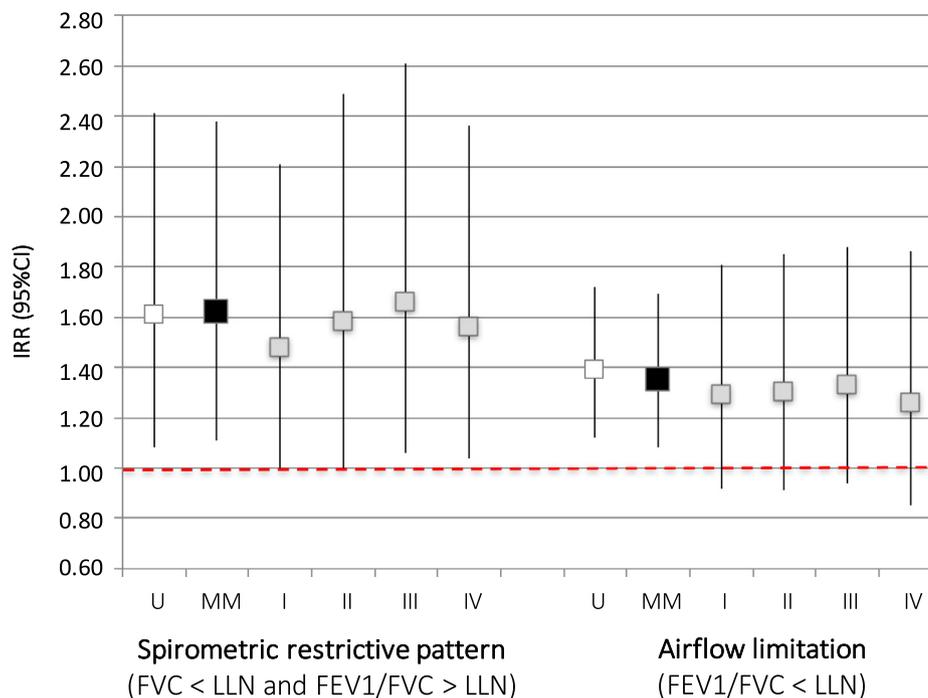

Fig. 2. Cross sectional associations of DHEA-S z-scores (low DHEA-S vs. higher) with the risk of airflow limitation (FEV1/FVC<LLN) and of restrictive pattern on spirometry (FVC<LLN and FEV1/FVC≥LLN). Results from main model (MM) and sensitivity analyses. Models: U: unadjusted; MM: adjusted for age, BMI, smoking history, passive smoke exposure, physical activity, schooling and periods (main model); I: MM with exclusion of corticosteroids users, II: MM with exclusion of women with asthma, III: MM with exclusion of current smokers, IV: MM with exclusion of women recruited in the ECRHS symptomatic sample.

Table 4

Longitudinal associations of DHEAS z-scores with incidence of spirometric restrictive pattern (FVC < LLN and FEV1/FVC ≥ LLN) and pre- and post-bronchodilator airway limitation (FEV1/FVC < LLN).

	Person-years	Incident cases	Rates x1000/year	Unadjusted IRR (95% CI)	p	Adjusted^ IRR (95% CI)	p
Spirometric restritive pattern							
Low DHEA-S*	1559	3	1.9	1.07 (0.31,3.71)	.916	1.40 (0.39,5.04)	.611
Higher DHEA-S	10,129	17	1.7	reference		reference	
Air flow limitation (pre-bronchodilator)							
Low DHEA-S*	1699	18	10.6	**3.38 (1.92,5.93)**	**<0.001**	**3.43 (1.91,6.14)**	**<0.001**
Higher DHEA-S	11,792	42	4.0	reference		reference	
Air flow limitation (post-bronchodilator)							
Low DHEA-S*	1665	6	3.6	**3.15 (1.21,8.21)**	**.019**	**2.81 (1.05,7.53)**	**.040**
Higher DHEA-S	10,277	16	1.6	reference		reference	

IRR: incidence rate ratio. ^IRR are adjusted for age and BMI at baseline, change in BMI, history of smoking between the two examinations. *z-score < −1.

airflow limitation at follow-up, either when assessed pre- or post-bronchodilator.

Our findings on airflow limitation are consistent with previous studies that reported an association between low DHEA-S and asthma, of which airway obstruction is one of the principal features [16−19]. Although the cross-sectional association of DHEA-S with airflow limitation was not significant after excluding women with asthma, the magnitude of the association in women without asthma was similar as in the total sample. Furthermore, the longitudinal association of baseline DHEA-S with airflow limitation incidence remained significant in women without asthma. DHEA-S might impact on airflow limitation through its immunomodulatory and anti-inflammatory properties. In vitro studies have shown that DHEA-S has direct effects on immune cells migration and inhibits the secretion of pro-inflammatory cytokines (namely IL-6, IL-2, IL-10, and TNF) from several cell types in humans [5−8]. The hypothetic mechanisms of action might be both via direct inhibition of NF-kB and indirectly via downstream testosterone and estrogens [5−8]. In addition, in vivo studies have shown that DHEA-S and testosterone can act as bronchoactive steroids, regulating airway contractility by relaxing the airway smooth muscles and preventing bronchospasm [28]. Together, these data suggest that DHEA-S may attenuate airway inflammation and bronchospasm and, *vice-versa*, low DHEA-S may increase the hyper-responsiveness of smooth muscle surrounding the airways and inflammation-driven loss of elastic recoil of lungs and large airways [29], contributing to the development of airflow limitation.

As low DHEA-S was prospectively associated also with incident fixed airflow limitation at follow-up, we hypothesize that low DHEA-S might be a risk factor for the development of COPD. Of note, a recent 6-year follow-up study reported that low DHEA-S levels were associated with greater mortality in COPD patients with acute exacerbation [30], suggesting that DHEA-S might be linked to both disease development and progression.

Previous reports showed that patients with asthma or using corticosteroids have low DHEA-S [16−19,31]. In our study, however, we found that the associations of DHEA-S with airflow limitation at follow-up were not limited to women having a respiratory disease or taking corticosteroids at baseline.

The strengths of our study include the large sample size, longitudinal design, and the use of a standardized protocol across centers, with spirometry and serum biomarkers collected in women from the general population.

We recognize that measurements of DHEA-S using two different methods is a potential limitation of our study. However through transformation of DHEA-S concentrations in z-scores we were able to harmonize and combine the measurements obtained by immunoassay and by mass spectrometry. Although the two methods have different sensitivity and specificity (with LC-MS/MS considered the gold standard for DHEA-S measurement), the associations found in the main analyses were overall confirmed when performing separate analyses for the measurements obtained by immunoassay and mass-spectrometry, supporting the validity of our findings.[Table E3] Our observation that DHEA-S seemed associated with incident airflow limitation especially at older ages suggests that there might be a threshold effect, which needs further investigation.

As only women were sampled, the results of this study may not be generalized to men. Furthermore, as our sample was selected from the general population and relatively young at baseline, the number of incident cases of airflow limitation and restrictive pattern on spirometry was limited. Although we do acknowledge these limits, this is the first study with lung function in women and the only study with longitudinal data on lung function decline. Furthermore, we found significant associations with the incidence of airflow limitation. Our study - with repeated measures of DHEA-S and spirometry data on such a large sample − provides valuable evidence on the association between DHEA-S and lung function decline.

Independently from smoking habits and use of corticosteroids, low DHEA-S was associated with impaired lung function in women and predicted airflow limitation. Our findings provide new evidence supporting a protective role of DHEA-S in respiratory health. It might be particularly relevant to investigate DHEA-S deficiency in non-smoking women with unexplained airflow restriction. Further studies are needed to assess these relationships in men and to evaluate if restoring DHEA-S to normal levels might improve respiratory function and decrease the risk of developing airflow limitation.

Authors' contributions

Dr Pesce and Dr Leynaert conceived and designed the study. Dr Pesce planned and performed the statistical analyses, and wrote the first draft of the manuscript. All authors contributed in the collection of data in/from original studies, discussion of the statistical analysis plan and interpretation of study results. All authors critically reviewed and approved the final version of the manuscript.

Declaration of interests

Dr Demoly reports presentation honoraria from Stallergène Greer, ALK, Mylan, Astra Zeneca, Bausch&Lomb and personal fees as board advisor from Chiesi, Sanofi, Thermo Fisher Scientific, outside the submitted work. Dr Jogi reports personal grants from the Estonian Research Council [personal research grant no.562], and reports travel grants and presentation honoraria from GSK, Novartis, Boehringer, outside the submitted work. Dr Pin reports travel grants and presentation honoraria from GSK, Novartis, Astra Zeneca, Zambon Pharma, outside the submitted work. All the authors declare no competing interests.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.eclinm.2020.100389.

References

[1] Townsend EA, Miller VM, Prakash YS. Sex differences and sex steroids in lung health and disease. Endocrine Rev 2012;33(1):1–47.
[2] Triebner K, Matulonga B, Johannessen A, et al. Menopause is associated with accelerated lung function decline. Am J Rrespir Crit Care Med 2017;195(8):1058–65.
[3] van der Plaat DA, Pereira M, Pesce G, et al. Age at menopause and lung function: a Mendelian Randomization study. Eur Respir J 2019; Oct 17;54(4) pii: 1,802,421. doi: 10.1183/13,993,003.02421–2018.
[4] Labrie F, Martel C, Belanger A, Pelletier G. Androgens in women are essentially made from DHEA in each peripheral tissue according to intracrinology. J Steroid Biochem Mol Biol 2017;168:9–18.
[5] Hazeldine J, Arlt W, Lord JM. Dehydroepiandrosterone as a regulator of immune cell function. J Steroid Biochem Mol Biol 2010;120:127–36.
[6] Choi IS, Cui Y, Koh YA, Hyun-Chul L, CHo YB, Won YH. Effects of dehydroepiandrosterone on Th2 cytokine production in peripheral blood mononuclear cells from asthmatics. Kor J Internal Med 2008;23:176–81.
[7] Koziol-White, et al., et al. DHEA-S inhibits human neutrophil and human airway smooth muscle migration. Biochim Biophys Acta 2012;1822(10):1638–42.
[8] Straub RH, et al. Serum dehydroepiandrosterone (DHEA) and DHEA sulfate are negatively correlated with serum interleukin-6 (IL-6), and DHEA inhibits IL-6 secretion from mononuclear cells in man in vitro: possible link between endocrinosenescence and immunosenescence. K Clin Endocrinol Metab 1998;83:2012–7.
[9] Robinzon B, Cutolo M. Should dehydroepiandrosterone replacement therapy be provided with glucocorticoids? Rheumatol 1999;38 848–495.
[10] Straub RH, Schoelmerich J, Zietz B. Replacement therapy with DHEA plus corticosteroids in patients with chronic inflammatory diseases – substitutes of adrenal and sex hormones. Z Rheumatol 2000;59(Suppl2):108–18.
[11] Ohlsson C, Vandenput L, Tivesten A. DHEA and mortality: what is the nature of the association? J Steroid Biochem Mol Biol 2015;145:248–53.
[12] Shufelt C, Bretsky P, Almeida CM, et al. DHEA-S Levels and Cardiovascular Disease Mortality in Postmenopausal Women: Results from the National Institutes of Health—National Heart, Lung, and Blood Institute (NHLBI)-Sponsored Women's Ischemia Syndrome Evaluation (WISE). J Clin Endocrinol Metab 2010;95(11):4985–92.
[13] Jimenez MC, Sun Q, Schurks M, et al. Low dehydroepiandrosterone sulfate is associated with increased risk of ischemic stroke among women. Stroke 2013;44:1784–9.
[14] Hedman M, Nilsson E, de la Torre B. Low blood and synovial fluid levels of sulpho-conjugated steroids in rheumatoid arthritis. Clin Exp Rheumatol 1992;10(1):25–30.
[15] Suzuki T, Suzuki N, Engleman EG, Mizushima Y, Sakane T. Low serum levels of dehydroepiandrosterone may cause deficient IL-2 production by lymphocytes in patients with systemic lupus erythematosus (SLE). Clin Exp Immunol 1995;99(2):251–5.
[16] Dunn PJ, Mahood CB, Speed JF, Jury DR. Dehydroepiandrosterone sulfate concentrations in asthmatic patients: pilot study. N Z Med J 1984;97:805–8.
[17] Weinstein RE, Lobocki CA, Gravett S, et al. Decreased adrenal sex steroid levels in the absence of glucocorticoid suppression in postmenopausal asthmatic women. J Allergy Clin Immunol 1996;97(1):1–8.
[18] Reinke SN, Gallart-Ayala H, Gomez C, et al. Metabolomics analysis identifies different metabotypes of asthma severity. Eur Respir J 2017;49 1,601,740.
[19] DeBoer MD, Phillips BR, Mauger DT, et al. Effects of endogenous sex hormones on lung function and symptom control in adolescents with asthma. BMC Pulmon Med 2018;18:58.
[20] Mannino DM, Buist AS, Petty TL, Enright PL, Redd SC. Lung function and mortality in the United States: data from the First National Health and Nutrition Examination Survey follow up study. Thorax 2003;58:388–93.
[21] Macsali F, Real FG, Plana E, et al. Early age at menarche, lung function, and adult asthma. Am J Respir Crit Care Med 2011;183(1) 8.14.
[22] European Community Respiratory Health Survey II Steering Committee. The European Community Respiratory Health Survey II. Eur Respir J 2002;20(5):1071–9.
[23] Quanjer pH, Hall GL, Stanojevic S, Cole TJ, Stocks J, Global Lungs Initiative. Age- and height-based prediction bias in spirometry reference equations. Eur Respir J 2012;40(1):190–7.
[24] Rohleder N, Kirschbaum C. The hypothalamic-pituitary-adrenal (HPA) axis in habitual smokers. Int J Psychophysiol 2006;59(3):236–43.
[25] Nagaya T, Kondo Y, Okinaka T. Serum dehydroepiandrosterone reflects age better than health status, and may increase with cigarette smoking and alcohol drinking in middle-aged men. Aging Clin Exp Res 2012;24(2):134–8.
[26] Svartberg J, Schirmer H, Medbø A, Melbye H, Aasebø U. Reduced pulmonary function is associated with lower levels of endogenous total and free testosterone. The Tromsø Study. Eur J Epidemiol 2007;22:107–12.
[27] Mohan SS, Knuiman MW, Divitini ML, et al. Higher serum testosterone and dihyrdrotestosterone, but not oestradiol, are independently associated with favourable indices of lung function in community-dwelling men. Clin Endocrinol 2015;83:268–76.
[28] Montaño LM, Espinoza K, Flores-Soto E, Chavez J, Perusquia M. Androgens are bronchoactive drugs that act by relaxing airway smooth muscle and preventing bronchospasm. J Endocrinol 2014;222:1–13.
[29] Tonga KO, Chapman DG, Farah CS, et al. Reduced lung elastic recoil and fixed airflow obstruction in asthma. Respirol 2019. doi: 10.1111/resp.13688.
[30] Zurfluh S, Nickler M, Ottiger M, et al. Association of adrenal hormone metabolites and mortality over a 6-year follow-up in COPD patients with acute exacerbation. Clin Chem Lab Med 2018;56(4):669–80.
[31] Cavkaytar O, Vuralli D, Arik Ylmaz E, et al. Evidence of hypothalamic-pituitary-adrenal axis suppression during moderate-to-high-dose inhaled corticosteroid use. Eur J Pediatr 2015;174(11):1421–31.