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RESEARCH ARTICLE

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Sex-related differences in pulmonary vascular volume distribution

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Abstract

Pulmonary arterial hypertension affects females more frequently than males, and there are known sex-related differences in the lungs. However, normal sex-related differences in pulmonary vascular structure remain incompletely described. We aimed to contrast computed tomography-derived pulmonary vascular volume and its distribution within the lungs of healthy adult females and males. From the CanCOLD Study, we retrospectively identified healthy never-smokers. We analyzed full-inspiration computed tomography images, using vessel and airway segmentation to generate pulmonary vessel volume, vessel counts, and airway counts. Vessels were classified by cross-sectional area >10, 5–10, and <5 mm^2 into bins, with volume summed within each area bin and in total. We included 46 females and 36 males (62 ± 9 years old). Females had lower total lung volume, total airway counts, total vessel counts, and total vessel volume (117 ± 31 vs. 164 ± 28 mL) versus males (all $p < 0.001$). Females also had lower vessel volume >10 mm^2 (14 ± 8 vs. 27 ± 9 mL), vessel volume 5–10 mm^2 (35 ± 11 vs. 55 ± 10 mL), and vessel volume <5 mm^2 (68 ± 18 vs. 82 ± 19 mL) (all $p < 0.001$). Normalized to total vessel volume, vessel volume >10 mm^2 (11 ± 4 vs. $16 \pm 4\%$, $p < 0.001$) and 5–10 mm^2 (30 ± 6 vs. $34 \pm 5\%$, $p = 0.001$) remained lower in females but vessel volume <5 mm^2 relative to total volume was 18% higher (59 ± 8 vs. $50 \pm 7\%$, $p < 0.001$). Among healthy older adults, pulmonary vessel volume is distributed into smaller vessels in females versus males.

KEYWORDS
aging, multislice computed tomography, pulmonary arterial hypertension, pulmonary circulation, sex characteristics

INTRODUCTION

Pulmonary arterial hypertension (**PAH**) involves pulmonary blood vessel constriction, obstruction, and loss,[1] leading to elevated pulmonary vascular resistance and arterial pressure, right ventricular dysfunction, and death. PAH has classically been viewed as primarily affecting females, and recent registries have reaffirmed a 2–3:1 predilection versus males.[2] Sex hormones influence PAH pathogenesis,[3] but it is plausible that inherent sex-related differences in pulmonary vascular structure also exist, which could influence hemodynamic reserve.

Females have differences in lung morphology compared to males. Females' lung volumes are smaller, even when adjusted for body size, and are more rectangular in shape compared to the triangular (i.e., wider in the lower vs. the upper lung) lungs of males.[4,5] Further, airways are smaller at a given generation, even if body height or lung volume are controlled for.[6,7] Airway differences could be reflected in the vasculature, since they develop in parallel[8] and the diameter of small airways and vessels are proportionate.[9] Indeed, females have smaller main pulmonary artery diameter[10] suggesting sex-related differences may occur across the vascular tree.

Computed tomography (**CT**) can quantitatively assess the lung vasculature.[11] This technique has been used to evaluate pulmonary macro-vascular volume (**VV**) in adults with chronic obstructive pulmonary disease (**COPD**),[12] nonsmokers,[13] and the Framingham cohort.[14] However, to our knowledge, pulmonary VV from CT has not been directly compared between sexes in healthy volunteers. Accordingly, we contrasted pulmonary VV and its distribution within the lungs of female and male never-smokers, without COPD or other relevant conditions, from the community. We hypothesized that compared to males, females would have relatively (1) less small pulmonary VV, and (2) based on the prismatic shape of female's lungs, more VV in the superior aspects, while (3) proportionality between airways and vessels would be conserved across sexes.

METHODS

Participants

We retrospectively analyzed data from the prospective, multicentre, population-based Canadian Chronic Obstructive Lung Disease (**CanCOLD**; NCT00920348) Study.[15] The study was approved by each site's research ethics board; this analysis was approved by the University of British Columbia research ethics board (H21-02749). In the original CanCOLD enrollment, adults >40 years old were randomly selected from the population, and ~1500 Canadians provided written, informed consent and enrolled between 2009 and 2015. To focus on normal lungs in health, we considered participants with spirometry at Visit 1 and excluded those with COPD or smoking history. We also excluded documented respiratory allergies or conditions (including asthma and pulmonary fibrosis), heart disease, hypertension, diabetes, cancer, cirrhosis, hepatitis, and then those with incomplete CT data. To account for potential lung size influences on the vessels, we (1) indexed values to total lung volume (**TLV**) or total VV (**TVV**) (detailed below) in the overall sample, and (2) matched females and males for age ±5 years and CT TLV ±5% in a subsample (Figure 1). When more than one case was

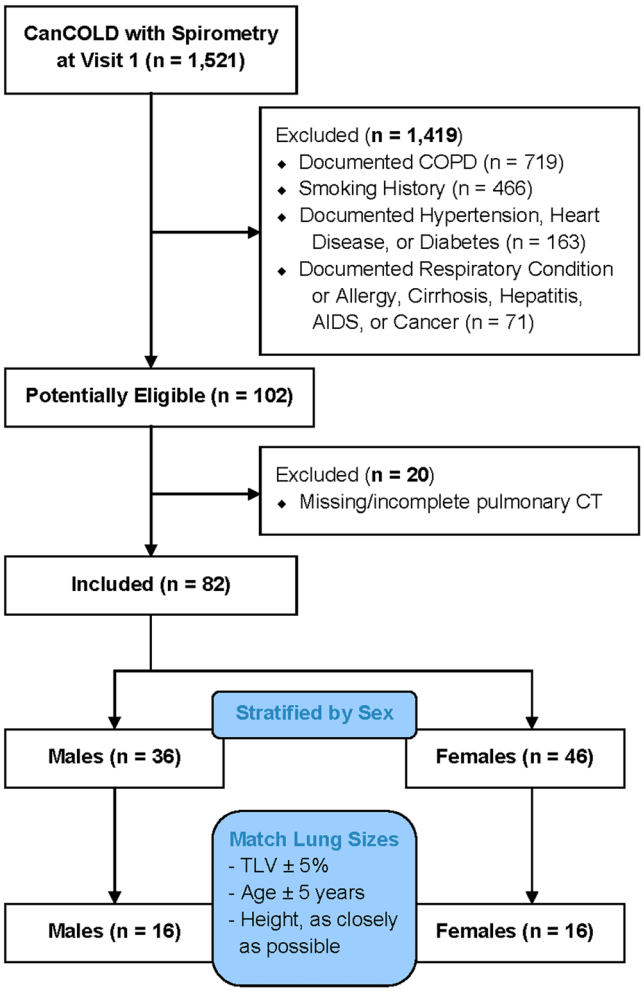

FIGURE 1 Consolidated Standards of Reporting Trials diagram of case selection. Of 1521 participants with spirometry at Visit 1, 1419 were removed for an exclusion criterion, and 20 were removed for incomplete CT data, leaving 82 healthy participants including 36 males and 46 females. From each group, 16 were matched based on total lung volume within 5%. We were able to match 16 of each group by age ±5 years, CT total lung volume ±5%, and height as closely as possible in a subsample.

eligible to be matched based on similar age and TLV, the female and male cases closest in height were chosen.

Measurements

CanCOLD Study methods have been detailed previously.[15] Participants' medical, smoking, and physical activity histories were systematically assessed, as were anthropometrics and blood pressure. Spirometry, plethysmography lung volumes, and lung diffusing capacity for carbon monoxide tests, as well as an incremental cardiopulmonary exercise test performed on a cycle ergometer and metabolic cart, were performed to American Thoracic Society/European Respiratory Society standards.

CT acquisition

Images were acquired using various systems, calibrated similarly, at each site. Participants were positioned supine, and lung images were acquired at full inspiration from the apex to the base as described previously.[15,16] Acquisition parameters were 100 kVp, 50 mAs, 0.5-s gantry rotation, pitch of 1.375, and 1.0- or 1.25-mm slice thickness, contiguous slices. The standard or soft tissue reconstruction kernel was used for quantitative analysis.

CT analysis

Images were analyzed using the VIDA Diagnostics, Inc. Apollo 2.0 software and clinical image analysis service that is ISO13485-certified for quality control. Vessel and airway segmentation was used to generate airway counts, vessel counts, and VV measures. Briefly, lung parenchymal measurements were generated for the whole lung to determine TLV. The airway tree was then segmented, and the airways were labeled from the trachea to the subsegmental bronchi; segmented airways were summed to determine total airway count as described previously.[16] The entire visible pulmonary vascular tree was segmented and vessels were sorted into bins based on 10 and 5 mm^2 cross-sectional area (**CSA**) thresholds, consistent with prior studies,[11–14] with the smallest vessels having an internal diameter ~1 mm. The number of vessels were counted, and their aggregate VV was determined in total and for each bin including CSA > 10, 5–10, and ≤5 mm^2. VV was expressed in absolute terms and relative to total vascular volume (**TVV**), TLV,[11] or height.[17] Our main endpoint was VV < 5 mm^2,[11–14] which reflects small macro-vascular blood vessels. The lungs were also divided into upper, middle, and lower thirds from the apex to base to explore VV craniocaudal regional distribution.

Statistical analysis

Statistical analyses were performed using SPSS v.28 (IBM Inc.). Normality was assessed using the Shapiro–Wilk test and Q–Q plots. Normally distributed data are presented as mean ± standard deviation; nonnormally distributed data are presented as median (25th–75th percentile). Between-group comparisons were made using independent *t*-tests or Wilcoxon Signed Rank tests, with effect sizes estimated using Cohen's *d*. Frequencies were compared using Chi-squared tests, and associations were explored using Pearson correlations. A two-tailed alpha level of 0.05 was considered significant.

RESULTS

Participants

Participant selection is depicted in Figure 1. Of 102 healthy participants who met study criteria, 82 had full CT data sets; characteristics are shown in Table 1. There were no differences in age (range: males = 43–91, females = 43–83 years) or body mass index. Forced expiratory volume in 1 s ($\mathbf{FEV_1}$)/forced vital capacity (FVC) was within normal ranges in both groups, and although there were absolute differences in spirometry and lung diffusing capacity, percent-predicted values were not different. Females had lower absolute, but higher percent-predicted, total lung capacity and residual volumes. Exercise habits were not different, but females had lower peak work-rate and relative aerobic capacity. We matched 16 of each group in a subsample, and similar trends persisted (Table 1).

Females have less pulmonary VV

First, we examined total counts and volumes in the segmented lungs. Total airway counts were not different between groups (Table 2). Females overall demonstrated lower absolute total vessel counts compared to males, although this difference was negated when adjusted for TLV. Individual TLV and TVV data are shown in Figure 2. Females had less TLV ($d = -1.415$) compared to males; when TLV was indexed to height, it remained smaller (29 ± 6 vs. 35 ± 5 mL/cm, $p < 0.001$). On group average, females also had 29% less TVV ($d = -1.594$); differences

TABLE 1 Characteristics of all healthy participants.

	Overall Sample, $N = 82$				Matched Subsample, $n = 32$		
Sample size, *n* (%)	**Overall 82 (100)**	**Males 36 (44)**	**Females 46 (56)**	***p* Value**	**Males 16 (50)**	**Females 16 (50)**	***p* Value**
General							
Age, years	62 ± 9	61 ± 10	62 ± 9	0.627	61 ± 8	60 ± 6	0.858
Height, cm	167 ± 10	175 ± 7	160 ± 7	<0.001	174 ± 6	164 ± 7	<0.001
Mass, kg	73 ± 14	81 ± 13	67 ± 13	<0.001	83 ± 10	69 ± 14	0.003
BSA, m^2	1.82 ± 0.21	1.96 ± 0.17	1.70 ± 0.17	<0.001	1.98 ± 0.12	1.75 ± 0.18	<0.001
BMI, kg/m^2	26 ± 4	26 ± 3	26 ± 4	0.781	27 ± 3	26 ± 5	0.266
SBP, mmHg	120 ± 15	122 ± 15	119 ± 14	0.453	121 ± 15	116 ± 12	0.318
DBP, mmHg	75 ± 10	75 ± 11	75 ± 10	0.817	74 ± 13	76 ± 9	0.600
Pulmonary function							
FEV1, L	2.98 ± 0.76	3.57 ± 0.62	2.53 ± 0.52	<0.001	3.38 ± 0.56	2.80 ± 0.52	0.005
FEV1, % predicted	105 ± 15	105 ± 15	105 ± 14	0.947	100 ± 16	107 ± 15	0.173
FVC, L	3.81 ± 1.04	4.61 ± 0.86	3.19 ± 0.68	<0.001	4.37 ± 0.76	3.64 ± 0.70	0.009
FVC, % predicted	102 ± 14	102 ± 15	102 ± 13	0.955	97 ± 15	108 ± 14	0.051
FEV1/FVC, %	79 ± 5	78 ± 4	79 ± 5	0.094	78 ± 4	77 ± 6	0.827
TLC, L	6.13 ± 1.37	7.03 ± 1.26	5.43 ± 0.99	<0.001	6.59 ± 1.35	6.04 ± 0.95	0.204
TLC, % predicted	106 ± 16	102 ± 17	110 ± 15	0.034	96 ± 20	115 ± 14	0.004
RV, L	2.13 ± 0.60	2.25 ± 0.58	2.04 ± 0.60	0.137	2.23 ± 0.55	2.24 ± 0.61	0.989
RV, % predicted	114 ± 30	106 ± 28	119 ± 31	0.032	108 ± 32	125 ± 26	0.109
DLCO, mL/min/mmHg	23.3 ± 6.3	27.4 ± 5.7	20.0 ± 4.6	<0.001	26.9 ± 5.2	22.3 ± 3.5	0.008
DLCO, % predicted	103 ± 20	104 ± 19	102 ± 20	0.647	102 ± 16	108 ± 17	0.318
Exercise parameters							
Exercise ≥3x/week, *n* (%)	17 (21)	6 (17)	11 (24)	0.422	1 (6)	3 (19)	0.600
Peak VO_2, mL/kg/min	25.6 ± 7.4	27.8 ± 7.6	23.9 ± 7.0	0.031	26.6 ± 8.7	25.6 ± 5.4	0.733
Peak WR, Watts	132 ± 48	160 ± 52	110 ± 31	<0.001	152 ± 57	119 ± 29	0.067

Note: Number (percentage) or mean ± standard deviation.

Abbreviations: BMI, body mass index; BSA, body surface area; DBP, diastolic blood pressure; DLCO, diffusing capacity of the lung for carbon monoxide; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; RV, residual volume; SBP, systolic blood pressure; TLC, total lung capacity; VO2, oxygen consumption; WR, work-rate.

persisted if TVV was indexed to height (−23%) or TLV (−7%, $d = -0.659$). In the subsample matched for TLV, TVV remained 7% less on group-average, yet was not statistically significant (Table 2).

Volume is distributed into smaller vessels in females

We then examined VV distribution across CSA bins. Overall, females demonstrated less absolute VV in all bins, as shown in the upper panels of Figure 3. In the matched subsample, females also demonstrated less absolute VV in the >10 and 5–10 mm^2 bins. However, the difference in VV in the <5 mm^2 bin was not significant and appeared paradoxically higher in females. Indeed, in the overall sample, when VV in each bin was expressed as a proportion of TVV, VV > 10/TVV ($d = -1.337$) and VV5–10/TVV ($d = -0.746$) were lower but VV < 5/TVV ($d = 1.139$ was *higher* in females compared to males (Figure 3, lower panels). The differences in VV distribution are graphically

TABLE 2 Computed tomography data from all participants stratified by sex and matched in a subsample.

	All participants				Matched subsample		
	Overall	**Males**	**Females**	***p* Value**	**Males**	**Females**	***p* Value**
Global measures							
TAC, *n*	211 ± 72	222 ± 77	202 ± 67	0.199	195 ± 84	219 ± 63	0.362
TVC, *n*	6446 ± 2183	7310 ± 2129	5770 ± 1998	0.001	6258 ± 2065	7050 ± 2156	0.297
TLV, mL	5235 ± 1256	6053 ± 975	4596 ± 1071	<0.001	5506 ± 903	5414 ± 939	0.779
TVV, mL	138 ± 38	164 ± 28	117 ± 31	<0.001	147 ± 28	137 ± 25	0.283
VV < 5, mL	74 ± 20	82 ± 19	68 ± 18	<0.001	71 ± 18	81 ± 17	0.134
VV5–10, mL	44 ± 15	55 ± 10	35 ± 11	<0.001	51 ± 9	39 ± 9	<0.001
VV > 10, mL	20 ± 11	27 ± 9	14 ± 8	<0.001	24 ± 7	17 ± 5	0.003
Regional measures							
Upper VV, mL	35 ± 10	41 ± 8	31 ± 9	<0.001	37 ± 8	36 ± 9	0.774
Upper VV < 5	22 ± 6	25 ± 6	21 ± 6	0.004	22 ± 6	25 ± 6	0.103
Upper VV5–10	10 ± 4	13 ± 3	8 ± 3	<0.001	12 ± 4	9 ± 3	0.011
Upper VV > 10	3 ± 2	4 ± 2	2 ± 3	<0.001	3 ± 1	2 ± 1	0.023
Middle VV, mL	77 ± 21	92 ± 16	65 ± 17	<0.001	82 ± 15	75 ± 12	0.139
Middle VV < 5	36 ± 9	39 ± 9	33 ± 8	<0.001	35 ± 8	38 ± 7	0.238
Middle VV5–10	26 ± 8	32 ± 5	21 ± 6	<0.001	29 ± 5	23 ± 5	0.003
Middle VV > 10	15 ± 8	21 ± 7	11 ± 6	<0.001	19 ± 6	14 ± 4	0.011
Lower VV, mL	26 ± 10	32 ± 9	21 ± 7	<0.001	27 ± 9	26 ± 6	0.580
Lower VV < 5	16 ± 6	19 ± 6	15 ± 5	0.002	15 ± 5	18 ± 5	0.002
Lower VV5–10	8 ± 4	11 ± 3	6 ± 2	<0.001	10 ± 3	7 ± 2	<0.001
Lower VV > 10	2 ± 1	3 ± 2	1 ± 1	<0.001	2 ± 1	1 ± 1	<0.001
Normalized measures							
TVC/TLV, *n*/mL	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	0.529	1.1 ± 0.3	1.3 ± 0.3	0.131
VV < 5/TVV, %	55 ± 9	50 ± 7	59 ± 8	<0.001	48 ± 7	59 ± 7	<0.001
VV5–10/TVV	32 ± 6	34 ± 5	30 ± 6	0.001	35 ± 5	29 ± 5	<0.001
VV > 10/TVV	14 ± 4	16 ± 4	11 ± 4	<0.001	17 ± 4	12 ± 3	<0.001
VV < 5/TLV, mL/L	14 ± 2	14 ± 2	15 ± 2	0.001	12 ± 2	14 ± 1	0.004
VV5–10/TLV	8 ± 2	9 ± 2	8 ± 2	<0.001	9 ± 2	7 ± 2	0.004
VV > 10/TLV	4 ± 1	4 ± 1	3 ± 1	<0.001	4 ± 1	3 ± 1	0.003
Upper VV/TVV, %	26 ± 4	25 ± 4	26 ± 3	0.084	25 ± 5	26 ± 3	0.524
Middle VV/TVV	56 ± 3	56 ± 2	56 ± 3	0.822	56 ± 2	56 ± 3	0.168
Lower VV/TVV	18 ± 4	19 ± 4	18 ± 4	0.133	19 ± 4	18 ± 3	0.753

Note: VV < 5, VV of vessels with a cross-sectional area <5 mm^2; VV5–10, VV of vessels with a cross-sectional area >5–10 mm^2; VV < 10, VV of vessels with a cross-sectional area >10 mm^2.

Abbreviations: TAC, total airway count; TLV, total lung volume; TVC, total vessel count; TVV, total vessel volume; VV, vascular volume.

summarized in Figure 4. Similar patterns were observed if VV was instead normalized to TLV, with females having lesser VV > 10/TLV and VV5–10/TLV, but greater VV < 5/TLV (Table 2). While females had less absolute VV in the upper, middle, and lower thirds of the lungs compared to males, there were no differences in the percentage of TVV contained within each (Table 2).

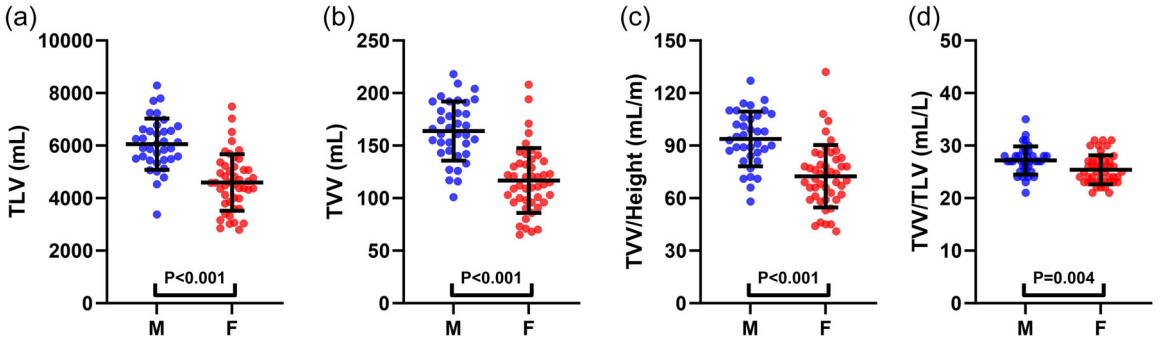

FIGURE 2 (a) Total lung volume (TLV) and total vascular volume (TVV) in males (M) and females (F). TVV is presented in absolute terms (b) and indexed to height (c) and TLV (d). TVV was lesser in females compared to males, even when controlling for their smaller statures and lung sizes. Mean and SD laid over individual data. $N = 82$.

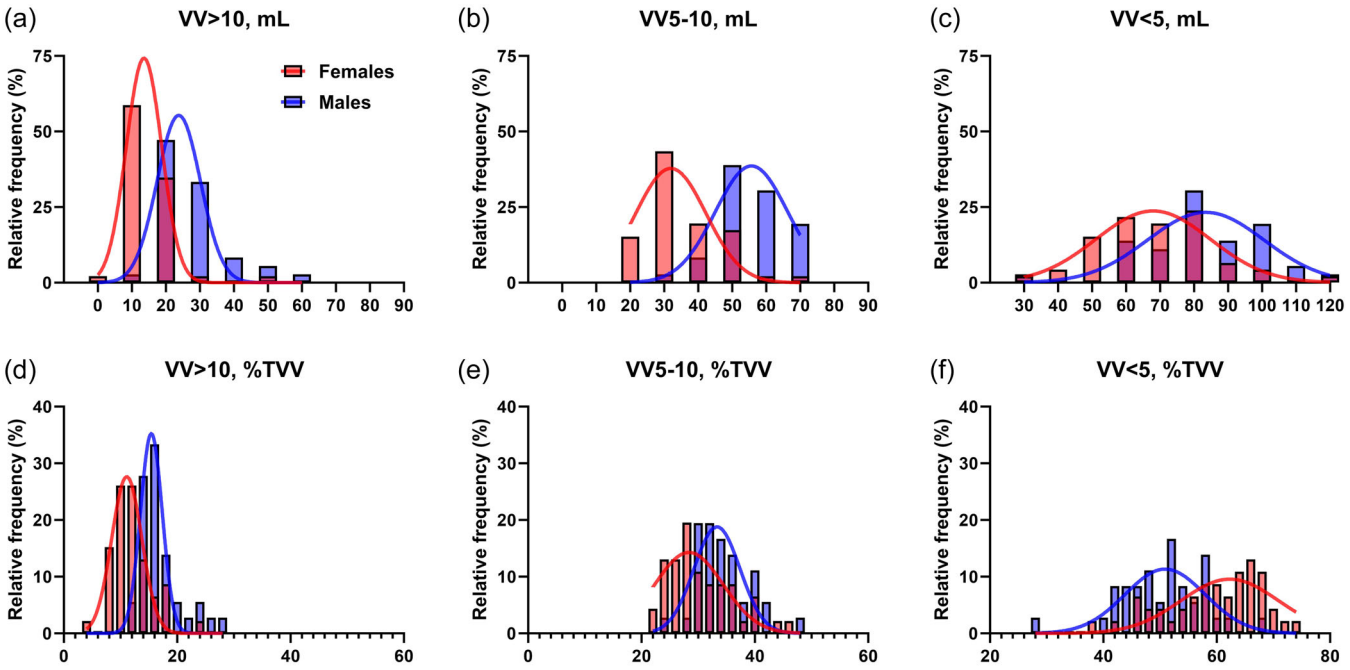

FIGURE 3 Relative frequency histograms of pulmonary vascular volume (VV). Females more frequently had lower absolute VV contained within vessels with cross-sectional areas (a) >10 mm^2 (**VV > 10**), (b) 5–10 mm^2 (**VV5–10**), and (c) <5 mm^2 (**VV < 5**). When VV in each cross-sectional area was expressed as a proportion of total vascular volume (**%TVV**), females more frequently had lower (d) >10 mm^2 and (e) 5–10 mm^2, but higher (f) <5 mm^2. $N = 82$.

Relationships between lung size, airway counts, and vessel counts

Relationships between total lung measurements are shown in Figure 5. Overall, total vessel count was strongly correlated with TLV ($r = 0.753$, $p < 0.001$); the relationship persisted within both females ($r = 0.816$, $p < 0.001$) and males ($r = 0.597$, $p < 0.001$). Conversely, total airway count was only weakly correlated with TLV ($r = 0.382$, $p < 0.001$); the relationship also persisted within both females ($r = 0.315$, $p = 0.033$) and males ($r = 0.444$, $p = 0.007$). Total vessel count and total airway count were moderately correlated ($r = 0.521$, $p < 0.001$). Overall, females had lower ratios of vessels to airways compared to males (30 ± 10 vs. 35 ± 9, $p = 0.031$), though in the matched subsample, this difference was negated (35 ± 10 vs. 33 ± 9, $p = 0.649$).

DISCUSSION

We used quantitative CT imaging to assess pulmonary vascular structure in healthy adults drawn randomly from the Canadian community and contrast VV and its

distribution within the lungs between females and males. This analysis yielded three main findings. First, TVV is lower in females and the difference persists when adjusted for height or lung size. Second, and contrary to our hypothesis, VV is distributed into smaller vessels in females, with a higher percentage of TVV located in vessels with a CSA < 5 mm^2. However, TVV distribution across the craniocaudal axis is not different. Third, total vessel count correlates with both TLV and total airway count in females and males. Together, our data indicates sex-related differences in pulmonary vascular structure that occur independent of lung size.

Females have less pulmonary VV

We studied never-smokers >40 years old who were randomly sampled from nine regions and rigorously screened for relevant conditions and risk factors. In these healthy adults, females had less TVV compared to males. This finding might appear intuitive; females, on average, are smaller than males, but there are also sex-related differences in thoracic geometry.[4] In turn, females have smaller lung volumes, however measured,[4,5] and this tendency persists if height or thoracic size are controlled for.[4] Prior studies have shown that females have smaller main pulmonary artery diameter.[10] In our healthy sample, we extended these findings by showing that females had lower TLV both in absolute terms and relative to height, as well as less TVV in absolute terms and relative to height or to TLV. These results indicate sex-related differences in TVV that could be considered inherent.

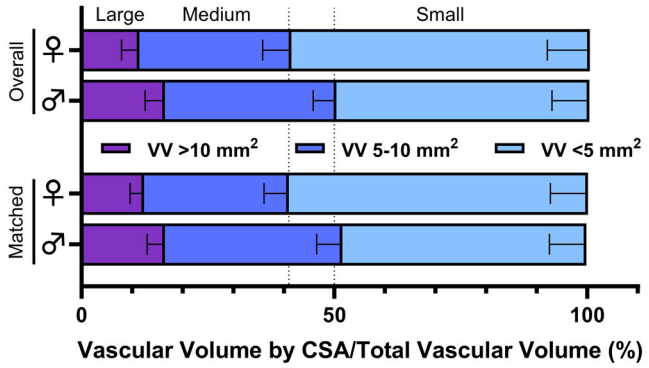

FIGURE 4 Pulmonary blood volume distribution across large, medium, and small vessels in females (♀) and males (♂) in the entire sample of $N = 82$ (upper), and in the matched subsample of $n = 32$ (lower). In females, the percentage of total vascular volume contained in small vessels was ~10% greater compared to males.

VV is distributed into smaller vessels in females

We also found differences in VV distribution, with a higher VV < 5/TVV in females. This finding is consistent with data from adults with heart failure, in which those with high %VV < 5 were more likely to be female,[18] while our overall 55% VV < 5/TVV is similar to other control groups.[19] We used an absolute CSA threshold to delineate "small" vessels as others have.[11–14] VV < 5, the aggregate volume of vessels with CSA < 5 mm^2, is determined by the number of vessels summed and the volume of each segment, which is in turn influenced by length and diameter. When indexed to or matched for TLV, females did not have fewer vessels counted. Here, we assumed that vessel count distribution across increasing branch orders is similar between males and females. If vessel diameters or CSA are then systematically smaller in females, less volume would be contained in the VV > 10 and VV5–10 bins, and a higher fraction of vessels would be classified as <5 mm^2, ultimately shifting TVV distribution toward VV < 5.

Dysanaptic, or unequal growth "between constituent parts of an organ while allowing normal physiological function of the whole,"[20] was used to reflect normal variance in the airway-to-lung size relationship. Sex-related

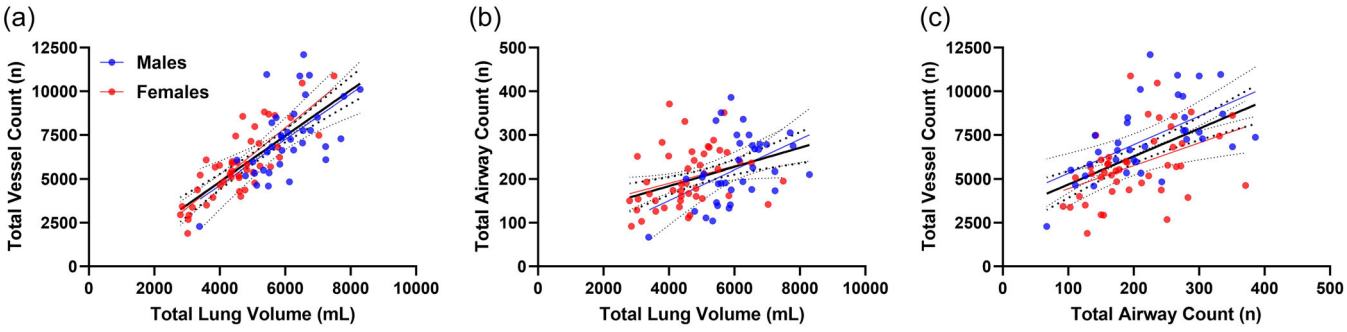

FIGURE 5 Relationships between (a) total vessel count and total lung volume ($r = 0.753$, $p < 0.001$), (b) total airway count and total lung volume ($r = 0.382$, $p < 0.001$), and (c) total vessel count and total airway count ($r = 0.521$, $p < 0.001$). $N = 82$.

dysanapsis has been described more recently, as females have smaller airways relative to lung size.[21] Our results suggest that sex-related dysanapsis in the lungs extends to the vasculature as females have smaller vessels relative to lung size. To confirm, further work is needed to contrast the distribution of vessel counts and size in females and males, and associate vessel sizes with their branch order position. Adult females' lungs are also shaped differently from males'.[21] Females tend to have more "prismatic" lungs compared to more "pyramidal" shaped lungs of males.[5] This may suggest that a greater portion of the vascular bed is higher in upright females' lungs, with altered blood flow distribution. However, dividing the lungs into vertical thirds did not signal any meaningful differences in VV distribution.

Potential inherent and acquired sex-related differences in the pulmonary vasculature

CanCOLD included adults >40 years old and 60% of our sample were over 60 years. These data are relevant to the period when chronic disease incidence accelerates. However, it has limited ability to delineate differences inherent from early development from those acquired in mid-life that may be associated with menopause, although there is evidence that both could be involved. Sex-related differences in lung and airway structure and function over the lifespan have been reviewed previously.[22] Across adulthood, healthy females' airways are smaller at a given generation, independent of height or lung size[6,7]; differences persist through at least the eighth branch generation in younger adults,[23] suggesting they are present before menopause. Since airway and lung vascular development occur in parallel,[8] sex-related differences in pulmonary vascular structure before menopause may be anticipated. In adult human lungs, the airways and blood vessels branch and run in parallel, the diameter of bronchioles and small pulmonary arteries are a consistent ratio,[9] and we observed a modest correlation between airway and vessel counts. Further, sex hormones' influence on the pulmonary circulation are important, complex, and becoming clearer.[3] Dehydroepiandrosterone, progesterone, and estrogen influence vascular tone, inflammation, and remodeling. In females, the hormonal milieu changes acutely over the menstrual cycle and chronically with menopausal status, and the age of menopause is associated with adverse changes in the pulmonary circulation.[24] This suggests that sex hormones also influence measured pulmonary VV. We show that healthy older adult females have less total pulmonary VV, and relatively more small vessel volume, than males. While we could not assess menstrual phase or menopausal status, systematically smaller vessels could be attributable to inherent differences present before, and accentuated by, menopause. Smaller airways have been implicated in the propensity for females to develop airflow limitation[25]; if differences in airway structure that influence the pressure-flow relationship are reflected in the blood vessels, they may influence hemodynamic reserve.

Sex-related structural differences could contribute to hemodynamic function

For individuals who develop pulmonary vascular disease, smaller vessels may accentuate the hemodynamic consequences. Vascular resistance is exquisitely sensitive to radius and CSA and the extensively branched pulmonary vascular network has substantial reserve.[26] Over 50% of the distal vasculature must be lost before pulmonary artery pressure rises,[26] and so it has been referred to as a vascular "quiet zone" analogous to small airway disease. In PAH, muscular arteries and arterioles from 0.50 to 0.02 mm become impacted by thrombosis, vascular proliferation and inflammation, and vasoconstriction. These insults narrow and obstruct the lumen, and elevate pulmonary vascular resistance.[1] If sex hormones that modify vascular remodeling in females[3] are superimposed upon inherently smaller vessels which may possess less CSA and reserve, these differences, along with age, may interactively influence the hemodynamic presentation of PAH. However, whether there are meaningful sex-related differences in the normal or abnormal pulmonary artery pressure response to increasing pulmonary blood flow remains unclear,[27] and how right ventricular functional reserve is influenced by both age and sex is incompletely understood; these topics require future study.

Relevance of CT VV < 5 to vessels involved in PAH

The pulmonary vasculature branches into 15–17 orders[28] with internal diameters that decrease from ~27 mm at the pulmonary trunk[10] to precapillary vessels ~0.02 mm. Our imaging had a resolution of ~1 mm, and VV determined by 3D reconstruction includes the vessel walls and luminal blood. Pulmonary vessel internal diameter is normally ~80% of the external.[29] This suggests that vessels with a CSA of 5 mm^2 have an internal diameter ~2 mm, assuming circularity, and the smallest vessels segmented have an internal diameter ~1 mm. As such, our analysis likely captured vessels in the first seven

orders (Strahler orders 9–15),[28] with VV < 5 reflecting smaller than subsegmental arteries[12] that remain >0.6 mm in diameter.[28] PAH predominately involves lesions in the distal vessels <0.5 mm.[1] This implies some spatial disconnect between the vessels resolvable by CT and those at risk to be impacted by PAH.[30] While the extent to which sex-related differences extend further into the vascular tree is unclear, changes in VV < 5 are associated with histological alterations,[11] suggesting that the differences detected in larger vessels may be reflected in smaller vessels and physiologically meaningful.

LIMITATIONS

Our study has limitations which merit discussion. The CanCOLD Study focuses on obstructive lung disease, and echocardiography and cardiac catheterization were not performed. As such, while cases with documented PAH were excluded, and the prevalence of PAH in the general population makes it unlikely, we cannot definitively rule out PAH presence in our sample. The mean age at PAH diagnosis ranges from 50 to 65 in modern registries.[2] Since CanCOLD enrolled individuals >40 years old, and the average age of females in our sample was 62, participants were relevantly aged, but likely postmenopausal. Studying postmenopausal females may have reduced physiologic variability in pulmonary vascular measures, since airway and vascular measurements vary over the menstrual cycle. However, this prevented us from studying whether the observed sex-related differences exist before menopause, and further work is needed to address the interrelated influences of age and sex hormones. CT images were captured at total lung capacity. Lung inflation impacts pulmonary blood volume and likely influences measured VV; future studies should compare VV at residual volume, functional residual capacity, and total lung capacity. The ~7 order range captured by current CT begins to approach cast techniques that can resolve to 0.1 mm. However, we could not tag vessel segments anatomically at known Strahler orders to make sex-based comparisons of vessel size at specific positions within the vascular tree, nor could we separate arteries and veins, which could influence the interpretation. Future studies should aim to attribute sex-related differences in VV to pre- or postcapillary vessels to better understand why females may be at greater risk for PAH.

CONCLUSIONS

Among healthy community-dwelling adults, females had lower TVV compared to males, independent of body habitus or lung size, and a higher percentage of TVV within vessels with CSA < 5 mm^2. These findings suggest normal sex-related differences in pulmonary vascular structure that occur independent of lung size.

AUTHOR CONTRIBUTIONS

Conception and design: Stephen P. Wright, Miranda Kirby, Wan C. Tan, Jean Bourbeau, and Neil D. Eves. *Data collection and analysis*: Stephen P. Wright, Miranda Kirby, Gaurav V. Singh, Wan C. Tan, and Jean Bourbeau. *Interpretation*: Stephen P. Wright, Miranda Kirby, and Neil D. Eves. *Manuscript drafting*: Stephen P. Wright, Miranda Kirby, and Neil D. Eves. All authors contributed to manuscript revision and approval.

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ETHICS STATEMENT

The study was approved by each site's research ethics board; this analysis was approved by the University of British Columbia research ethics board (H21-02749).

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