Computed tomography reveals hypertrophic remodelling of the diaphragm in cystic fibrosis but not in COPD

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Shareable abstract (@ERSpublications)
Computed tomography detects distinctive signatures of skeletal muscle remodelling in CF and COPD, consisting of greater dimensions of the respiratory muscles in CF and changes suggesting increased lipid content of the nonrespiratory muscles in COPD https://bit.ly/44c4ljh

Cite this article as: Ostadan F, Donovan AA, Matouk E, *et al*. Computed tomography reveals hypertrophic remodelling of the diaphragm in cystic fibrosis but not in COPD. *ERJ Open Res* 2023; 9: 00282-2023 [DOI: 10.1183/23120541.00282-2023].

Received: 1 May 2023
Accepted: 5 July 2023

Abstract

Background Computed tomography (CT) is increasingly used for assessing skeletal muscle characteristics. In cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD), reduced limb muscle mass predicts poor clinical outcomes. However, the degree to which quantity or quality of respiratory and nonrespiratory muscles is affected by these diseases remains controversial.

Methods Thoracic CT images of 29 CF, 21 COPD and 20 normal spirometry control subjects were analysed to measure indices of muscle quantity (volume or cross-sectional area) and quality (radiodensity) in respiratory (diaphragm, abdominal) and nonrespiratory (pectoralis, lumbar paraspinal) muscles. Multivariable linear regression assessed relationships of CT measurements with body mass index (BMI), forced expiratory volume in 1 s (FEV_1) % pred, inflammation and infection biomarkers, nutritional status and CF genotype.

Results Diaphragm volume in CF was significantly higher than in COPD (by 154%) or controls (by 140%). Abdominal muscle area in CF was also greater than in COPD (by 130%). Nonrespiratory muscles in COPD had more low radiodensity muscle (marker of lipid content) compared to CF and controls. In CF but not COPD, higher BMI and FEV_1 % pred were independently associated with higher diaphragm and/or abdominal muscle quantity indices. Serum creatinine also predicted respiratory and nonrespiratory muscle quantity in CF, whereas other biomarkers including genotype correlated poorly with muscle CT parameters.

Conclusions Our data suggest that the CF diaphragm undergoes hypertrophic remodelling, whereas in COPD the nonrespiratory muscles show altered muscle quality consistent with greater lipid content. Thoracic CT can thus identify distinctive respiratory and nonrespiratory muscle remodelling signatures associated with different chronic lung diseases.

Introduction

Low skeletal muscle mass (sarcopenia) and reduced strength are risk factors for early death in numerous chronic illnesses as well as within the general population [1–3]. In both cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD), reduced skeletal muscle mass is an independent predictor of mortality [4, 5]. Patients with CF and COPD share a number of characteristics implicated in the development of skeletal muscle wasting and weakness. These common features include chronic airflow obstruction, reduced physical activity, poor nutritional status, chronic inflammation and intermittent hypoxia [6, 7]. However, the age of onset of CF and COPD (paediatric *versus* adult), as well as the prevalence of other comorbid conditions, differs between the two diseases.

We previously made the discovery that the cystic fibrosis transmembrane conductance regulator (CFTR) protein is normally expressed in skeletal muscles (both mouse and human) of healthy subjects, which raises the possibility that CFTR mutations in CF patients might directly contribute to skeletal muscle abnormalities [8]. Most recent studies of skeletal muscle properties in CF and COPD have focused on the lower limb musculature. For example, the quadriceps muscle in COPD often exhibits atrophy along with reduced oxidative capacity and a greater proportion of more fatigable fast-twitch fibres [9]. The quadriceps in CF similarly demonstrates significant atrophy and weakness in many patients [7]. In both CF and COPD, limb muscle weakness has primarily been attributed to decreased muscle mass, since in most studies the lower muscle strength compared to control subjects disappeared after correcting for differences in muscle size [7, 9, 10].

The effects of CF and COPD on the diaphragm are controversial, with opposing findings in the literature. For example, although muscle fibre atrophy was reported in diaphragm biopsies from small numbers of COPD patients [11], other investigations found no evident alteration of diaphragm mass [12–14]. Pressure-generating capacity of the diaphragm in severe COPD is decreased [15]. However, it is greater than expected for operating lung volume [16], suggesting that adaptive changes such as a decreased number of sarcomeres in series are able to partially compensate for chronic hyperinflation [17, 18]. The data on functional properties of the respiratory muscles in CF are also inconsistent, with different studies reporting that pressure-generating capacity is either decreased, normal or even supranormal [19]. An increase of fatigue-resistant slow-twitch fibres was observed in COPD diaphragm biopsies, which suggests a respiratory workload-induced training effect despite the simultaneous presence of fibre atrophy [11]. To our knowledge, there are no comparable human biopsy data available for CF patients in either respiratory or nonrespiratory muscles.

The controversies regarding effects of CF and COPD on the diaphragm are likely due in part to the fact that diaphragm muscle size and other characteristics are difficult to accurately assess *in vivo* because of its relatively inaccessible location. Over the past decade there has been rapidly growing interest in the ability of computed tomography (CT) to assess body composition, including for skeletal muscle, in different clinical conditions. This approach was initially pioneered in the cancer cachexia literature, where skeletal muscle and adipose tissue quantification by CT were found to be important prognostic indicators [3, 20]. Reduced skeletal muscle quantity on CT images is a powerful predictor of poor prognosis, and an increase in the relative amount of low CT radiodensity muscle is also strongly correlated with adverse clinical outcomes [21–23]. At the tissue level, greater intramuscular lipid accumulation has been confirmed in skeletal muscle with lower CT radiodensity [24, 25], and this finding is generally considered a marker of "poor quality" muscle. This is supported by the association of low radiodensity skeletal muscle with decreased muscle strength [26] and its reversibility by exercise training [27].

The ability of chest CT images to simultaneously measure indices of muscle dimensions and quality represents a largely unexploited opportunity to better understand how the diaphragm responds to different disease states. In this regard, CT imaging offers: 1) an objective and quantifiable method for evaluating the diaphragm in its natural state *in situ*; and 2) a convenient resource for making comparisons of different muscle groups (respiratory and nonrespiratory) within the same subjects. Accordingly, the primary objective of the present study was to determine whether chest CT imaging can identify distinctive skeletal muscle remodelling signatures in the diaphragm associated with either CF or COPD. Secondly, we sought to compare CT-determined patterns of altered skeletal muscle properties in the diaphragm to expiratory respiratory muscles (abdominal) as well as nonrespiratory muscles (pectoralis and lumbar paraspinal) within the same patients. Thirdly, we explored the relationship of CT-determined skeletal muscle remodelling signatures with clinical features and biomarkers reflecting patient adiposity, nutritional status, pulmonary function, inflammation and the CFTR genotype in CF patients.

Methods

Study cohorts and data collection

The study is a cross-sectional retrospective evaluation of: 1) patients from the Adult CF Clinic (CF group) of the Montreal Chest Institute at the McGill University Health Centre; and 2) subjects with either normal lung function (control group) or airflow obstruction (COPD group) as defined by spirometry. The control and COPD subjects were both recruited at the same centre from the Canadian Cohort Obstructive Lung Disease (CanCOLD) study (ClinicalTrials.gov NCT00920348). CanCOLD is an observational cohort study that recruited its participants using random sampling from the general population rather than clinical settings to avoid selection bias in assessing the prevalence of COPD [28]. The diagnosis of COPD was based on the Global Initiative for Chronic Obstructive Lung Disease (GOLD) spirometry definition as previously described by others [28–30]. Control subjects were defined by normal spirometry

(post-bronchodilator forced expiratory volume in 1 s/forced vital capacity (FEV_1/FVC) ratio ⩾0.70 and FEV_1 ⩾80% pred) as well as normal Medical Research Council Dyspnoea Scale (1.1±0.24) and normal COPD Assessment Test (3.8±2.9) scores. Standard CanCOLD assessment includes age (⩾40 years old), sex, height, weight, body mass index (BMI) and pulmonary function. Further details concerning the CanCOLD study design and eligibility criteria have been previously published [28, 31]. The current study was approved by the McGill University Health Centre Research Ethics Board. Protection of the privacy of the research participants and confidentiality of the data were ensured.

The hospital electronic medical record was used to identify 29 CF patients >18 years of age who had undergone thoracic CT imaging without intravenous contrast enhancement for a clinical indication during the years 2010–2020 (inclusive). All contrast-enhanced scans were excluded due to potential effects on muscle density measurements. Among non-CF subjects from the CanCOLD study, 20 control and 21 COPD subjects who underwent thoracic CT imaging without contrast enhancement were also identified. Full lung thoracic CT imaging was performed at suspended maximal inspiration on a helical scanner (matrix 512×512, field of view 286–436 mm, 120 kVp, slice thickness 1.25 mm). Patients with known neuromuscular disorders or inadequate clinical data were excluded. Age, sex, height, weight, BMI and spirometry data (FEV_1, FVC and FEV_1/FVC ratio) were obtained. In addition, for CF patients blood test indicators of inflammation and nutritional status (C-reactive protein, leukocyte count, creatinine, haemoglobin, albumin, iron, ferritin, vitamin D, total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, triglycerides, haemoglobin A1C) obtained during a period of clinical stability within 6 months of the CT scan date were retrieved from the medical record. Diabetes (defined by regular home medication use), systemic corticosteroid use and airway bacterial colonisation with *Pseudomonas aeruginosa* within the same time period, as well as CFTR genotype, were also determined.

CT image analysis

Pre-determined Hounsfield unit (HU) thresholds for assessing different tissue types [32] were applied to all thoracic CT images: –29 to +150 HU for skeletal muscle; –190 to –30 HU for subcutaneous adipose tissue; and –150 to –50 HU for visceral adipose. Skeletal muscle "quality" was evaluated based on the measurement of radiodensity, with low-density muscle (LDM) defined as being within the –29 to +29 HU range as previously described [33–35]. To measure skeletal muscle and adipose tissue characteristics, the Osirix open-source software (version 8.5.1; Pixmeo, SARL, Geneva, Switzerland) was applied to chest CT images as previously described in detail [31]. The left hemidiaphragm was mapped on continuous axial slices and segmented into a 3-dimensional image to determine tissue volume (the right hemidiaphragm was not used because of the similar attenuation values of the apposed liver [31]). Diaphragm configuration was also analysed by measuring dome height, which was determined by multiplying CT axial slice thickness by the number of axial slices between the inferior-most axial image of the lung containing the diaphragm to the superior-most axial image containing the central tendon region of the diaphragm as previously described [31]. For all other muscles, a single axial image at the superior border of the aortic arch (for pectoralis muscles) or first lumbar (L1) vertebral body (for lumbar and abdominal muscles) was selected to measure muscle tissue cross-sectional area (see figure 1) [36, 37]. Subcutaneous and visceral adipose tissue cross-sectional areas were also quantified at the L1 level. Skeletal muscle and adipose tissue quantity (volume for diaphragm, cross-sectional area for all others) were normalised to patient stature (height in m^2) to calculate the skeletal muscle index (SMI) and adipose tissue index (ATI), respectively, as previously described [38]. All reported measurements were performed by a single rater. The reproducibility of diaphragm dimension measurements was confirmed on a blinded replicate mapping of 26% of the sample by two blinded raters (A.A. Donovan and F. Ostadan) unaware of other participant information (inter-rater intraclass correlation coefficients: 0.96, $p<0.001$).

Statistical analysis

Participant characteristics are presented as mean±SD or proportion values unless otherwise indicated. Sample size was estimated from a pilot study of the three subject groups (n=9 per group). Using normalised diaphragm volume as the primary outcome, a sample size requirement of 63 subjects was calculated (α=0.05, power=0.8, effect size=0.4) using G*Power (version 3.1) software [39]. Mean differences between the groups were detected using ANOVA or ANCOVA with *post hoc* application of Tukey or Bonferroni tests, respectively, to correct for multiple comparisons. A sensitivity analysis was also performed in which subsets of the subjects were matched for age and sex. Associations of skeletal muscle and adipose tissue morphology parameters with individuals' demographics, clinical characteristics, pulmonary function and blood biomarker status were assessed using Pearson correlation or multivariate regression analyses. Interaction terms in the multivariate regression analyses evaluated between-group differences. The blood markers were explored as continuous variables. Diabetes, systemic corticosteroid

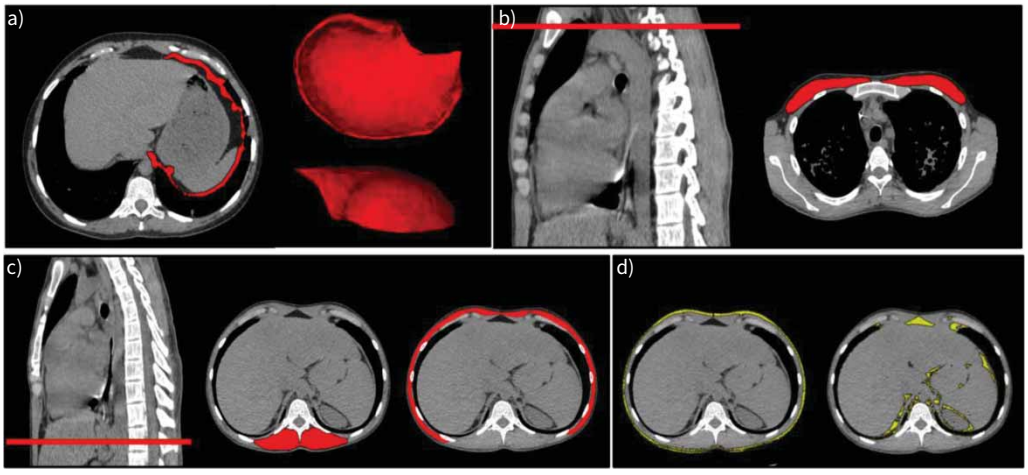

FIGURE 1 Representative computed tomography (CT) images of skeletal muscles and adipose tissue. Red and yellow shading indicate the CT image segmentation of skeletal muscle and adipose tissues, respectively. a) Single axial (left) and 3-dimensional reconstruction (right) images of the left hemidiaphragm. b) Aortic arch level on the sagittal view (left) and corresponding axial image of the pectoralis muscles (right). c) L1 vertebra level on the sagittal view (left) and corresponding axial images of the lumbar paraspinal (centre) and abdominal (right) muscles. d) Axial images of subcutaneous (left) and visceral (right) adipose tissue at the L1 level.

use and airway colonisation with *P. aeruginosa* were analysed as categorical variables. The above analyses were performed using R (version 2021.09.2 for Mac) or GraphPad Prism (version 9.3.1) software. Statistical significance was set at $p<0.05$ (two-sided) for all outcomes.

Results

Table 1 summarises anthropometric and clinical characteristics of the 70 participants included in the study. As expected, based on the epidemiology of the two diseases under study, the CF patients were younger and tended to have a lower proportion of males compared to the COPD subjects. Body weight and BMI were also lower in the CF patients in comparison to the control and COPD groups. The CF patients had lower values for FEV_1 and FVC (both absolute and % pred) than the COPD group but with a higher FEV_1/FVC ratio. Diabetes was present in 41%, prior systemic corticosteroid use in 54% and positive sputum cultures for *P. aeruginosa* in 57% of CF patients for whom this information was available (based on n=27, 24 and 28 CF patients, respectively). Furthermore, 41% of all CF patients were homozygous for the dF508 mutation in the *CFTR* gene.

TABLE 1 Anthropometric and clinical characteristics of the study subject groups

	Control	CF	COPD
Subjects n	20	29	21
Age years	54±6	34±14$^{\#}$	56±8¶
Male sex %	64	52	57
Height cm	169±8	165±8	169±9
Weight kg	78±15	61±16$^{\#}$	76±20¶
BMI kg·m^{-2}	27±5	22±5$^{\#}$	26±6¶
FEV_1 L	3.2±0.7	1.7±1.0$^{\#}$	2.5±0.8$^{\P,+}$
FEV_1 % pred	97±8	51±26$^{\#}$	77±18$^{\P,+}$
FVC L	4.3±0.9	2.5±1.1$^{\#}$	4.2±1.3
FVC % pred	102±9	65±23$^{\#}$	100±20¶
FEV_1/FVC ratio	74±4	67±14	59±7$^{\P,+}$

Participant characteristics are presented as mean±SD unless otherwise indicated. Control: normal spirometry control group; CF: cystic fibrosis group; COPD: chronic obstructive pulmonary disease group; BMI: body mass index; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity. p-values were determined by ANOVA with adjustment for multiple comparisons. $^{\#}$: $p<0.05$ for comparisons of control with CF; ¶: $p<0.05$ for comparisons of CF with COPD; $^{+}$: $p<0.05$ for comparisons of control with COPD.

Indices of skeletal muscle and adipose tissue quantity

Figure 1 shows representative skeletal muscle and adipose tissue CT images, while figure 2 depicts skeletal muscle and adipose tissue quantity measurements normalised to stature (SMI and ATI, respectively). After adjusting for age and sex, diaphragm (figure 2a) volume was significantly greater in CF patients compared to the control and COPD groups. Diaphragm dome height was reduced in CF (57±14 mm; p=0.02) compared to the control group (65±14 mm), and a similar trend was observed in COPD (60±10 mm). The abdominal muscle (figure 2b) cross-sectional area in CF did not differ from controls but was higher than in COPD. On the other hand, cross-sectional area for pectoralis muscle (figure 2c) was lower in CF relative to controls, and for lumbar muscle (figure 2d) there were no differences among the three groups. Total and visceral adipose tissue areas did not differ among the three groups of subjects, although subcutaneous adipose tissue area was lower in CF compared to COPD (figure 2e–g).

Relationships between BMI and absolute (non-normalised) muscle quantity measurements for all subjects are shown in figure 3. In CF patients, significant correlations between BMI and muscle quantity were found for diaphragm, abdominal muscles and lumbar muscles. In control subjects, BMI was only correlated with abdominal muscle area. In the COPD group, no significant relationships between BMI and muscle quantity were observed. Relationships between BMI and absolute (non-normalised) adipose tissue quantity measurements were also examined (supplementary figure S1). This revealed that in all three

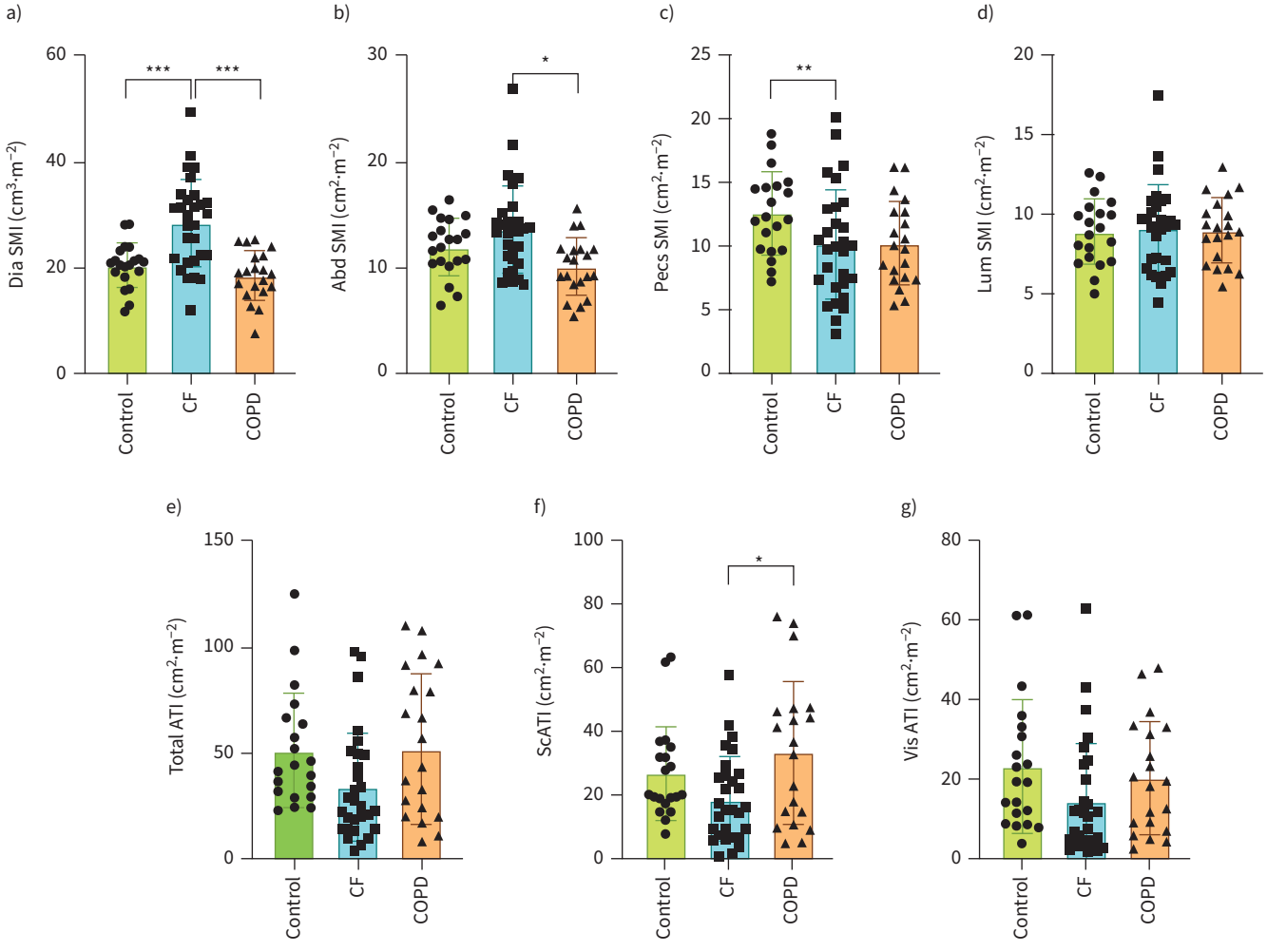

FIGURE 2 Age- and sex-adjusted comparisons of skeletal muscle and adipose tissue quantity indices. a–d) Skeletal muscle and e–g) adipose tissue quantity measurements normalised to height squared are shown. Control: normal spirometry control group; CF: cystic fibrosis group; COPD: chronic obstructive pulmonary disease group; Dia: diaphragm; Abd: abdominal; Pecs: pectoralis; Lum: lumbar paraspinal; SMI: skeletal muscle index; ATI: adipose tissue index; ScATI: subcutaneous adipose tissue index; VisATI: visceral adipose tissue index. Values are mean±SD. p-values were obtained from ANCOVA with adjustment for multiple comparisons. *: p<0.05; **: p<0.01; ***: p<0.001.

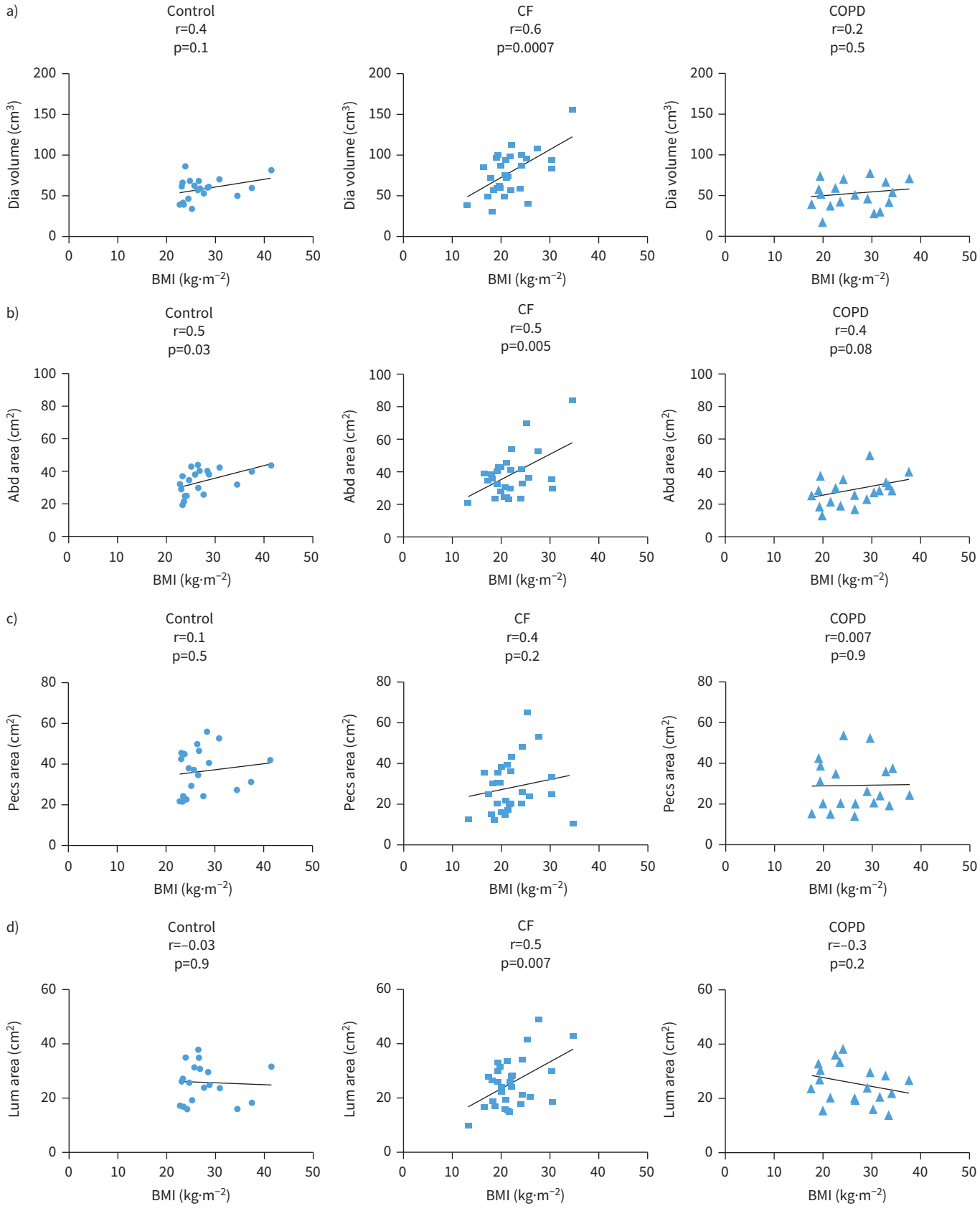

FIGURE 3 Scatter plots of relationships between body mass index (BMI) and skeletal muscle quantity indices. Data from all subjects for a) diaphragm (Dia), b) abdominal (Abd), c) pectoralis (Pecs) and d) lumbar (Lum) muscles are shown. Control: normal spirometry control; CF: cystic fibrosis; COPD: chronic obstructive pulmonary disease. p- and r-values were obtained from Pearson correlation.

subject groups, adipose tissue cross-sectional area values were highly correlated with BMI. Finally, in CF patients the FEV_1 % pred correlated with absolute abdominal (p=0.04) and lumbar (p=0.05) muscle area measurements, and a similar trend was found for diaphragm volume (p=0.07). In contrast, FEV_1 % pred did not correlate with skeletal muscle quantity measurements for any of the muscles in either the control or COPD groups.

Skeletal muscle radiodensity

The percentage of low radiodensity muscle (LDM –29 to +29 HU) was evaluated as a potential indicator of disease-associated alterations in skeletal muscle quality. We first compared the LDM% within each muscle across the three groups of subjects (figure 4a–d). After adjusting for age and sex, there were no differences in LDM% among subject groups for the respiratory muscles (diaphragm and abdominal). However, for the nonrespiratory muscles (pectoralis and lumbar) significantly higher LDM% values were observed in the COPD group compared to both control and CF subjects. To assess whether LDM% differs among the individual muscles, a comparison between the four muscles within each group of subjects was also performed (figure 4e–g). In control and CF subjects, a general pattern was observed in which the two

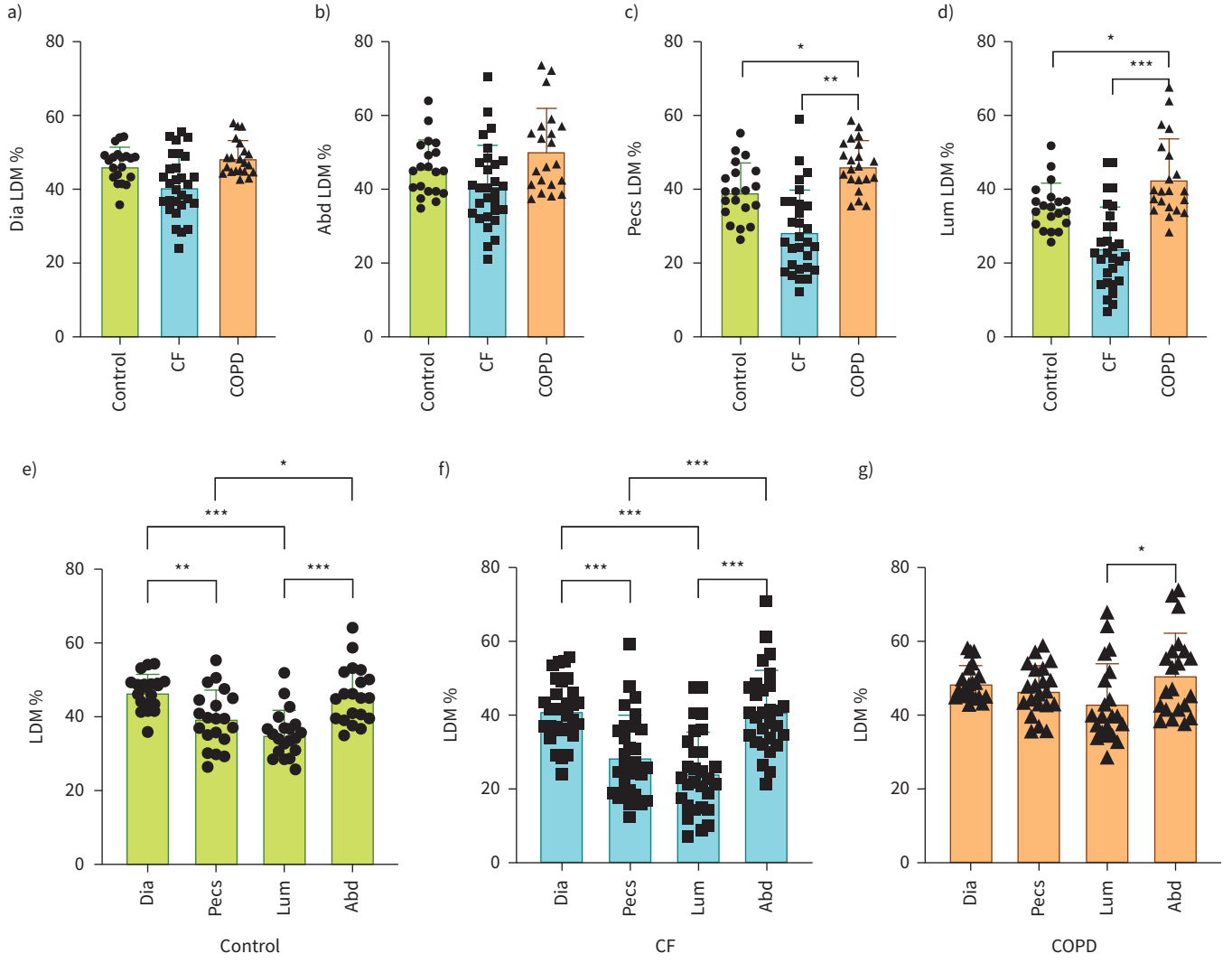

FIGURE 4 Low radiodensity skeletal muscle comparisons between and within study subject groups. Age- and sex-adjusted analyses were made for a–d) between-group comparisons of low radiodensity muscle percentage (LDM%) for the same skeletal muscle, and e–g) within-group comparisons of LDM% for the different skeletal muscles. Control: normal spirometry control group; CF: cystic fibrosis group; COPD: chronic obstructive pulmonary disease group; Dia: diaphragm; Abd: abdominal; Pecs: pectoralis; Lum: lumbar paraspinal. Values are means±SD. p-values in a–d and e–g were obtained from ANCOVA and ANOVA, respectively, with adjustment for multiple comparisons. *: p<0.05; **: p<0.01; ***: p<0.001.

respiratory muscles exhibited higher LDM% than the two nonrespiratory muscles. However, in COPD the LDM% was more uniform across muscle groups, and the only observed difference was between abdominal and lumbar muscles.

Relationships between BMI and LDM% for all subjects are shown in figure 5. In CF patients, greater BMI was positively associated with higher LDM% in both diaphragm and pectoralis. In control subjects, there was also a positive correlation between BMI and LDM% in pectoralis muscle. In COPD subjects, however, no significant relationships between BMI and LDM% were observed except in abdominal muscle where greater BMI was negatively correlated with LDM%. With respect to pulmonary function, FEV_1 % pred did not correlate with LDM% for any of the muscles in the control, CF or COPD groups.

Sensitivity analysis (age- and sex-matching)

Because the CF cohort was younger and contained a lower proportion of males, we further validated the above findings by performing a subgroup sensitivity analysis in which nine CF patients underwent ∼1:2 matching with the other groups (14 control and 18 COPD subjects) based on age (within 5 years) and sex. After this matching, anthropometric characteristics did not differ between the three subgroups while spirometry measures remained more reduced in CF patients (supplementary table S1).

Overall, the results of the sensitivity analysis were in line with the age- and sex-adjusted comparisons for the entire study population (figure 6). In particular, diaphragm volume remained significantly elevated in the CF group compared to control (p=0.003) and COPD (p=0.00007) subjects. Muscle radiodensity values for diaphragm and abdominal muscle did not differ among the three groups, but higher LDM% was observed in nonrespiratory muscles of COPD subjects compared to both control (p=0.03 for lumbar muscle) and CF (p=0.02 and 0.004 for pectoralis and lumbar muscles, respectively). Adipose tissue quantity values did not differ between the three groups in the sensitivity analysis (supplementary figure S2).

BMI and FEV_1 as predictors of skeletal muscle quantity and quality

Multivariable linear regression was next employed to further compare the three groups of subjects with respect to the independent effects of BMI and FEV_1 % pred on muscle parameters. After adjusting for age, sex and height, the effect of BMI as a predictor of diaphragm volume was significantly greater in CF patients than in control (p=0.03) or COPD (p=0.003) subjects (supplementary table S2). The positive relationship of BMI to abdominal and lumbar muscle area was also greater in CF compared to controls and/or COPD. Higher FEV_1 % pred was associated with greater diaphragm volume in CF (p=0.049), but the relationship of FEV_1 % pred with diaphragm volume did not differ between the three groups. The FEV_1 % pred was positively associated with abdominal muscle quantity in CF (p=0.005) and differed from COPD (p=0.02). The independent effects of BMI and FEV_1 % pred as predictors of skeletal muscle quality (LDM%) were similarly assessed (supplementary table S3). BMI was positively associated with LDM% of diaphragm in CF (p=0.03) and differed significantly from COPD (p=0.02). The FEV_1 % pred was not significantly associated with LDM% for any of the muscles in control, CF or COPD subjects.

Associations with blood, microbiological and genetic biomarkers in the CF cohort

Supplementary table S4 presents data for the blood biomarkers which were clinically available in the majority of CF patients. After adjusting for age and sex, inflammation biomarkers (leukocyte count and C-reactive protein) did not show relationships with muscle quantity or radiodensity. Independent of age, sex and height, higher creatinine was associated with greater muscle quantity in diaphragm, abdominal and lumbar muscles (diaphragm: β=0.2, 95% CI 0.006–0.4, p=0.04; abdominal: β=0.4, 95% CI 0.1–0.7, p=0.006; lumbar: β=0.2, 95% CI 0.1–0.4, p=0.005) as well as greater adiposity (subcutaneous: β=1.2, 95% CI 0.3–2.2, p=0.01; visceral: β=1.1, 95% CI 0.2–2.0, p=0.02). In addition, higher haemoglobin was associated with greater pectoralis muscle quantity (β=0.5, 95% CI 0.06–0.83, p=0.02). Abnormally elevated haemoglobin A1C did not predict muscle quantity but was associated with higher LDM% in diaphragm and pectoralis (diaphragm: β=11.7, 95% CI 5.3–18.0, p=0.001; pectoralis: β=13.9, 95% CI 4.4–23.5, p=0.006). None of the other nutritional biomarkers showed significant relationships to muscle radiodensity. Use of systemic corticosteroids was not associated with muscle measurements but correlated with the presence of diabetes (r=0.44, 95% CI 0.04–0.71, p=0.02). CFTR genotype was not a predictor of muscle quantity or LDM% after adjusting for age, sex and height. Colonisation with *P. aeruginosa* similarly had no association with skeletal muscle parameters.

Discussion

The main findings of our investigation, which remained robust after accounting for age, sex and anthropometric measures, can be briefly summarised as follows: 1) CF patients had significantly greater diaphragm volume than either control or COPD subjects, and abdominal muscle cross-sectional area was

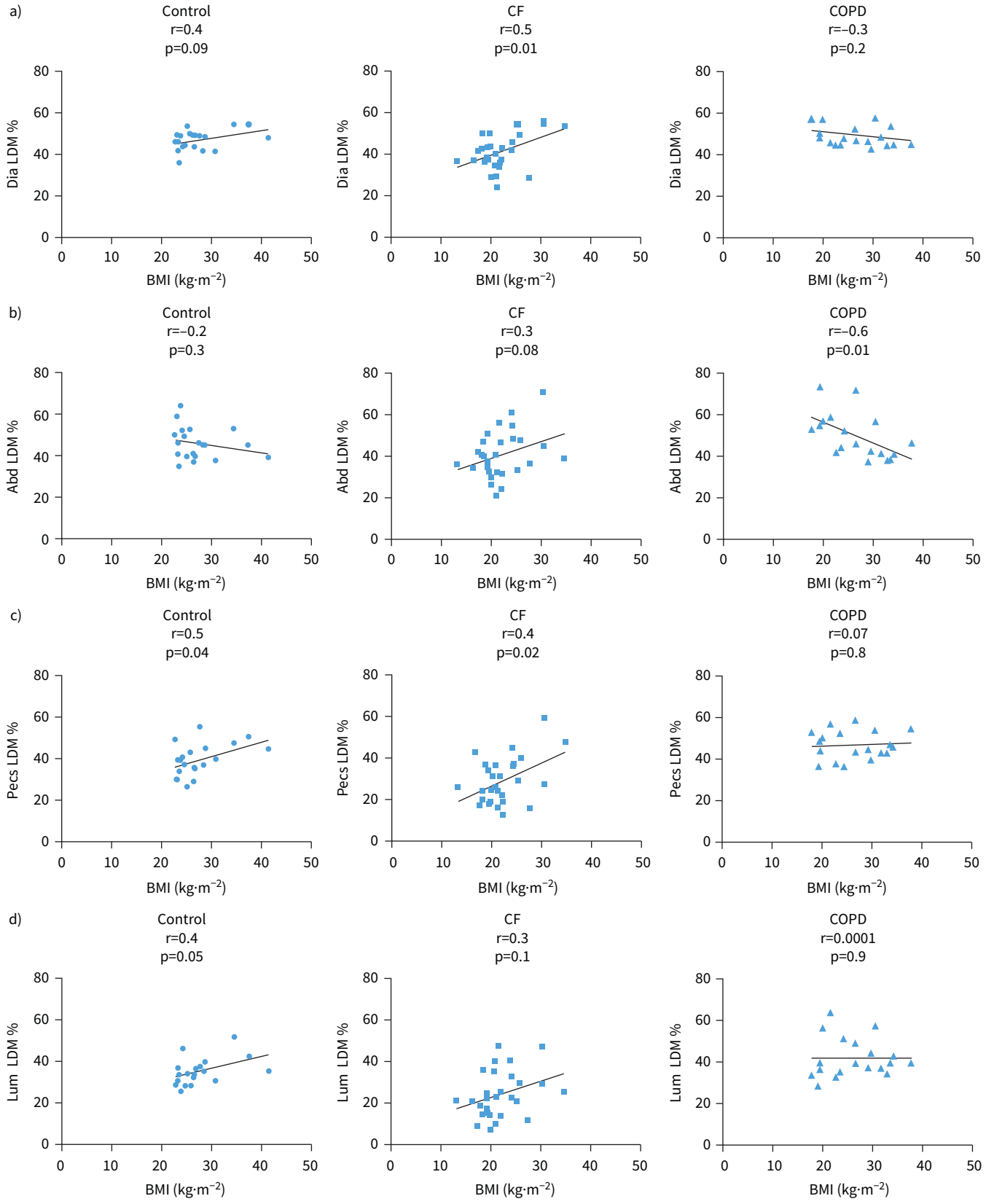

FIGURE 5 Scatter plots of relationships between body mass index (BMI) and the percentage of low radiodensity muscle (LDM%). Data from all subjects for a) diaphragm (Dia), b) abdominal (Abd), c) pectoralis (Pecs) and d) lumbar (Lum) muscles are shown. Control: normal spirometry control; CF: cystic fibrosis; COPD: chronic obstructive pulmonary disease. p- and r-values were obtained from Pearson correlation.

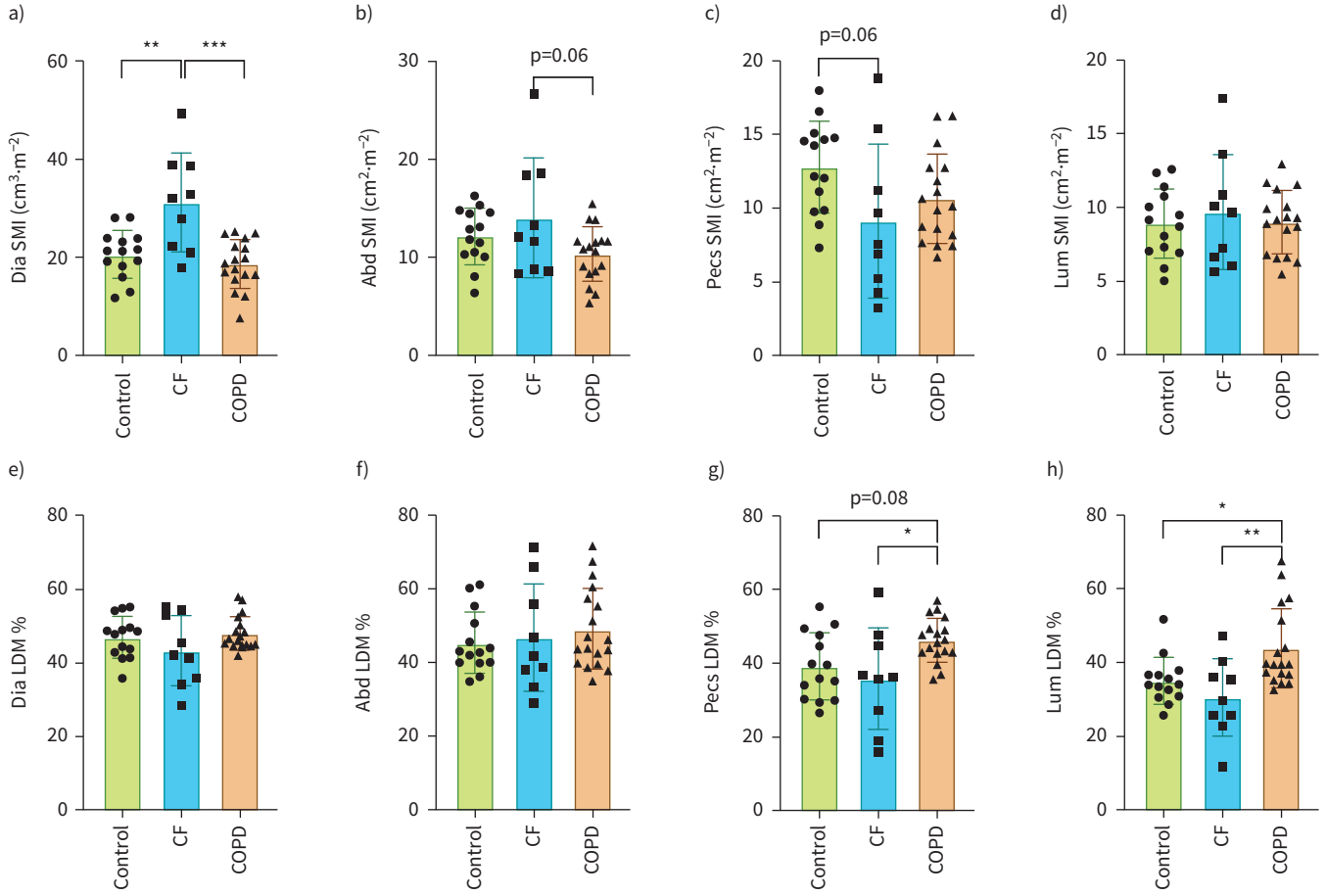

FIGURE 6 Sensitivity analysis comparing age- and sex-matched subjects from each group. a–d) Skeletal muscle quantity and e–h) low radiodensity muscle percentage (LDM%) from the three subgroups of subjects are shown. Control: normal spirometry control group; CF: cystic fibrosis group; COPD: chronic obstructive pulmonary disease group; Dia: diaphragm; Abd: abdominal; Pecs: pectoralis; Lum: lumbar paraspinal; SMI: skeletal muscle index. Values are means±SD. p-values were obtained from ANOVA with adjustment for multiple comparisons. *: p<0.05; **: p<0.01; ***: p<0.001.

also higher in CF compared to COPD; 2) in contrast, the cross-sectional area of nonrespiratory muscles was either lower in CF than in controls (pectoralis) or did not differ among the three groups (lumbar); 3) the LDM% of diaphragm and abdominal muscles did not differ among the three study subject groups; 4) in contrast, the LDM% of nonrespiratory muscles (pectoralis and lumbar) was significantly greater in COPD than in controls or CF. These data collectively suggest that thoracic CT imaging can reveal distinctive skeletal muscle remodelling signatures (summarised in supplementary table S5). These signatures differed according to both the type of muscle (respiratory *versus* nonrespiratory) and the nature of the underlying obstructive lung disease (CF *versus* COPD).

We used 3-dimensional reconstruction from contiguous axial CT images to measure diaphragm volume, as recently described in patients with sepsis [40] and in COPD [31]. As a method for determining overall diaphragm dimensions, CT has several advantages over the more commonly used technique of ultrasound [41]. Firstly, muscle thickness is affected by the degree of muscle relaxation and absolute muscle length, both of which require complex methods to be accurately controlled for when assessing the human diaphragm *in vivo*. Muscle volume, on the other hand, should remain constant irrespective of changes in muscle relaxation or length [42]. Secondly, the known regional variations in diaphragm dimensions [43] can be captured by CT whereas ultrasound can only evaluate a limited portion of the muscle [41]. Thirdly, CT image acquisition is less operator-dependent than ultrasound and high levels of interobserver agreement for axial image volume, thickness and dome height measurements of the diaphragm have been reported, with intraclass correlation coefficients ranging from 0.84 to 0.96 [31, 44], which is similar to the values obtained in the present investigation.

In interpreting our results, certain technical issues should be considered. The thoracic CT images in our study were obtained at suspended maximal inspiration, which entails contraction and shortening of the diaphragm that maximises its thickness. Airway wall thickness or diameter changes in the same range as diaphragm thickness alterations can be readily detected on thoracic CT images [45]. Thoracic CT has also been shown to accurately identify hemidiaphragm atrophy (reduced thickness) associated with fluoroscopically confirmed unilateral paralysis of the muscle [46]. One limitation of thoracic CT is that the images cannot easily discriminate between the pleural and muscle tissue components of the diaphragm. In our study, signs of pleural thickening were found in only three CF patients, and this was limited to the apical regions of the thorax. A further caveat is that to our knowledge there has not been an autopsy study performed to externally validate the relationship between diaphragm volume or diaphragm lipid content and CT-determined measurements.

At the tissue level, biopsies performed in both healthy volunteers and cancer patients have validated the fact that lower CT radiodensity of skeletal muscle is associated with greater triglyceride content as measured biochemically [24, 25], and this has often been considered an indicator of "poor quality" muscle. In principle, it is possible that other conditions such as increased intramuscular oedema could also affect CT radiodensity values. Although low radiodensity skeletal muscle has been defined in the range of 0 to +29 HU or −29 to +29 HU in most studies [47], there is no clearly established threshold for defining pathology. Furthermore, in our study the respiratory muscles had higher LDM% than nonrespiratory muscles in all subject groups. This suggests that absolute LDM% values are muscle-specific and could reflect their distinct metabolic properties and physiological roles. For all of these reasons, caution must be applied in inferring the presence of pathology based on muscle radiodensity values.

Nevertheless, many studies have reported that CT-derived estimations of reduced skeletal muscle mass and/or low skeletal muscle radiodensity are associated with adverse clinical outcomes [22, 23, 32–35, 48, 49]. Much of this literature has been based on muscle images acquired at the third lumbar vertebra (L3) level [32]. Because the L3 level was not consistently present on thoracic CT images, we employed the L1 level, which has also been shown to have significant prognostic value [36, 37]. In a similar fashion, low radiodensity of the pectoralis muscle was previously observed in COPD and linked to more severe pulmonary function impairment [48, 49]. We previously reported that COPD patients with severe disease (GOLD grade 4: FEV_1 <30% pred) had higher LDM values in the diaphragm than mild COPD subjects (GOLD grade 1: FEV_1 ⩾80% pred) [31]. The COPD subjects in the present study were at the mild-to-moderate end of the disease spectrum, and in these individuals the LDM% of diaphragm and abdominal muscles did not differ from control or CF subjects. On the other hand, the LDM% of nonrespiratory muscles (pectoralis and lumbar) in our COPD subjects was higher than in the control and CF groups. The latter finding is consistent with previous studies in which low radiodensity of these nonrespiratory muscles was reported in COPD [31, 48]. The results of the present study are also consistent with previous necropsy data [12], as well as older generation CT imaging [13] and ultrasound studies [14], which collectively suggest that overall diaphragm size is unaltered in COPD patients relative to normal subjects matched for age, sex, height and weight. In contrast, after adjustment for these factors we found that CT-determined diaphragm volume in CF patients remained significantly elevated compared to both control and COPD subjects. However, in opposition to our initial hypothesis, we did not observe any significant relationships between skeletal muscle CT parameters and biomarkers of inflammation and infection, or the prior use of corticosteroids in CF patients.

There are conflicting data in the literature regarding the effects of CF on respiratory muscle function [19]. For example, studies have reported that the pressure-generating capacity of inspiratory (including but not limited to the diaphragm) and/or expiratory muscles is either reduced [50–54], normal [55–58] or above normal values [59–61] in clinically stable CF patients. Among the small number of studies that have specifically evaluated the diaphragm in CF patients, Pradal *et al.* [50] found that maximal transdiaphragmatic pressure decreased with greater airflow obstruction and especially with reductions in the body weight/height ratio. Two other studies employed twitch stimulation of the phrenic nerves as a non-volitional test of diaphragm function and came to similar conclusions [62, 63]. Pinet *et al.* [63] attempted to indirectly estimate diaphragm mass by combining measurements of its thickness in a single region (the zone of apposition) by ultrasound and the overall diaphragm surface area by thoracic CT. These investigators reported that absolute diaphragm muscle bulk did not differ between CF and age/sex-matched control subjects. However, for a given lean body mass level, diaphragm and abdominal muscle bulk measurements were both higher in CF patients and also better preserved than in the limb muscle (quadriceps) of the same patients [63].

Several limitations of our study design should be acknowledged. First, we used a convenience sample of CF patients from our clinic population who had undergone CT imaging for various reasons and may be biased towards more complex disease, which could affect the generalizability of our observations. Second, the use of a convenience sample prevented us from obtaining certain information of interest which was not part of the routine clinical evaluation, such as lean body mass, tests of muscle strength and more detailed pulmonary function tests. Third, our sample size was modest due to the requirement for non-contrast thoracic CT imaging, and the CF sample was also younger than the other cohorts. To address the latter issue, we adjusted for age in the entire study population and also performed a separate sensitivity analysis using a subgroup of age-matched CF, COPD and control subjects. Importantly, the sensitivity analysis led to the same general conclusions as obtained for the overall study population. Finally, the CF patients had a lower FEV_1 % pred value than the COPD group, and hence it is possible that different results would be obtained in COPD patients with more severe disease. However, we believe this is unlikely since we did not find significant differences in diaphragm volume between patients with mild and severe COPD in a previous study [31]. In addition, in multivariate regression analyses diaphragm volume in COPD was not associated with FEV_1 % pred in either our previous study [31] or the current investigation.

The results of our study provide new insights into the diverse nature of skeletal muscle changes associated with chronic respiratory diseases. Mechanistically, our findings also raise the intriguing question of why fundamental differences in diaphragm remodelling should exist between CF and COPD. One possibility is that differences in the mechanical properties of the respiratory system in CF and COPD, such as higher airway resistance caused by mucus impaction in CF, or different levels of hyperinflation which is linked to sarcomere loss in COPD [17, 18], could be playing a role. In addition, we propose that a plausible hypothesis lies with the paediatric onset of the disease in CF. In contrast to COPD, the respiratory muscles of CF patients are faced with an increased workload from early in life, and airway resistance rises steeply from ~5 years of age to adolescence in CF [64]. Skeletal muscle plasticity and growth responses are greater during youth for both animals and humans, due to a combination of local muscle cell factors and circulating systemic mediators [65–68]. If the above hypothesis is correct, this could have implications for childhood respiratory diseases beyond CF, and one would predict greater hypertrophic remodelling of the diaphragm after respiratory muscle training in paediatric *versus* adult patients. Finally, it will be of interest to determine whether CT-determined diaphragm volume can serve as a biomarker or prognostic indicator in CF patients which can be used to assess various therapies such as CFTR-modulating drugs. Additional studies will be required to further explore these questions.

Provenance: Submitted article, peer reviewed.

Acknowledgements: We thank Jonathan Afilalo (Department of Medicine, Cardiology Division, McGill University) for helpful advice on the methodology of this study.

Conflict of interest: B.J. Petrof is an associate editor of this journal. The remaining authors have nothing to disclose.

Support statement: This study was supported by Cystic Fibrosis Canada grant 3308. Funding information for this article has been deposited with the Crossref Funder Registry.

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