Fibrinogen does not relate to cardiovascular or muscle manifestations in chronic obstructive pulmonary disease (COPD): cross-sectional data from the ERICA study

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Online Supplement

Methods

Study Design

ERICA is a longitudinal, observational study with phenotypic assessments being conducted at baseline, and follow-up via 6-monthly questionnaires for a period of 3 years. The data presented here is cross-sectional data from the baseline assessments.

The study was powered on the basis of a tertile analysis of aPWV and QMVC. Assuming an average aPWV of 10 (standard deviation (SD) 1.0m/s) and a minimal clinically relevant difference of 0.4m/s, 230 patients per tertile provide 90% power at $p<0.01$ to detect this difference between the top and bottom quartiles. Assuming an average QMVC of 32 (SD 8kg, 220 patients per tertile will provide 90% power at a significance of $p <0.01$ in order to detect the minimum clinical difference of 3kg between the top and bottom tertiles. Allowing for a 10% dropout rate and incomplete datasets, approximately 800 patients were required.

Study Assessments

We measured demographics, height, weight, fat-free mass (single-frequency (50kHz) bioelectrical impedance analysis via TANITA BC 418MA (Tanita Corporation, Tokyo, Japan), post-bronchodilator spirometry, health-related quality of life questionnaires (Medical Research Council dyspnoea score, St George's Respiratory Questionnaire and COPD Assessment Test), peripheral blood pressure and Short Physical Performance Battery (SPPB).

SPPB is A composite assessment of lower extremity function comprising standing balance, 4 meter gait speed and sitting-to-standing speed, with a maximum possible total score of 12 (1). Lower scores indicate increasing frailty. Six minute walk distance was measured in accordance with the guidelines of the American Thoracic Society (2) except that a practice walk was not performed due to time constraints. Subjects fasted for >4 hours and refrained from medications for >6 hours prior to aPWV measurements and venepuncture, the latter being used for plasma fibrinogen, white cell count (WCC) and high sensitivity C-reactive protein (hsCRP) measurements.

Fibrinogen

Whole blood was collected into a vacutainer tube (sodium citrate as the anticoagulant), and plasma fibrinogen was measured in fresh plasma samples using an automated, modified Clauss method (HemosIL Fibrinogen-C XL, Instrumentation Laboratories(3)). The assay method is a direct measurement of functional fibrinogen and is the method most commonly used in clinical laboratories.

Aortic Pulse Wave Velocity (aPWV)

Following 10 minutes of supine rest, brachial blood pressure was measured three times, and an average of the final readings used for analysis. APWV was measured via the SphygmoCor device (AtCor, West Ryde, Australia), between the carotid and femoral arteries, using a piezoelectric tonometer placed over the artery and ECG gating, as previously described in detail (4).

Quadriceps Maximal Volitional Contraction

QMVC force was measured using the technique of Edwards *et al (5)* and expressed as a percentage of predicted values using the equations developed by Seymour and co-workers (6). Patients were verbally encouraged to make a maximal contraction by pushing against an inextensible strap placed above the ankle. The manoeuvre was repeated six times with a minimum 20 second interval between efforts. We used the highest value of contraction which could be sustained for 1 second for analysis.

Exacerbations and Medications

For this study, we have defined exacerbations as self-reported increase in COPD symptoms that required treatment with antibiotics and/ or steroids and severe exacerbations as those that require hospital admission.

There are no prohibited medications in the study. All subjects continued their routine prescribed medications throughout the study and the patient's physician may offer treatments (e.g. medication change, rehabilitation) in line with the patients' needs.

Statistical analysis

Linear regression analyses were used to estimate the differences between the mean in the overall study population and the mean in each group, for continuous outcome variables. To compare the results of binary outcome variables across the phenotypic groups, logistic regression was used to estimate odds ratios. To compare the MRC score across the

phenotypic groups, proportional odds logistic regression (POLR) was used to estimate odds ratios. When estimating odds ratios, the group with neither muscle weakness nor high cardiovascular risk was taken as the baseline group. Correction was not made for multiple comparisons.

Multiple linear regression analyses were conducted to estimate the dependence of QMVC and aPWV on each inflammatory biomarker, with adjustment for known or expected confounders. Non-normally distributed variables were log transformed prior to analysis. The full study population was included in the main analysis.

Sensitivity analyses were carried out that excluded subjects with simultaneously elevated hsCRP (>20 mg/L) and WCC (>11.0 x 10^9/L), to exclude the possibility that data were skewed by participants with subclinical exacerbations.

Results

eTable 1 Variables with missing data

Variable	Number of subjects with missing data
Smoking history	11
Height	6
Weight	12
Body mass index	12
Fat free mass	35
Forced expiratory volumes in 1 second	4
Forced vital capacity	4
Quadriceps maximal volitional contraction	32
Short physical performance battery	12
6 minute walk distance	36
Supine heart rate	56
Supine mean arterial pressure	15
Pulse wave velocity	65
Fibrinogen	13
High sensitivity-C-Reactive Protein	20
White cell count	15
Medical Research Council dyspnoea score	10
COPD Assessment Test score	10
SGRQ total score	24
Exacerbation history	104
Diabetes history	8
Antihypertensive therapy	11
Cholesterol lowering therapy	15
Previous history of myocardial infarction	10

eTable 1: Number of subjects with missing data per variable in total ERICA cohort (n = 729)

eTable 2 Aortic pulse wave velocity and fibrinogen (n=635)

Variable	Estimate	Standard error	P value	95% Confidence Interval
Log fibrinogen	0.18	0.37	0.64	-0.54, 0.90
Age (years)	0.13	0.01	<0.0001	0.11, 0.16
Gender	0.45	0.20	0.028	0.06, 0.86
Supine heart rate	0.05	0.01	<0.0001	0.17, 1.56
Supine mean arterial pressure	0.06	0.01	<0.0001	-0.14, 1.00
Current smoker	-0.19	0.20	0.33	-1.63, -0.22
Smoking pack year history	0.001	0.003	0.65	-0.03, 1.58
FEV_1 (L)	-0.13	0.20	0.52	0.03, 0.06
Diabetes	0.90	0.29	<0.01	0.04, 0.08
Previous heart attack	-0.003	0.34	0.99	-0.56, 0.21
Anti-hypertensive therapy	-0.02	0.20	0.92	-0.005, 0.007
Cholesterol lowering therapy	0.31	0.22	0.14	0.38, 1.52
Site 2 vs site 1	0.86	0.35	0.03	-0.61, 0.75
Site 3 vs site 1	0.46	0.29	0.12	-0.50, 0.26
Site 4 vs site 1	-0.92	0.36	0.01	-0.40, 0.38
Site 5 vs site 1	0.77	0.41	0.06	-0.16, 0.67

eTable 2: Linear regression analysis for associations with cardiovascular manifestation, as measured by aortic pulse wave velocity, in subjects with COPD (n=635). Age, gender, supine heart rate, supine mean arterial pressure and the presence of diabetes are significantly associated with aortic pulse wave velocity ($p < 0.0001$, $p = 0.028$, $p<0.0001$, $p<0.0001$ and $p<0.01$ respectively), but fibrinogen is not associated with aortic pulse wave velocity ($p = 0.64$). Site 1 is set as the index, comparator site for remaining sites.

eTable 3 Quadriceps maximal volitional contraction and fibrinogen (n=656)

Variable	Estimate	Standard error	P value	95% Confidence Interval
Log fibrinogen	-0.33	1.47	0.82	-3.41, 2.24
Age (years)	-0.17	0.05	<0.001	-0.26, -0.08
Gender	6.4	1.05	<0.0001	4.20, 8.30
Current smoker	-0.17	0.78	0.79	-3.77, 1.71
Fat free mass	0.37	0.05	<0.0001	-4.93, -0.34
Height	-0.12	0.06	0.061	-2.66, 2.82
FEV_1	4.7	0.83	<0.0001	-3.45, 2.87
Site 2 vs site 1	-1.14	1.4	0.42	-1.73, 1.31
Site 3 vs site 1	-2.68	1.17	0.023	0.27, 0.48
Site 4 vs site 1	0.07	1.40	0.96	-0.25, 0.01
Site 5 vs site 1	-0.32	1.61	0.84	3.09, 6.31

eTable 3: Linear regression analysis for determinants of quadriceps maximal volitional contraction in subjects with COPD (n=656). Age, gender, fat-free mass and FEV_1 emerge as significantly associated with quadriceps weakness ($p < 0.001$, $p < 0.0001$, $p < 0.0001$ and $p<0.0001$ respectively), with fibrinogen not emerging as a significant association ($p=0.82$). Site 1 is set as the index, comparator site for remaining sites.

eTable 4 Pulse wave velocity and hsCRP (n = 631)

Variable	Estimate	Standard error	P value
Log hsCRP	0.08	0.08	0.31
Age (years)	0.14	0.01	<0.0001
Gender	0.46	0.20	0.03
Supine heart rate	0.05	0.01	<0.0001
Supine mean arterial pressure	0.06	0.01	<0.0001
Current smoker	-0.18	0.20	0.36
Smoking pack year history	0.001	0.003	0.78
FEV_1 (L)	-0.15	0.20	0.45
Diabetes	0.85	0.30	0.005
Previous heart attack	-0.01	0.35	0.99
Anti-hypertensive therapy	0.03	0.2	0.87
Cholesterol lowering therapy	0.33	0.22	0.13
Site 2	0.76	0.36	0.03
Site 3	0.35	0.29	0.22
Site 4	-0.98	0.37	0.01
Site 5	0.68	0.41	0.10

eTable 4: Linear regression analysis for determinants of arterial stiffness, as measured by pulse wave velocity, with hs-CRP as the marker of systemic inflammation. Site 1 is the index, comparator site for remaining sites.

eTable 5 Pulse wave velocity and white blood count (n=636)

Variable	Estimate	Standard error	P value
Log WCC	0.36	0.33	0.27
Age (years)	0.14	0.01	<0.0001
Gender	0.44	0.21	0.03
Supine heart rate	0.05	0.01	<0.0001
Supine mean arterial pressure	0.06	0.01	<0.0001
Current smoker	-0.17	0.20	0.41
Smoking pack year history	0.001	0.003	0.74
FEV_1 (L)	-0.16	0.20	0.41
Diabetes	0.86	0.29	0.002
Previous heart attack	-0.03	0.34	0.93
Anti-hypertensive therapy	-0.01	0.20	0.99
Cholesterol lowering therapy	0.36	0.22	0.09
Site 2	0.75	0.35	0.03
Site 3	0.35	0.29	0.22
Site 4	-1.00	0.36	0.01
Site 5	0.72	0.41	0.08

eTable 5: Linear regression analysis for determinants of arterial stiffness, as measured by pulse wave velocity, with white cell count as the marker of systemic inflammation. Site 1 is the index, comparator site for remaining sites.

eTable 6 Quadriceps maximal volitional contraction and hsCRP (n=648)

Variable	Estimate	Standard error	P value
Log hsCRP	-0.54	0.32	0.09
Age (years)	-0.19	0.05	<0.0001
Gender	6.50	1.04	<0.0001
Current smoker	0.02	0.77	0.98
Fat free mass	0.39	0.05	<0.0001
Height	-0.13	0.06	0.04
FEV_1	4.23	0.82	<0.0001
Site 2	-0.99	1.38	0.48
Site 3	-2.57	1.16	0.03
Site 4	0.28	1.39	0.84
Site 5	-0.24	1.59	0.88

eTable 6: Linear regression analysis for determinants of quadriceps weakness, as measured by quadriceps maximal volitional contraction, with hs-CRP as the marker of systemic inflammation. Site 1 is the index, comparator site for remaining sites.

eTable 7 Quadriceps maximal volitional contraction and white blood count (n=657)

Variable	Estimate	Standard error	P value
Log WCC	-0.99	1.28	0.44
Age (years)	-0.18	0.05	<0.001
Gender	6.52	1.05	<0.0001
Current smoker	-0.08	0.79	0.92
Fat free mass	0.37	0.06	<0.0001
Height	-0.13	0.06	0.05
FEV_1	4.67	0.82	<0.0001
Site 2	-1.14	1.39	0.97
Site 3	-2.71	1.16	0.02
Site 4	0.05	1.39	0.97
Site 5	-0.15	0.79	0.92

eTable 7: Linear regression analysis for determinants of quadriceps weakness, as measured by quadriceps maximal volitional contraction, with white cell count as the marker of systemic inflammation. Site 1 is the index, comparator site for remaining sites.

eTable 8 Pulse wave velocity and fibrinogen in sub-population (n=630)

Variable	Estimate	Standard error	P value
Log fibrinogen	0.32	0.38	0.39
Age (years)	0.14	0.01	<0.0001
Gender	0.44	0.20	0.03
Supine heart rate	0.05	0.01	<0.0001
Supine mean arterial pressure	0.06	0.01	<0.0001
Current smoker	-0.17	0.20	0.40
Smoking pack year history	0.002	0.003	0.62
FEV_1 (L)	-0.09	0.20	0.66
Diabetes	0.88	0.29	0.002
Previous heart attack	0.004	0.34	0.99
Anti-hypertensive therapy	-0.02	0.20	0.94
Cholesterol lowering therapy	0.29	0.22	0.19
Site 2	0.85	0.35	0.02
Site 3	0.44	0.29	0.13
Site 4	-0.85	0.36	0.02
Site 5	0.81	0.41	0.05

eTable 8: Linear regression analysis for determinants of arterial stiffness, as measured by pulse wave velocity, with fibrinogen as the marker of systemic inflammation in a sub-population where subjects with simultaneously elevated hs-CRP of >20 mg/L and WCC of >11 x 10^9/L were excluded from the analysis. Site 1 is the index, comparator site for remaining sites.

eTable 9 Pulse wave and hsCRP in sub-population (n=626)

Variable	Estimate	Standard error	P value
Log hsCRP	0.15	0.09	0.08
Age (years)	0.14	0.01	<0.0001
Gender	0.45	0.21	0.03
Supine heart rate	0.05	0.01	<0.0001
Supine mean arterial pressure	0.06	0.01	<0.0001
Current smoker	-0.15	0.20	0.46
Smoking pack year history	0.001	0.003	0.76
FEV_1 (L)	-0.12	0.20	0.56
Diabetes	0.82	0.30	0.007
Previous heart attack	-0.005	0.34	0.99
Anti-hypertensive therapy	-0.03	0.20	0.89
Cholesterol lowering therapy	0.30	0.22	0.16
Site 2	0.74	0.36	0.04
Site 3	0.34	0.29	0.25
Site 4	-0.90	0.37	0.02
Site 5	0.73	0.41	0.08

eTable 9: Linear regression analysis for determinants of arterial stiffness, as measured by pulse wave velocity, with hsCRP as the marker of systemic inflammation in a sub-population where subjects with simultaneously elevated hs-CRP of >20 mg/L and WCC of >11 x 10^9/L were excluded from the analysis. Site 1 is the index, comparator site for remaining sites.

eTable 10 Pulse wave velocity and white cell count in sub-population (n=631)

Variable	Estimate	Standard error	P value
Log WCC	0.52	0.34	0.12
Age (years)	0.14	0.01	<0.0001
Gender	0.43	0.21	0.04
Supine heart rate	0.05	0.01	<0.0001
Supine mean arterial pressure	0.06	0.01	<0.0001
Current smoker	-0.15	0.20	0.46
Smoking pack year history	0.001	0.003	0.70
FEV_1 (L)	-0.13	0.20	0.51
Diabetes	0.83	0.29	0.005
Previous heart attack	-0.02	0.34	0.96
Anti-hypertensive therapy	0.001	0.20	1.00
Cholesterol lowering therapy	0.33	0.22	0.12
Site 2	0.74	0.35	0.04
Site 3	0.35	0.29	0.23
Site 4	-0.92	0.36	0.01
Site 5	0.78	0.42	0.06

eTable 10: Linear regression analysis for determinants of arterial stiffness, as measured by pulse wave velocity, with white cell count as the marker of systemic inflammation in a sub-population where subjects with simultaneously elevated hs-CRP of >20 mg/L and WCC of >11 x 10^9/L were excluded from the analysis. Site 1 is the index, comparator site for remaining sites.

eTable 11 Quadriceps maximal volitional contraction and fibrinogen (n=648) in sub-population

Variable	Estimate	Standard error	P value
Log fibrinogen	-0.21	1.50	0.89
Age (years)	-0.18	0.05	<0.0001
Gender	6.48	1.06	<0.0001
Current smoker	-0.23	0.79	0.77
Fat free mass	0.37	0.06	<0.0001
Height	-0.13	0.06	0.05
FEV_1	4.60	0.83	<0.0001
Site 2	-1.23	1.41	0.38
Site 3	-2.77	1.18	0.02
Site 4	-0.03	1.42	0.98
Site 5	-0.36	1.64	0.83

eTable 11: Linear regression analysis for determinants of quadriceps weakness, as measured by quadriceps maximal volitional contraction, with fibrinogen as the marker of systemic inflammation in a sub-population where subjects with simultaneously elevated hs-CRP of >20 mg/L and WCC of >11 x 10^9/L were excluded from the analysis. Site 1 is the index, comparator site for remaining sites.

eTable 12 Quadriceps maximal volitional contraction and hsCRP (n=640) in sub-population

Variable	Estimate	Standard error	P value
Log hsCRP	-0.55	0.34	0.10
Age (years)	-0.19	0.05	<0.0001
Gender	6.56	1.05	<0.0001
Current smoker	-0.05	0.78	0.95
Fat free mass	0.39	0.06	<0.0001
Height	-0.14	0.06	0.04
FEV_1	4.14	0.83	<0.0001
Site 2	-1.05	1.39	0.45
Site 3	-2.63	1.17	0.02
Site 4	0.14	1.42	0.92
Site 5	-0.32	1.63	0.84

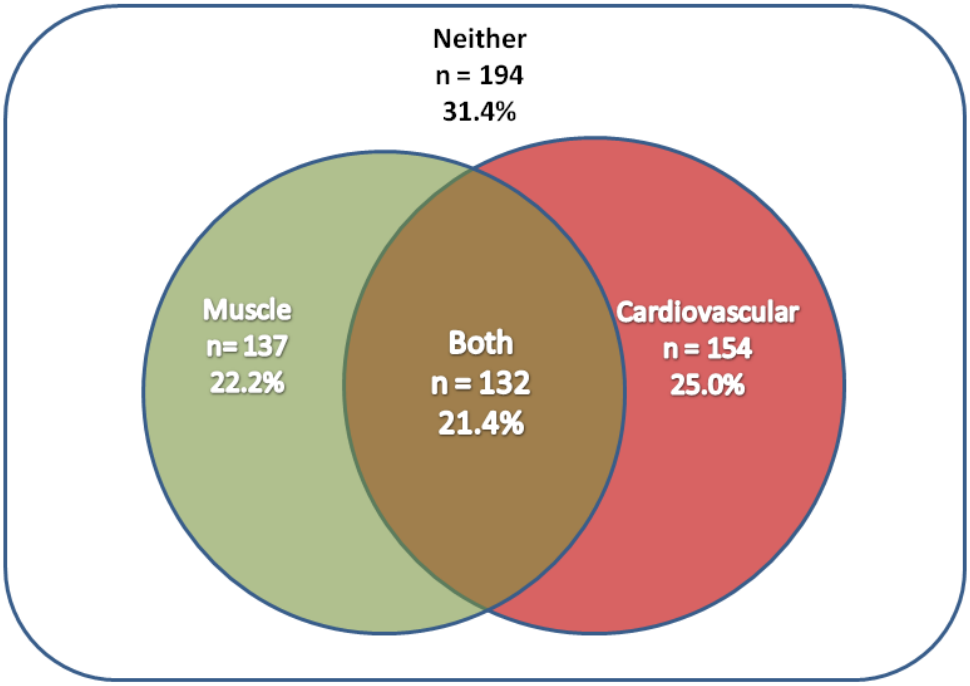
eTable 12: Linear regression analysis for determinants of quadriceps weakness, as measured by quadriceps maximal volitional contraction, with hsCRP as the marker of systemic inflammation in a sub-population where subjects with simultaneously elevated hs-CRP of >20 mg/L and WCC of >11 x 10^9/L were excluded from the analysis. Site 1 is the index, comparator site for remaining sites.

eTable 13 Quadriceps maximal volitional contraction and white cell count (n=649) in sub-population

Variable	Estimate	Standard error	P value
Log WCC	-0.80	1.33	0.55
Age (years)	-0.18	0.05	<0.0001
Gender	6.58	1.06	<0.0001
Current smoker	-0.16	0.80	0.84
Fat free mass	0.37	0.06	<0.0001
Height	-0.13	0.06	0.04
FEV_1	4.59	0.83	<0.0001
Site 2	-1.21	1.40	0.39
Site 3	-2.77	1.17	0.02
Site 4	-0.05	1.41	0.97
Site 5	-0.19	1.65	0.91

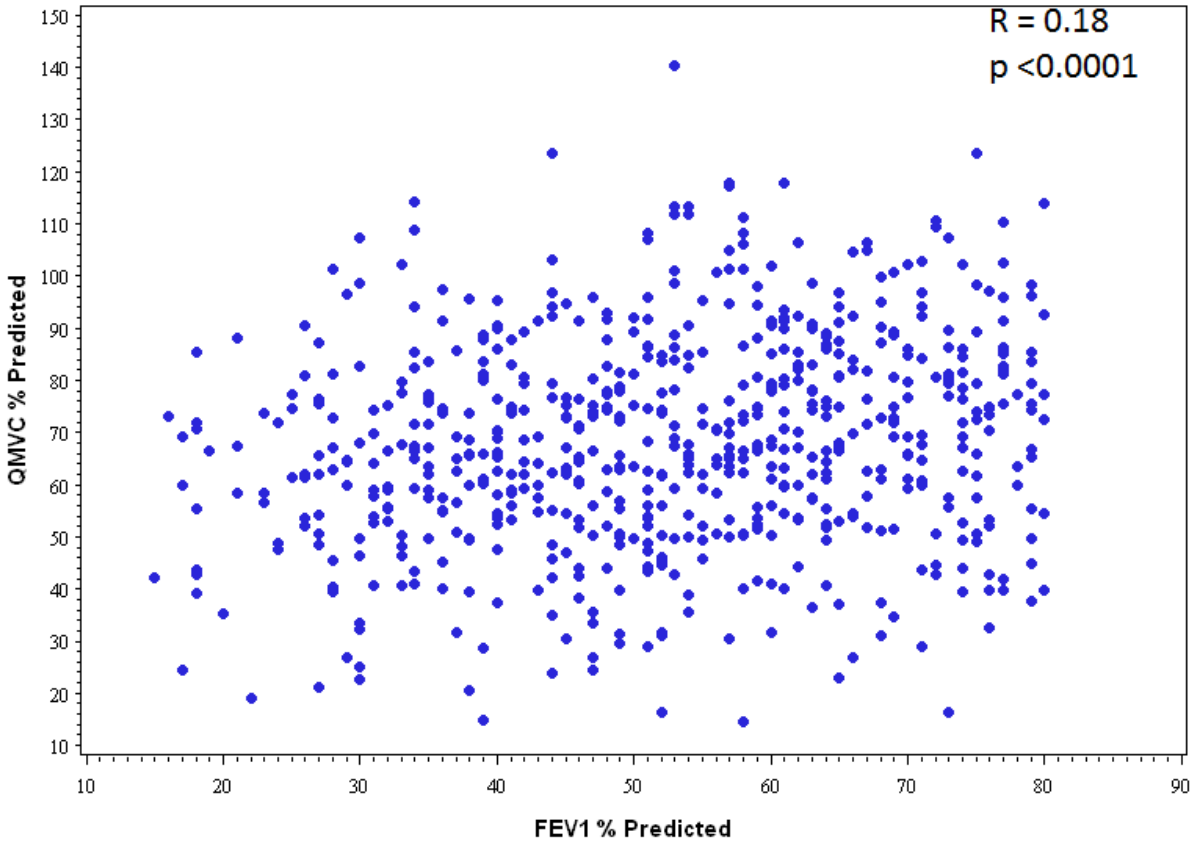
eTable 13: Linear regression analysis for determinants of quadriceps weakness, as measured by quadriceps maximal volitional contraction, with white cell count as the marker of systemic inflammation in a sub-population where subjects with simultaneously elevated hs-CRP of >20 mg/L and WCC of >11 x 10^9/L were excluded from the analysis. Site 1 is the index, comparator site for remaining sites.

eFigure 1 Distribution of cardiovascular and muscular manifestations in COPD



eFigure 1 Distribution of cardiovascular and muscular manifestations in COPD (total n=617). Group 'Neither' (n=194) represents subjects with neither elevated aortic pulse wave velocity nor skeletal muscle weakness. Group 'Muscle' (n=137) represents subjects with skeletal muscle weakness alone. Group 'Cardiovascular' (n=154) represents subjects with cardiovascular manifestation, defined as elevated aortic stiffness, alone, and group 'Both' (n=132) contains subjects demonstrating both skeletal muscle weakness and elevated aortic stiffness. Skeletal muscle weakness was defined according to predicted quadriceps maximal volitional contraction, based on equations developed by Seymour et al [2] and elevated arterial stiffness was defined as an aortic pulse wave velocity of >10 m/s.

eFigure 2 Relationship between quadriceps strength and airflow limitation in COPD subjects



eFigure2 Relationship between quadriceps strength and airflow limitation in COPD subjects (n = 694)

Scatterplot showing a weak but significant correlation between % predicted quadriceps maximal volitional contraction (QMVC % predicted) and % predicted forced expiratory volume in 1 second (FEV1 % Predicted) in COPD subjects in the ERICA cohort

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