Determinants of endothelial function in patients with COPD

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ABSTRACT   Chronic obstructive pulmonary disease (COPD) is associated with increased cardiovascular mortality. Endothelial dysfunction may underpin this association. This cross-sectional study aimed to determine the impact of airflow obstruction, systemic inflammation, oxidative stress, sympathetic activation, hypoxaemia and physical activity on endothelial function in COPD.

In stable COPD patients, assessments of endothelial function by flow-mediated dilatation (FMD), cardiovascular risk (Pocock score), airflow obstruction (forced expiratory volume in 1 s (FEV1)), systemic inflammation (high-sensitivity C-reactive protein and interleukin-6), oxidative stress (malondialdehyde), sympathetic activation (baroreflex sensitivity), hypoxaemia (arterial oxygen tension), hypercapnia (arterial carbon dioxide tension (P_{aCO_2})), physical activity (steps per day) and exercise capacity (6-min walking distance) were performed. Associations between FMD and potential determinants were assessed in univariate and multivariate analyses.

106 patients (Global Initiative for Chronic Obstructive Lung Disease stage I/II 35%, stage III 25% and stage IV 40%) were included. In multivariate analysis FEV1 was positively associated with FMD, independent of other significant FMD determinants from univariate analysis (sex, smoking, combined inhaled long-acting β-adrenergic and steroid medication, heart rate, baroreflex sensitivity and P_{aCO_2}) and adjusted for potential confounders (cardiovascular risk and age). In addition, the FMD and FEV1 association was modified by physical activity.

The findings of this study demonstrate that the severity of airflow obstruction is a significant determinant of endothelial function in patients with COPD. A high level of physical activity seems to have a favourable effect on this association.

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In COPD, the association between airflow obstruction and impaired endothelial function is modified by physical activity http://ow.ly/o84r9

Introduction

Cardiovascular disease plays an important role concerning morbidity and mortality in patients with chronic obstructive pulmonary disease (COPD) [1]. In prospective population-based studies, the degree of airflow limitation was an independent predictor of cardiovascular events, implying a causal relationship between airflow obstruction and cardiovascular disease [2, 3]. Although smoking is an established risk factor for both atherosclerosis and COPD, epidemiological studies have revealed that the increased cardiovascular risk in COPD patients seems to be independent of smoking habits [3, 4]. The mechanisms underlying the relationship between COPD and cardiovascular disease are currently unclear. Multiple causal factors leading to vessel wall damage and atherosclerotic plaques have been suggested, including hypoxia [5], concurrent systemic inflammation and oxidative stress [6, 7], sympathetic activation and physical inactivity [8].

Of these determinants, the level of physical activity seems to play a critical role. COPD patients who are regularly physically active seem to have a lower risk of both COPD-related hospital admissions and mortality [9]. Physical activity measured objectively by a multisensory armband has been shown to strongly predict all-cause mortality in COPD [10] and patients with a low level of physical activity were at higher risk for being readmitted to hospital [11]. Thus, physical inactivity is not only a disease manifestation of COPD, but also seems to play an important role in disease progression and the development of comorbidities [9]. Thus, we postulated *a priori* a modifying effect of physical activity on the relationship between COPD and endothelial function.

The impairment of endothelial function represents a potential pathophysiological link between COPD and cardiovascular disease. Endothelial function as assessed by flow-mediated dilatation (FMD) of the brachial artery has been shown to provide predictive information concerning the future occurrence of cardiovascular events [12, 13]. Therefore, noninvasive measures of endothelial function are of major interest, with the anticipation that patients at risk could be identified early in the absence of clinically apparent vascular disease [14, 15].

In the present study, our objective was to investigate potential determinants of endothelial function, including hypoxia, systemic inflammation, oxidative stress, sympathetic activation and physical activity in a cohort of patients with COPD.

Methods

Subjects

216 patients were screened for eligibility and recruited during ambulatory visits at Zurich University Hospital (Zurich, Switzerland). Consecutive patients aged 40–75 years with objectively confirmed COPD according to Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines [16] were included.

Patients were excluded if they had suffered from an exacerbation of COPD within the last 6 weeks, had a malignancy or a coexisting pulmonary or systemic inflammatory disease. Patients were also excluded if they were taking oral corticosteroids or if they suffered from mental or physical disability precluding informed consent or compliance with the protocol.

The study was conducted in accordance with the Declaration of Helsinki of the World Medical Association. The research ethics committee of the University Hospital of Zurich approved the study (EK-ZH-NR: 1734) and all subjects gave written informed consent to participate.

Study protocol

For ethical reasons neither inhaled nor systemic medication was witheld during the study. Patients were asked to abstain from alcohol, tobacco and caffeine on the day the measurements were performed. Use of inhaled short-acting β-agonists was discouraged before measurements of cardiovascular function were taken on the day of the study.

The studies were undertaken in a temperature-controlled room with the study subject in a resting supine position for ⩾10 min before measurements began. First, arterial blood pressure and baroreflex sensitivity were measured. Assessment of FMD was performed thereafter (after ⩾20–30 min of rest), followed by lung function testing.

Measurements

Assessment of endothelial function by FMD of the brachial artery

FMD measurements were performed by ultrasonography according to the method originally described by CELERMAJER *et al.* [14]. Longitudinal images of the brachial artery were obtained with a high-frequency (10.0 MHz) ultrasound scanning probe proximal to the antecubital fossa. Two-dimensional images,

acquired with ECG gating, were obtained at baseline using Doppler ultrasound imaging to assess arterial diameter and flow velocity. Reactive hyperaemia was then induced by the inflation of a pneumatic tourniquet around the forearm to 200 mmHg for 5 min, and repeated arterial diameter and flow velocity measurements were made at maximal dilatation 60 s after cuff deflation. To assess endothelial-independent vasodilatation, the maximal diameter of the brachial artery 3 min after a single sublingual dose of nitroglycerin (NTG) (0.5 mg) was measured. All measurements were stored digitally and analysed offline. Brachial artery diameter was measured automatically at the onset of the R wave using dedicated software (Vascular Research Tools 5; Medical Imaging Applications LLC, Coralville, IA, USA). The mean values of at least three cardiac cycles were averaged for each time point and results of endothelial-dependent (FMD) and endothelial-independent (NTG) vasodilatation were expressed as percentage change in arterial diameter from the baseline diameter.

Blood pressure, heart rate and baroreflex sensitivity

Blood pressure and heart rate were measured in triplicate separated by 1-min intervals after resting in a supine position for 5 min with a validated, semi-automated oscillometric device (Omron Healthcare, Kyoto, Japan). Baroreflex sensitivity is a measure of the capability to increase parasympathic tone and to decrease sympathetic activity in response to an increase in blood pressure. To assess baroreflex sensitivity, noninvasive continuous beat-to-beat finger blood pressure and heart rate were measured over 5 min in the supine position and recorded with a Finapres device (Finometer-Midi; Finapres Medical Systems, Amsterdam, the Netherlands). Baroreflex sensitivity was automatically quantified by analysis of the changes in pulse rate in response to changes in blood pressure with designated software (BeatScope, Version 1.1a; Finapres Medical Systems), as previously described [17].

Blood analysis

Measurements of high-sensitivity C-reactive protein (hsCRP), interleukin (IL)-6 and malondialdehyde were performed on plasma samples that were stored at -80°C. Particle-enhanced immunonephelometry was used to measure hsCRP. IL-6 and malondialdehyde were measured using ELISA kits (see the online supplementary material).

Assessment of cardiovascular risk

A cardiovascular risk score (Pocock score) was used to assess objectively each individual's 5-year risk of death due to cardiovascular events (see the online supplementary material) [18].
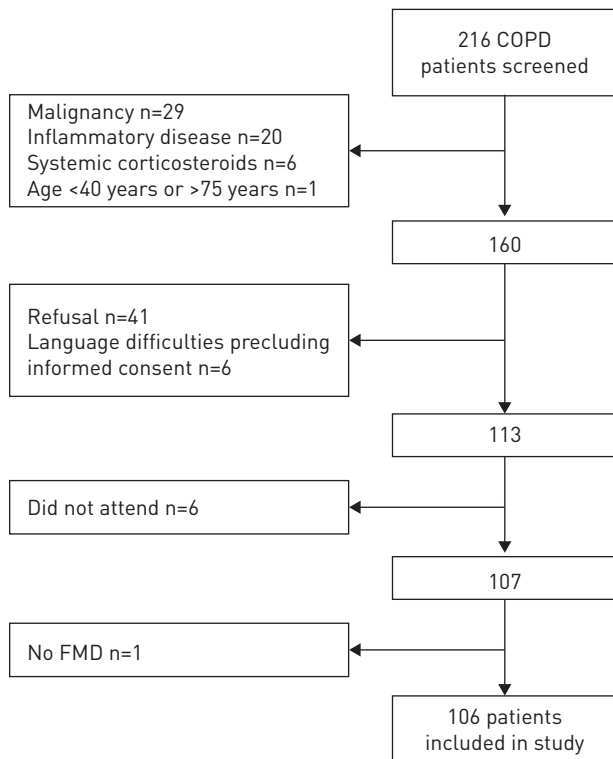

FIGURE 1 Study flow. COPD: chronic obstructive pulmonary disease; FMD: flow-mediated dilatation.

TABLE 1 Characteristics of patients with mild to very severe chronic obstructive pulmonary disease

	All patients	GOLD stage		
		I/II	III	IV
Subjects n	106	38	26	42
Clinical characteristics				
Age years	61.3±7.7	61.2±8.0	59.8±9.4	62.3±6.2
Male/female n	70/36	27/11	14/12	29/13
Body mass index kg·m^{-2}	26.9±6.9**	30.2±7.5	26.4±6.5	24.2±5.2
Smoking pack-years	40±24	38±26	37±26	44±24
Current smokers	21 (20)**	14 (36)	2 (8)	5 (12)
Antihypertensive drug	51 (48)	19 (50)	11 (42)	21 (50)
β-blocker	15 (14)	9 (24)	1 (4)	5 (12)
Cholesterol-lowering drug	29 (27)	13 (34)	4 (15)	12 (29)
Combined inhaled LABA and steroid	65 (61)**	13 (34)	16 (62)	36 (85)
Arterial hypertension[#]	45 (42)	20 (53)	12 (46)	13 (31)
Diabetes	11 (10)	4 (11)	4 (15)	3 (7)
Coronary artery disease	20 (19)	7 (18)	4 (15)	9 (21)
Peripheral artery disease	13 (12)	2 (5)	3 (12)	8 (19)
Pocock risk score[¶]	2.1±2.1	2.2±2.5	1.7±1.5	2.2±2.0
Lung function				
FEV1 % pred	44.6±22.3**	71.5±12.1	36.8±5.6	25.0±4.9
FVC % pred	78.4±18.3**	92.9±13.3	79.8±15.2	64.5±12.8
TLC % pred	112.8±24.8**	95.4±15.6	120.7±22.9	122.8±24.6
RV/TLC ratio	55.7±13.4**	41.8±7.3	58.0±9.3	66.1±8.4
DLCO % pred	49.7±25.9**	76.3±23.9	42.0±12.7	32.3±12.2
Blood gas analysis				
PaO$_2$ kPa	9.2±1.85**	10.1±1.9	8.8±1.3	8.7±1.9
PaCO$_2$ kPa	5.1±0.70**	4.7±0.8	5.0±0.6	5.5±0.7
Sympathetic activity				
Heart rate L·min^{-1}	81.4±12.8**	75.6±11.4	83.0±8.3	85.6±14.6
Systolic blood pressure mmHg	125.2±15.9	127.4±15.2	122.2±12.2	125.1±18.3
Diastolic blood pressure mmHg	78.6±10.6	77.7±11.6	79.9±9.7	78.7±10.9
Baroreflex sensitivity ms·mmHg^{-1}	4.2±2.9**	5.5±3.4	4.1±3.1	3.1±1.7
Laboratory parameters				
Total cholesterol mmol·L^{-1}	5.2±1.1	4.9±1.1	5.3±1.1	5.3±1.1
hsCRP mg·L^{-1}	2.9±2.9	3.3±3.4	2.9±3.1	2.2±2.2
Interleukin-6 pg·mL^{-1}	3.4±3.3	3.4±3.3	2.5±2.4	4.0±3.8
Malondialdehyde nmol·mL^{-1}	2.7±5.9	1.8±4.0	3.6±6.6	3.0±6.9
Haemoglobin g·dL^{-1}	14.4±1.5	14.3±1.8	14.5±1.4	14.4±1.4
Physical activity				
6-min walking distance m	398±141**	493±132	377±98	328±128
Steps per 24 h[+]	5442±3989**	7744±4908	4798±2492	3800±2684

Data are presented as mean±SD or n (%), unless otherwise stated. GOLD: Global Initiative for Chronic Obstructive Lung Disease; LABA: long-acting β$_2$-agonist; FEV1: forced expiratory volume in 1 s; % pred: % predicted; FVC: forced vital capacity; TLC: total lung capacity; RV: residual volume; DLCO: diffusing capacity of the lung for carbon monoxide; PaO$_2$: arterial oxygen tension; PaCO$_2$: arterial carbon dioxide tension; hsCRP: high-sensitivity C-reactive protein. [#]: blood pressure ⩾140/90 mmHg; [¶]: approximated percentage risk of 5-year mortality from cardiovascular disease; [+]: mean number of steps walked per 24 h in seven consecutive days. **: p<0.01 for comparison of differences between groups.

Physical activity

Performance-based daily physical activity (steps per 24 h) was assessed using a multisensory armband (SenseWear-Pro armband; BodyMedia, Inc., Pittsburgh, PA, USA), which was worn on the upper arm for seven consecutive days except when the device had to be taken off for showering/bathing. The activity monitor has previously been used in similar settings [19, 20]. The armband incorporates a biaxial accelerometer that records the number of steps per day [21]. Exercise capacity was assessed by the 6-min walk test (6MWT), performed according to American Thoracic Society guidelines [22].

Data analysis and statistics

Results are shown as mean±SD, unless otherwise stated. Differences in baseline characteristics between groups were assessed by F-test and Chi-squared tests as appropriate. Univariate regression was used to

investigate relationships between FMD and airflow obstruction as well as associations with the postulated underlying mechanisms. Further multivariate analysis involved regression of variables that showed a univariate p-value of <0.1 with adjustment for potential confounders (age, Pocock score and baseline brachial artery diameter). Multiple imputation with chained equations were used to handle missing values (<5% for all variables, except malondialdehyde 17%, steps per 24 h 13% and baroreflex sensitivity 10%). An indicator variable was created for malondialdehyde as a marker of oxidative stress. To investigate a modifying effect of physical activity on the association between FMD and forced expiratory volume in 1 s (FEV1), an interaction term (physical activity × FEV1) was included in the regression analysis. Beside this *a priori*-stated hypothesised interaction, we did not assess any other interactions.

Results

Study participants
Of the 216 patients screened for eligibility, 106 entered the analysis (fig. 1). Patient characteristics are shown in table 1. The prevalence of cardiovascular comorbidities was high (53%).

Endothelial function and airflow obstruction in COPD
Endothelial function as assessed by FMD was associated with post-bronchodilator FEV1 % predicted ($\beta=0.04$, $p<0.01$) (fig. 2a). Mean ± SD FMD in patients with GOLD stage I/II was 4.3 ± 2.0% pred and was progressively impaired in patients with stage III (2.8 ± 1.5% pred) and stage IV (2.0 ± 1.3% pred) (table 2).

Potential determinants influencing endothelial function
Sympathetic activation
Impaired baroreflex sensitivity was associated with FMD ($\beta=0.15$, $p=0.03$), whereas the association between heart rate and FMD did not reach statistical significance ($\beta= -0.02$, $p=0.09$). Baroreflex sensitivity showed an association with FEV1 % pred ($\beta=0.37$, $p<0.01$) (fig. 3).

Effect of arterial blood gases
Arterial carbon dioxide tension ($P_{a}CO_2$) was inversely associated with FMD ($\beta= -0.74$, $p<0.01$), whereas no association was found between hypoxaemia and FMD.

Systemic inflammation and oxidative stress
Levels of hsCRP, IL-6 and malondialdehyde were not statistically significantly associated with FMD (table 3).

Physical activity and endothelial function in COPD
Exercise capacity assessed by the 6MWT was statistically significantly associated with FMD ($\beta=0.004$, $p<0.01$), and daily physical activity (steps per 24 h) showed an association at $p<0.1$ ($\beta=8.52 \times 10^{-5}$, $p=0.067$).
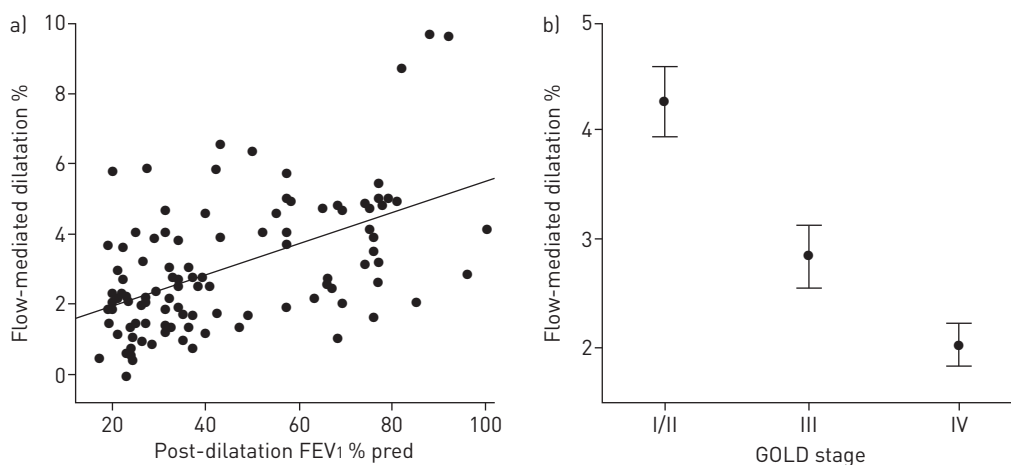

FIGURE 2 a) Univariate regression between flow-mediated dilatation (FMD) and forced expiratory volume in 1 s (FEV1) % predicted (% pred); b) FMD measurements of all patients with chronic obstructive pulmonary disease grouped according to Global Initiative for Chronic Obstructive Lung Disease (GOLD) stages. Bars represent SEM.

TABLE 2 Endothelial-dependent and -independent vasodilatation in chronic obstructive pulmonary disease patients

	All patients	GOLD stage		
		I/II	III	IV
Subjects n	106	38	26	42
Baseline brachial artery diameter mm	3.7±0.7	3.9±0.7	3.6±0.7	3.9±0.7
Baseline brachial artery flow m·s^{-1}	0.10±0.12	0.09±0.05	0.08±0.05	0.13±0.17
Post-occlusion brachial artery flow m·s^{-1}	0.57±0.24	0.54±0.22	0.55±1.8	0.62±0.30
FMD %	3.0±1.9	4.3±2.0	2.8±1.5	2.0±1.3
NMD %	12.6±5.7	12.1±4.8	12.9±7.4	12.9±5.6

Data are presented as mean±SD, unless otherwise stated. GOLD: Global Initiative for Chronic Obstructive Lung Disease; FMD: flow-mediated dilatation; NMD: nitroglycerine-mediated dilatation.

Multiple regression model of all postulated mechanisms

The multivariate model (table 4) included variables showing at least a moderate association (defined as p<0.1 in the univariate analysis). To further control for potential confounding, conventional cardiovascular risk factors were additionally included in the final model regardless of the univariate results. In this multivariate model, airflow obstruction, physical activity and their interaction, as well as brachial artery diameter, were the only variables independently associated with FMD.

A stratified analysis was performed as the significant interaction term (number of steps per 24 h × FEV1 %) assumed a modifying effect of physical activity on the association between FEV1 and FMD. Patients were categorised according to the median number of steps per 24 h and denominated either as physically active or inactive patients. Results of the covariance analysis demonstrating the association between FEV1 and predicted FMD (adjusted for age, sex, smoking, use of combined inhaled steroids and long-acting β-adrenergic drugs, PaCO$_2$, baroreflex-sensitivity, heart rate, 6MWT, Pocock score and brachial artery diameter) within subgroups are displayed graphically in figure 4. Compared to the active patient group, results in inactive patients showed a stronger association between FEV1 and FMD.

Discussion

This study investigated potential determinants of endothelial function in COPD patients. We demonstrated a strong cross-sectional relationship between endothelial function measured by FMD and the severity of airflow obstruction across a heterogeneous group of COPD patients. Analysis of possible underlying factors revealed that only FEV1 and physical activity showed an independent effect on endothelial function. Subgroup analysis suggested a more pronounced effect of FEV1 on endothelial function in physically less active patients.

There is preliminary evidence that COPD is associated with endothelial dysfunction [23, 24]. Hence, impairment of endothelial function may represent a mechanism through which COPD leads to
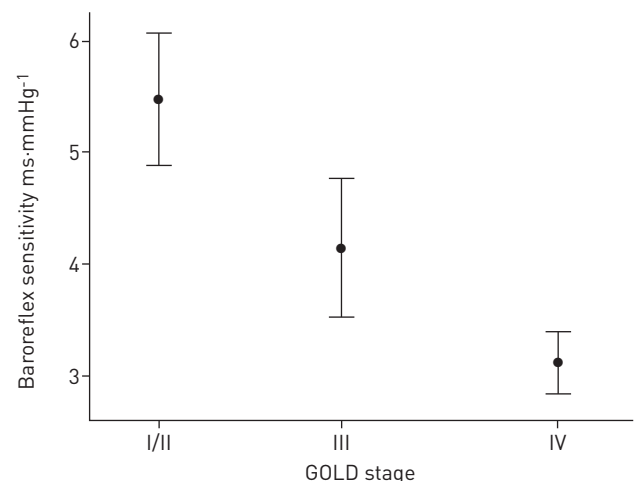

FIGURE 3 The relationship between baroreflex sensitivity and airflow obstruction (forced expiratory volume in 1 s). Baroreflex sensitivity was nearly normal in chronic obstructive pulmonary disease patients with Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage I/II, but was progressively impaired in patients with stage III and stage IV.

TABLE 3 Univariate regression analysis of factors potentially associated with endothelial function (measured by flow-mediated dilatation)

	β-coefficient	95% CI	p-value
Clinical characteristics			
Age years	-0.32	-0.08–0.01	0.173
Male	-0.74	-1.4–0.01	0.055
Body mass index kg·m^{-2}	0.01	-0.05–0.06	0.818
Smoking pack-years	-0.01	-0.02–0.01	0.361
Current smoking	1.23	0.36–2.10	0.006
Antihypertensive drug	-1.57	-0.89–0.57	0.669
β-blocker	-0.18	-1.20–0.84	0.726
Cholesterol-lowering drug	-0.15	-0.96–0.66	0.718
Combined inhaled LABA and steroid	-0.78	-1.52––0.43	0.038
Total cholesterol mmol·L^{-1}	-0.04	-0.38–0.30	0.827
Pocock risk score[#]	-0.12	-0.30–0.06	0.186
Lung function			
FEV_1 % pred	0.04	0.03–0.06	<0.01
FVC % pred	0.04	0.02–0.06	<0.01
TLC % pred	-0.01	-0.02–0.01	0.389
RV/TLC ratio %	-0.05	-0.07––0.02	<0.01
D_{LCO} % pred	0.02	0.01–0.03	<0.01
Blood gases			
P_{aO_2} kPa	0.10	-0.10–0.30	0.331
P_{aCO_2} kPa	-0.74	-1.23––0.24	<0.01
Sympathetic activity			
Heart rate L·min^{-1}	-0.02	-0.05–0.00	0.088
Systolic blood pressure mmHg	0.00	-0.03–0.02	0.776
Diastolic blood pressure mmHg	0.00	-0.03–0.03	0.983
Baroreflex sensitivity ms·mmHg^{-1}	0.15	0.02–0.28	0.027
Systemic inflammation and oxidative stress			
hsCRP mg·L^{-1}	0.01	-0.01–0.04	0.270
Interleukin-6 pg·mL^{-1}	0.02	-0.09–0.13	0.664
Malondialdehyde[¶] nmol·mL^{-1}	-0.33	-1.08–0.41	0.376
Physical activity			
6-min walking distance m	0.004	0.00–0.01	0.001
Steps per 24 h[+]	8.52×10^{-5}	-6.23×10^{-6}–1.77×10^{-4}	0.067

LABA: long-acting β$_2$-agonist; FEV_1: forced expiratory volume in 1 s; % pred: % predicted; FVC: forced vital capacity; TLC: total lung capacity; RV: residual volume; D_{LCO}: diffusing capacity of the lung for carbon monoxide; P_{aO_2}: arterial oxygen tension; P_{aCO_2}: arterial carbon dioxide tension; hsCRP: high-sensitivity C-reactive protein. [#]: approximated percentage risk of 5-year mortality from cardiovascular disease; [¶]: implemented as an indicator variable with a threshold of 0.16 nmol·mL^{-1}; [+]: average number of steps walked per 24 h in a measurement period of seven consecutive days.

cardiovascular disease. However, data on potential biological factors underpinning the association between COPD and endothelial function, such as hypoxia, systemic inflammation, oxidative stress, sympathetic activation and physical activity, are mostly lacking. Therefore, our aim was to identify alterations in endothelial function in COPD and characterise the impact of various mechanisms potentially underpinning this association.

BARR et al. [24] investigated 107 former smokers, of whom 40% suffered from COPD. The authors observed a relationship between FMD and FEV_1, as well as with percentage of lung emphysema. In a study by EICKHOFF et al. [25], 60 patients with mild-to-moderate COPD were compared to smoking and nonsmoking control subjects without COPD. The authors found that FMD was significantly impaired in patients with COPD compared to smoking and nonsmoking control subjects without COPD. In contrast to our study, FMD values in patients with COPD were considerably higher in the study by EICKHOFF et al. [25], which might be due to differences in the measurement techniques, as the sphygmanometer cuff was placed around the upper arm instead of the forearm as recommended [26]. In contrast to our findings and those of EICKHOFF et al. [25], MACLAY et al. [27] presented a case–control study that assessed vascular function in 18 COPD patients and smoking control subjects without COPD. The authors of the latter small study did not

TABLE 4 Multivariate analysis of determinants of flow-mediated dilatation and potential confounders

	β-coefficient	95% CI	p-value
Age years	-2.0×10^{-3}	-0.06–0.05	0.941
Male	-0.07	-0.90–0.77	0.877
Current smoking	0.63	-0.30–1.56	0.182
Combined inhaled LABA and steroid	0.08	-0.66–0.81	0.840
Pocock risk score[#]	2.4×10^{-3}	-0.22–0.22	0.983
FEV_1 % pred	0.08	0.04–0.11	<0.01
Pa_{CO_2} kPa	-0.23	-0.75–0.29	0.387
Heart rate $L \cdot min^{-1}$	0.01	-0.02–0.04	0.388
Baroreflex sensitivity $ms \cdot mmHg^{-1}$	0.09	-0.7–0.26	0.268
6-min walking distance m	-1.3×10^{-3}	-4.6×10^{-3}–1.9×10^{-3}	0.409
Steps per 24 h[¶]	3.5×10^{-4}	7.3×10^{-5}–6.1×10^{-4}	0.014
Steps per 24 h $\times$ FEV_1 %	-5.9×10^{-6}	-1.1×10^{-5}–-7.7×10^{-7}	0.025
Brachial artery diameter mm	-0.68	-1.25–-0.11	0.019

LABA: long-acting β_2-agonist; FEV1: forced expiratory volume in 1 s; % pred: % predicted; Pa_{CO_2}: arterial carbon dioxide tension. [#]: approximated percentage risk of 5-year mortality from cardiovascular disease; [¶]: average number of steps walked per 24 h in seven consecutive days.

observe differences in endothelial function between the two groups. However, MACLAY et al. [27] used venous occlusion plethysmography with infusion of endothelium-dependent vasodilators as an assessment of endothelial function, so the studies are not directly comparable. In addition, the study by MACLAY et al. [27] may not have been appropriately powered to exclude clinically significant differences in endothelial function between the studied groups.

Our results extend the findings of previous studies by rigorously analysing potential determinants underpinning the relationship between impaired endothelial function and COPD. EICKHOFF et al. [25] reported that higher levels of CRP, but not IL-6, were associated with a lower FMD in a group of 60 COPD patients, suggesting that systemic inflammation may play a role in the pathogenesis of endothelial dysfunction in COPD. In the present larger study, hsCRP and IL-6 did not affect the relationship between FMD and airflow obstruction in COPD patients. A possible explanation for the conflicting findings could be that patients included in the study by EICKHOFF et al. [25] were free from comorbidities, whereas we included typical COPD patients, some of whom had diabetes and cardiovascular disease, factors that are known to be associated with systemic inflammation, and thus may have masked an effect of COPD on inflammatory markers [28].

COPD has been shown to be associated with increased oxidative stress markers such as reactive oxygen species [29]. Oxidative activity can be determined by measuring oxidative products of lipid peroxidation, such as malondialdehyde, which is considered to be a reliable measure of oxidative stress [30]. However, to date there have been no data from clinical studies investigating the relationship between oxidative stress burden and endothelial function in patients with COPD. In the present study, levels of malondialdehyde showed no association with FMD, suggesting that oxidative stress assessed in blood may not play a predominant role in determining endothelial function in patients with COPD.

Consistent with autonomic sympathetic activation, elevated heart rate [31], reduced heart rate variability [32] and depressed baroreflex sensitivity [33] have been described in COPD patients. However, there have been no data as to whether sympathetic activation leads to an impairment of endothelial function in COPD. In the present study, elevated heart rate and reduced baroreflex sensitivity both showed a significant relationship with the severity of airflow obstruction. Although we could demonstrate a moderate association between baroreflex sensitivity and FMD in the univariate analysis, no significant independent association remained after implementation in the multivariate analysis. Therefore, sympathetic activity seems not to be a predominant determinant of endothelial function in COPD.

Patients with COPD often develop hypoxaemia, which may be present intermittently (e.g. during exercise or sleep) or may be sustained in more severe cases. It has been suggested that hypoxia is associated with a number of pro-atherogenic effects, including systemic inflammation [5], oxidative stress [34] and an increase of blood pressure [35]. However, the effects of hypoxia on endothelial function in patients with COPD have not been studied. Our results imply that hypoxia seems not to promote impairment of
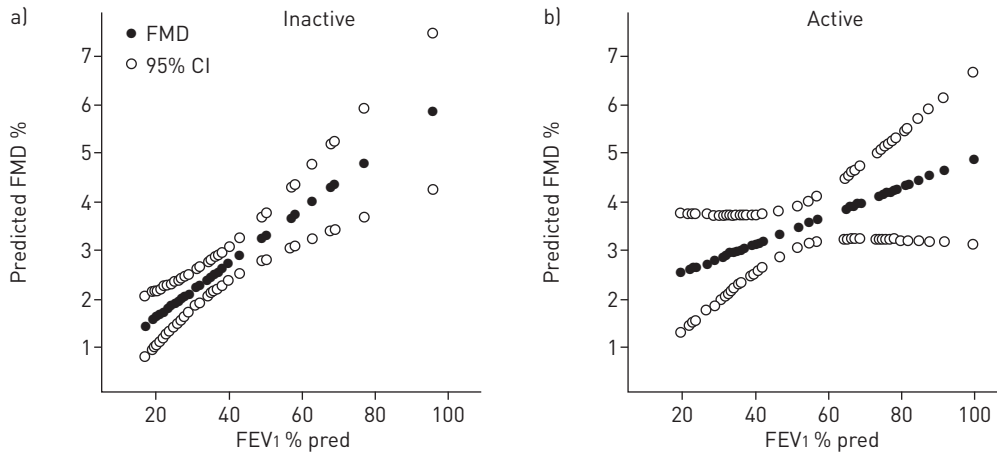
FIGURE 4 Predicted flow-mediated dilatation (FMD) within 95% CI in relation to the forced expiratory volume in 1 s (FEV1) % predicted (% pred) for a) inactive and b) active patients. Patients were classified according to their level of daily physical activity: below and above the median number of steps per day were classified as inactive and active, respectively. Analysis of covariance was used to predict FMD after controlling for diameter of the brachial artery, age, sex, current smoking, use of combined inhaled steroids and long-acting β-adrenergic drugs, arterial carbon dioxide tension, baroreflex sensitivity, heart rate, 6-min walk test and Pocock score. There was a significant association between FMD and FEV1 in inactive patients (adjusted β=0.06, p<0.01) compared to the active patients (adjusted β=0.03, p=0.11), suggesting a modifying effect of physical fitness on the association between FMD and FEV1.

endothelial function in COPD. However, hypercapnia showed a relationship with reduced FMD in univariate analysis, although this effect disappeared after correction for airflow obstruction.

Various studies in animal models and humans demonstrated the impact of physical activity on endothelial function [36, 37]. It has been postulated that physical activity leads to shear stress on the arterial wall, causing increased expression of endothelial nitric oxide synthase, thus improving endothelial vasomotor function. In the current study, a significant modifying effect of physical activity on endothelial function was found (fig. 4). Patients with severe airflow obstruction who were physically active were less prone to more severe impairment of FMD, compared to inactive COPD patients with severe airflow obstruction. Of the studied determinants with potential impact on the association of airflow obstruction and FMD, only physical activity demonstrated an independent effect. These results suggest that physical activity may attenuate the progression of vascular dysfunction in COPD. However, this needs to be proven in well-designed interventional studies.

Although there is accumulating evidence from large epidemiological studies that impaired lung volumes may be important predictors of future cardiovascular events [2, 3], this has not been widely recognised and thus lung volumes have not been implemented in cardiovascular risk scores such as the Pocock score. In the current study, there was no relationship between the Pocock risk score and FMD. Thus, it seems that FEV1 and the level of daily physical activity may be more important determinants of endothelial function in patients with COPD than the combined conventional factors implemented in the Pocock score.

The main limitation of the present study is the cross-sectional design, which does not allow the establishment of a causal relationship. A prospective cohort study would provide stronger evidence because the temporal relationship as a key element of causal inference could be determined. A randomised trial may be more difficult, as both physical activity and lung function are difficult to modify, at least in the short term. However, it may be that future large and long-term randomised trials that aim at modifying physical activity will provide more evidence. A further limitation is that we had three variables with >5% missing values, including malondialdehyde (17%), steps per 24 h (13%) and baroreflex sensitivity (10%). Finally, for ethical reasons, (inhaled) drugs were not withheld during the study; therefore, we cannot entirely exclude that the relationship between FEV1 and FMD is driven by differences in β-agonist use rather than the severity of airflow limitation. However, by including the use of β-agonists and baroreflex sensitivity as covariates in the multivariate analysis, appropriate correction for this possible bias can be assumed.

In conclusion, the findings of this study demonstrate that the severity of airflow obstruction is a significant determinant of endothelial function in patients with COPD. A high level of physical activity seems to have a favourable effect on this association.

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