J Bras Pneumol. 2020;46(5):e20200007
https://dx.doi.org/10.36416/1806-3756/e20200007



# Changes in endothelial cell-derived extracellular vesicles after acute exercise in patients with COPD: a pilot study

Dario Nieri[1,2], Tommaso Neri[1], Sabrina Santerini[3], Stefania Lombardi[4], Alessandro Celi[1]

## TO THE EDITOR:

Endothelial dysfunction and peripheral muscle impairment are known to be extrapulmonary manifestations of COPD.[1,2] Quantification of endothelial cell-derived extracellular vesicles (EEV) has been proposed as a possible method to evaluate endothelial function.[3] In addition, EEV could be markers of emphysema and predictors of prognosis in COPD.[4] Although physical activity is currently recommended for COPD patients,[2] the effect of a single bout of exercise on endothelial response in such patients is unknown. It has been proposed that vascular function after an acute bout of exercise in healthy individuals follows a biphasic pattern (a transient decrease followed by normalization or even improvement).[5] However, to our knowledge, this has yet to be studied in patients with COPD. We conducted a pilot study to evaluate whether EEV might be considered as a marker of endothelial response after acute exercise in patients with COPD. We hypothesized that there would be an increase in EEV counts after acute exercise.

We enrolled patients with a confirmed diagnosis of COPD in accordance with current guidelines,[2] a post-bronchodilator $FEV_1$ between 30% and 70% of the predicted value, and no severe comorbidities. A group of healthy subjects was enrolled as controls. The study was approved by the local research ethics committee, in compliance with the Declaration of Helsinki, and all participants gave written informed consent. After clinical evaluation, all participants were submitted to biochemical blood testing and pulmonary function tests (visit 0). At visit 1, all subjects underwent an incremental, symptom-limited cardiopulmonary exercise test (CPET) on a cycle ergometer, using an incremental ramp protocol based on their baseline pulmonary function in order to complete the test in 6-10 min. During CPET, data on metabolic, ventilatory, and central hemodynamic variables were collected (Table 1). At visit 2, participants underwent a constant-load CPET after a 3-min warm-up period and at 70% of the maximum workload achieved during the incremental test. In order to measure plasma EEV, peripheral blood samples were collected at rest (T0), at peak of exercise (Tmax, i.e., immediately after the subject stopped pedaling because of exhaustion), and at recovery (Trec, i.e., 1 h after completion of CPET). Both incremental and constant-load CPETs were performed in the morning, approximately 2 h after a light breakfast (excluding coffee), with an interval of 5-7 days between tests. Smokers were asked to refrain from smoking three days prior to the tests. Platelet-poor plasma was obtained by two rounds of centrifugation (1,500 *g* for 15 min and 13,000 *g* for 2 min). For the characterization of plasma extracellular vesicles (EV), multiparametric flow cytometry was performed using a BD FACS Canto flow cytometer (BD Biosciences, San Jose, CA, USA) as described in one study[6] with modifications. EEV were initially discriminated by size as events (light scatter distribution within the 0.5- to 0.9-μm bead range) and then specifically identified as positive events for peridinin chlorophyll protein complex-labeled annexin V, carboxyfluorescein diacetate succinimidyl ester, allophycocyanin-labeled anti-CD62e, and phycoerythrin-labeled anti-CD31.

Data are presented as medians and interquartile ranges. The two-sample Kolmogorov-Smirnov test and the Friedman test for repeated measures were used. All statistical analyses were performed with the IBM SPSS Statistics software package, version 20.0 (IBM Corporation, Armonk, NY, USA).

A total of 17 subjects (12 COPD patients and 5 controls) were enrolled. The anthropometric characteristics were similar between the groups. The COPD group showed moderate airflow obstruction and a moderate decrease in DLCO on spirometry (Table 1). EEV response differed between the two groups. No differences were found in the control group regarding EEV levels at T0, Tmax, and Trec—11.8 (37.6) events/min; 14.8 (31.1) events/min; and 19.6 (30.1) events/min, respectively ($p = 0.678$). In the COPD group, we found a significant decrease in EEV levels at Tmax—3.4 (14.1) events/min—when compared with those at T0—6.7 (16.9) events/min ($p = 0.024$)—and those at Trec—7.8 (35.1) events/min ($p = 0.002$). No difference was found between T0 and Trec. We arbitrarily assigned to T0 an EEV level of 100% and calculated EEV levels at Tmax and at Trec, as well as relative baseline variations. No differences were found in the control group. In the COPD group, however, EEV levels at Tmax were significantly lower than at T0—62.3% (27.6%) vs. 100.0% (0.0%); $p = 0.024$—and at Trec—62.3% (27.6%) vs. 109.5% (156.7%); $p = 0.002$. This decrease in EEV levels at Tmax, with a return to baseline levels at Trec, was observed in 11 of the 12 COPD patients.

1. Centro Dipartimentale di Biologia Cellulare Cardiorespiratoria; Dipartimento di Patologia Chirurgica, Medica, Molecolare e dell'Area Critica, Università degli Studi di Pisa, Pisa, Italia.
2. SC Pneumologia, USL Toscana Nordovest, Lucca, Italia.
3. Sezione di Fisiopatologia Respiratoria e Riabilitazione Respiratoria Universitaria, Dipartimento CardioToraco Vascolare, Azienda Ospedaliero Universitaria Pisana, Pisa, Italia.
4. SSD Analisi ChimicoCliniche ed ImmunoAllergologia, USL Toscana Nordovest, Massa e Carrara, Italia.

**Table 1.** Anthropometric measurements, clinical features, pulmonary function results, together with metabolic, respiratory, and central hemodynamic variables collected during cardiopulmonary exercise testing.[a]

| Variable | Group | | p |
|---|---|---|---|
| | COPD (n = 12) | Control (n = 5) | |
| Age, years | 70.5 (7.0) | 65.0 (9.0) | N/S |
| Male/female, n/n | 8/4 | 4/1 | N/S |
| Smoking status, n | | | |
| Current smoker | 2 | 0 | N/A |
| Former smoker | 10 | 5 | |
| Never smoker | 0 | 0 | |
| Smoking history, pack-years | 35.0 (36.8) | 4.0 (18.0) | 0.005 |
| Dyspnea, mMRC scale score | 2.0 (1.0) | 0.0 (0.0) | 0.005 |
| BMI, kg/m$^2$ | 24.2 (7.9) | 25.9 (5.5) | N/S |
| $FEV_1$, L | 1.20 (0.35) | 3.38 (1.01) | 0.002 |
| $FEV_1$, % predicted | 56.5 (22.0) | 110.0 (23.0) | 0.002 |
| $FEV_1$/FVC | 0.45 (0.17) | 0.76 (0.07) | 0.002 |
| TLC, L | 6.44 (2.53) | 7.48 (1.30) | N/S |
| TLC, % predicted | 114.0 (25.0) | 106.0 (16.0) | N/S |
| DLCO, mL · min$^{-1}$ · mmHg$^{-1}$ | 13.0 (9.9) | 28.2 (7.8) | 0.002 |
| DLCO, % predicted | 58.0 (28.8) | 104.0 (20.0) | 0.002 |
| HR at rest, bpm | 84.0 (14.0) | 68.0 (23.5) | N/S |
| Blood pressure at rest, mmHg | 132 (10)/74 (7) | 117 (23)/78 (17) | N/S |
| $VO_2$peak[b], mL/kg × min | 16.3 (10.1) | 22.3 (7.9) | N/S |
| $VO_2$peak, % predicted | 74.0 (49.0) | 82.0 (21.5) | N/S |
| Maximum workload[b], W | 79.0 (52.0) | 169.0 (63.5) | 0.022 |
| Maximum workload, % predicted | 70.0 (46.0) | 104.0 (31.5) | 0.038 |
| Oxygen pulse[b], mL/beat | 15.2 (4.1) | 17.4 (7.4) | N/S |
| Breathing reserve[b], L | 9.7 (18.4) | 85.1 (39.9) | 0.002 |
| Endurance time[c], s | 227.5 (132.5) | 380.0 (157.5) | 0.053 |
| $VO_2$peak[c], mL/kg × min | 14.6 (6.2) | 18.5 (12.3) | N/S |
| $VO_2$peak, % pred | 60.0 (33.0) | 71.0 (40.5) | N/S |
| Hemoglobin, g/dL | 14.7 (2.0) | 15.2 (2.0) | N/S |
| White blood cell count, cells/mm$^2$ | 7,735 (2,412.5) | 5,310 (3,545) | N/S |
| Serum creatinine, mg/dL | 0.91 (0.35) | 0.81 (0.32) | N/S |
| Alanine aminotransferase, U/L | 16.0 (7.5) | 26.0 (16.5) | N/S |
| Aspartate aminotransferase, U/L | 19.0 (7.0) | 22.0 (7.5) | N/S |
| NT-proBNP, pg/mL | 95.0 (108.3) | 56.0 (27.5) | 0.038 |
| CRP, mg/dL | 0.24 (0.30) | 0.12 (0.49) | N/S |
| Fibrinogen, mg/dL | 333.0 (100.0) | 288.0 (74.0) | N/S |

N/S: not significant; N/A: not applicable; mMRC: modified Medical Research Council; BMI: body mass index; % pred: % of the predicted value; $VO_2$: oxygen consumption; NT-proBNP: N-terminal pro-brain natriuretic peptide; and CRP: C-reactive protein. [a]Values expressed as median (interquartile range), except where otherwise indicated. [b]Data collected during incremental cardiopulmonary exercise testing. [c]Data collected during constant-load cardiopulmonary exercise testing.

To our knowledge, no studies have investigated the behavior of circulating EEV after exercise in COPD patients. Although circulating EV levels are expected to increase in healthy subjects, results vary with regard to the cellular origin of EV and changes in EV levels after exercise.[7] One study[8] found an increase in apoptotic EEV levels in a sample of 17 patients with COPD after endothelial stimulation induced by shear stress; therefore, it is difficult to compare those results with ours. Decreased circulating EEV levels after exercise appear to be characteristic of the pathophysiology of COPD and might represent exaggerated EEV clearance at peak of exercise in such patients. In athletes, physical exercise has been reported to protect the vascular system and promote an in-vitro uptake of EEV into endothelial cells that is associated with a protection of target cells against apoptosis.[9] Similarly, it is possible that acute exercise could cause (either protective or detrimental) endothelial activation only in patients with COPD: the decrease in EEV we observed could be the result of exaggerated EEV clearance by the endothelium; because the pathogenesis of COPD involves the pulmonary capillary bed,[10] we can speculate about an activation of an altered pulmonary endothelium

specifically during exercise in these patients (whereas in controls, in whom pulmonary function is expected to be normal, we found no significant decrease in EEV). Whether or not this phenomenon actually involves the pulmonary vascular bed specifically should be the topic of further investigations using the von Willebrand factor as an endothelial-cell marker to determine the origin of EEV.

## AUTHOR CONTRIBUTIONS

DN, TN, and AC: study conception and design; DN, TN, SS, and SL: laboratory analysis and tests; DN, TN, and AC: data analysis; DN, TN, and AC: preparation of the manuscript; DN, TN, SS, SL, and AC: review and approval of the manuscript.

## REFERENCES


1. Schivo M, Albertson TE, Haczku A, Kenyon NJ, Zeki AA, Kuhn BT, et al. Paradigms in chronic obstructive pulmonary disease: phenotypes, immunobiology, and therapy with a focus on vascular disease. J Investig Med. 2017;65(6):953-963. https://doi.org/10.1136/jim-2016-000358
2. Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, et al. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease 2017 Report. GOLD Executive Summary. Am J Respir Crit Care Med. 2017;195(5):557-582. https://doi.org/10.1164/rccm.201701-0218PP
3. Lekakis J, Abraham P, Balbarini A, Blann A, Boulanger CM, Cockcroft J, et al. Methods for evaluating endothelial function: a position statement from the European Society of Cardiology Working Group on Peripheral Circulation. Eur J Cardiovasc Prev Rehabil. 2011;18(6):775-789. https://doi.org/10.1177/1741826711398179
4. Nieri D, Neri T, Petrini S, Vagaggini B, Paggiaro P, Celi A. Cell-derived microparticles and the lung. Eur Respir Rev. 2016;25(141):266-277. https://doi.org/10.1183/16000617.0009-2016
5. Dawson EA, Green DJ, Cable NT, Thijssen DH. Effects of acute exercise on flow-mediated dilatation in healthy humans. J Appl Physiol (1985). 2013;115(11):1589-1598. https://doi.org/10.1152/japplphysiol.00450.2013
6. Neri T, Tavanti L, De Magistris S, Lombardi S, Romei C, Falaschi F, Paggiaro P, Celi A. Endothelial Cell-Derived Extracellular Vesicles as Potential Biomarkers in Chronic Interstitial Lung Diseases. Ann Clin Lab Sci. 2019;49(5):608-610. https://doi.org/10.1183/13993003.congress-2019.PA2253
7. Highton PJ, Martin N, Smith AC, Burton JO, Bishop NC. Microparticles and Exercise in Clinical Populations. Exerc Immunol Rev. 2018;24:46-58.
8. Barak OF, Mladinov S, Hoiland RL, Tremblay JC, Thom SR, Yang M, et al. Disturbed blood flow worsens endothelial dysfunction in moderate-severe chronic obstructive pulmonary disease. Sci Rep. 2017;7(1):16929. https://doi.org/10.1038/s41598-017-17249-6
9. Wahl P, Jansen F, Achtzehn S, Schmitz T, Bloch W, Mester J, et al. Effects of high intensity training and high volume training on endothelial microparticles and angiogenic growth factors. PLoS One. 2014;9(4):e96024. https://doi.org/10.1371/journal.pone.0096024
10. Kanazawa H, Asai K, Hirata K, Yoshikawa J. Possible effects of vascular endothelial growth factor in the pathogenesis of chronic obstructive pulmonary disease. Am J Med. 2003;114(5):354-358. https://doi.org/10.1016/S0002-9343(02)01562-0