

Differences in Overexpression of Hypoxia-induced Transcription Factors and Associated Biomarkers in Three Different Types of Chronic Hypoxia



Diferencias en la sobreexpresión de biomarcadores asociados a la hipoxia en tres tipos de hipoxia diferentes

Dear Editor,

Research into the impact of hypoxia has focused on chronic continuous hypoxia (CCH), characteristic of COPD¹ and chronic intermittent hypoxia (CIH), characteristic of sleep apnea–hypopnea syndrome (OSA).^{2,3} The co-existence of CCH and CIH results in a third type of hypoxia, “Overlap Syndrome”.⁴ The mechanisms of adaptation to these different types of hypoxia may be different, with changes on the cardiovascular system or the peripheral musculature, with a dose-response associated with hypoxia.⁵

We hypothesized that the three models of hypoxia behave differently in the expression of hypoxia-induced transcription factors (factor inducible hypoxia 1–2 and factor nuclear Kappa-B, NF-κB), as well as inflammatory biomarkers including tumor necrosis factor α (TNF-α), interleukin (IL)-8, IL-6, vascular endothelial growth factor (VEGF), vascular cell adhesion protein 1 (VCAM-1), and biomarkers of oxidative stress including superoxide dismutase (SOD) and catalase activities (CAT), both systemically and in the peripheral muscle. Our objective was to compare the mechanisms of adaptation in patients with three types of hypoxia, on the expression of transcription factors sensitive to hypoxia, the expression of systemic inflammation, oxidative stress level and antioxidant capacity, at the systemic and at the muscle levels.

This prospective, observational, cross-sectional study was conducted in male patients over 18 years, diagnosed with COPD, OSA or Overlap Syndrome, further a control group. All subjects under-

went a clinical evaluation, spirometry with bronchodilator testing and a home respiratory polygraphy (Embletta Gold PG System).

The COPD patients had FEV₁ < 80% in post-bronchodilator spirometry, a daytime arterial partial pressure of oxygen (PaO₂) < 70 mmHg and an apnea–hypopnea index (AHI) < 5. The OSA patients had FEV₁ > 80%, a PaO₂ > 80 mmHg, an AHI > 15 and had not received CPAP treatment previously. The overlap group had FEV₁ < 80%, a PaO₂ < 70 mmHg and an AHI > 15. The control group had a normal spirometry with an AHI < 5.

Blood samples were collected by venipuncture and were immediately centrifuged, and plasma aliquots were stored at –80 °C. Muscle biopsy samples were obtained from all patients (not in control group) from the vast lateral of the quadriceps muscle, frozen in liquid nitrogen and preserved at –80 °C. For the analysis in plasma, we selected hypoxia-associated transcription factors, inflammatory, oxidative stress and antioxidants biomarkers. In the muscle biopsies we analyzed the gene expression of the inflammatory markers using reverse transcription quantitative polymerase chain reaction (RT-qPCR).

The study included 34 subjects: 4 with COPD, 12 with OSA, 13 with overlap and 5 controls.

The plasma concentration of transcription factors, inflammation and oxidative stress markers presented significant differences in the three groups of patients compared to the controls (Table 1). In plasma, the NF-κB1 was higher and SOD was significantly decreased in the OSA group compared with COPD and Overlap Syndrome.

As regards the muscle gene expression of transcription factors (Table 1), we found that in COPD patients the expression of VCAM-1 was higher and SOD was lower than in OSA and overlap groups.

In the OSA group we found a significant correlation ($r=0.579$; $P=.04$) between the levels of NF-κB1 obtained in plasma and in muscle, besides a significant correlation between the inflammatory and oxidative biomarkers in plasma and in muscle: plasma

Table 1

Plasma Concentration of Transcription Factors, Inflammation and Oxidative Stress Markers, and Muscle Gene Expression of Transcription Factors, Inflammation and Oxidative Stress Parameters.

Plasma Biomarkers	COPD	OSA	Overlap	Controls
HIF-1α (pg/ml)	254.8 ± 83.6*	210.2 ± 124.8*	178.2 ± 157.1*	22.8 ± 35.6
HIF-2α (pg/ml)	3 ± 3.4	15.7 ± 29.3	8.8 ± 29.0	7.7 ± 8.0
NF-κB1 (pg/ml)	8.5 ± 2.6†	13.8 ± 4.3*	11.2 ± 6.4	7.8 ± 3.4
IL-6 (pg/ml)	2.1 ± 1.2	1.6 ± 1	1.8 ± 0.7	0.9 ± 0.8
IL-8 (pg/ml)	8.1 ± 0.4*	8.2 ± 1.6*	8.2 ± 0.7*	1.2 ± 1.7
TNF-α (pg/ml)	0.6 ± 0.1	0.5 ± 0.2	0.7 ± 0.26	0.5 ± 0.6
VEGF (pg/ml)	43.6 ± 53.2	50.7 ± 29.1	49.2 ± 47.6	34.1 ± 55.1
VCAM-1 (pg/ml)	42.4 ± 39.1	29.5 ± 21.3	36.9 ± 25.4	24.5 ± 10.5
CAT (nmol/min/ml)	80.2 ± 30.3*	59.9 ± 25.4*	67.5 ± 14.5*	5.0 ± 1.5
SOD (U/ml)	1.3 ± 0.1*†	1.0 ± 0.2*†	1.3 ± 0.5*†	2.5 ± 0.9
Muscle biomarkers	COPD	OSA	Overlap	
HIF-1α (pg/ml)	0.052 ± 0.078	0.05 ± 0.034	0.058 ± 0.05	
HIF-2α (pg/ml)	0.13 ± 0.157	0.17 ± 0.09	2.33 ± 6.72	
NF-κB1 (pg/ml)	0.38 ± 0.733	0.065 ± 0.062	0.062 ± 0.049	
IL-6 (pg/ml)	0.007 ± 0.009	0.007 ± 0.008	0.006 ± 0.006	
IL-8 (pg/ml)	0.022 ± 0.02	0.463 ± 1.4	0.372 ± 0.8	
TNF-α (pg/ml)	0.03 ± 0.06	0.002 ± 0.003	0.005 ± 0.009	
VEGF (pg/ml)	0.165 ± 0.31	0.126 ± 0.08	0.218 ± 0.43	
VCAM-1 (pg/ml)	0.105 ± 0.03†	0.05 ± 0.04	0.09 ± 0.08	
CAT (nmol/min/ml)	1.432 ± 2.2	0.733 ± 0.3	1.295 ± 1.4	
SOD (U/ml)	4.577 ± 3.6†	13.856 ± 9.87	15.954 ± 17.5	

Abbreviations: COPD: chronic obstructive pulmonary disease; OSA: sleep apnea–hypopnea syndrome; HIF-1α: hypoxia inducible factor 1, subunit α; HIF-2α: hypoxia inducible factor 2, subunit α; NF-κB1: nuclear factor kappa-B 1; IL-6: interleukin-6; IL-8: interleukin-8; TNF-α: tumor necrosis factor alpha; VEGF: vascular endothelial growth factor; VCAM-1: vascular cell adhesion protein 1; CAT: catalase activity; SOD: superoxide dismutase activity.

* Significant differences ($p < 0.05$) versus controls.

† Significant differences ($p < 0.05$) versus SAHS.

‡ Significant differences ($p < 0.05$) versus overlap.

IL8-muscle SOD ($r=0.69$; $P=.01$); plasma endothelin-muscle CAT ($r=0.61$; $P=.03$); plasma ICAM-muscle IL8 ($r=0.71$; $P=.009$) and plasma ICAM-muscle CAT ($r=0.80$; $P=.002$).

In COPD group there was an increase in muscle expression of VCAM-1 compared to OSA, and a decreased in SOD compared with the other two groups.

The three groups of hypoxemic patients show an overexpression of hypoxia-sensitive transcription factors, primarily HIF-1 α , compared to control subjects. In OSA there was an increase in plasma levels of NF- κ B1 compared to COPD group. In situations of CIH, overexpression of NF- κ B1 in plasma has been demonstrated in a cell culture model⁶ with a selective activation of inflammatory pathways, unlike CCH hypoxia of patients with COPD and overlap, where the activation of adaptive pathways predominates. Oxidative stress driven by CIH may initiate systemic inflammation in OSA,⁷ which inflammatory response in turn promoting the development of cardiovascular morbidities.^{8,9} We have found a decrease in the plasma of SOD in OSA patients. In situations with a chronically oxidative stress due to overexpression of NF- κ B1, there may be a decrease in SOD by a saturation of the antioxidant systems.

We found no differences between the three patient groups in the expression of transcription factors in peripheral musculature. However, in COPD there is a higher level of VCAM and lower SOD than in OSA, reflecting a more intense inflammatory state at the muscle, besides a lower ability to nullify the deleterious effect of free radicals. These findings match with the findings of other authors¹⁰ and agree with that muscle dysfunction in COPD is clinically important. In patients with CCH, inflammatory and oxidative processes in plasma and in muscle fiber seem to be independent. In OSA, there is a correlation between the levels of the NF- κ B in plasma and in muscle fiber, as well as between parameters of inflammatory and antioxidant status in plasma and peripheral muscle, suggesting that inflammation and oxidative stress at the systemic levels and in the muscle are interrelated processes.

In summary, the three groups of hypoxemic patients show an overexpression of hypoxia-sensitive transcription factors, with a higher expression of NF- κ B in OSA. In plasma, an increase in oxidative stress is evident in the three groups, but the deficit in antioxidant substances is greater in OSA, which may be due to the overexpression of NF- κ B. At peripheral muscle, there were no differences in inflammatory parameters, unlike the response to oxidative stress, with an increase in the production of free radicals and a decrease in antioxidant substances in COPD patients versus OSA patients. Inflammation and oxidative stress in plasma and muscle in COPD and COPD-OSA seem to be independent phenomena. In OSA, inflammatory and oxidative stress levels in muscle fiber are related to systemic levels.

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Conflicts of Interest

None declared.

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¿Es útil una aplicación móvil para los pacientes con asma moderada-grave?



Is a Mobile Application Useful for Patients with Moderate-Severe Asthma?

Estimado Director:

En España, hasta un 3,9% de los asmáticos graves no están controlados¹⁻³, en parte debido al mal seguimiento de los planes

de automanejo o a la baja adherencia al tratamiento (50-67%)^{4,5}, conllevando mayores costes⁶.

La telemedicina se ha postulado como herramienta potencialmente útil en el manejo del asma^{7,8}, si bien puede presentar dificultades que pueden influir en su utilidad en la práctica clínica diaria. La pandemia actual por COVID-19 ha puesto de manifiesto la necesidad de telemonitorización de pacientes que, dada la situación, no podían acudir a consultas presenciales. Esta ofrece comodidad, evita desplazamientos, aumenta la eficiencia del sistema sanitario y en determi-