UNIVERSITAT DE BARCELONA

Caracterización del Estrés Oxidativo Pulmonar en Ejercicio Aeróbico Prolongado, usando el método de Aire Exhalado Condensado

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"Luego de haber alcanzado el objetivo, tengo la sensación de que el recorrido fue el momento más sublime"

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Lista de Abreviaturas

8-OHdG: 8-hidroxi-2' -deoxiguanosina
ADN: Ácido desoxirribonucleico
AE: Aire exhalado
AEC: Aire exhalado condensado
AGPI: Ácidos grasos poli-insaturados
ATP: Adenosín trifosfato
CAT: catalasa
CD_4^+: Linfocitos T que ayudan a coordinar la respuesta inmune
$-CH_2-$: grupo metileno
EI: Esputo inducido
EPOC: Enfermedad pumonar obstructiva crónica
ERO: Especies reactivas de oxpigeno
ERN: Especies reactivas de nitrógeno
Fe^{2+}: Ion ferroso
Fe^{3+}: Ion férrico
GR: Glutatión reductasa
GSH: Glutatión reducido
GSH-Px: Glutatión peroxidasa
GSSG: Glutatión oxidado
GST: Glutatión s-transferasa
G-6PD: Glucógeno 6 fosfato deshidrogenasa
H^+: Catión de hidrógeno
H_2O: Agua
H_2O_2: Peróxido de hidrógeno
$HO_2\cdot$: Radical hidroperoxilo
IL-1: Interleuquina 1
IL-6: Interleuquina 6
IL-8: Interleuquina 8
TNF-a: Factor de necrosis tumoral alfa
LTs: Leucotrienos
MAPK: Proteínas quinasas activadas por mitogen
MDA: Malondealdeido
NADH: Nicotinamida adenina dinucleótido deshidrogenasa

NADPH: Nicotinamida adenina dinucleótido fosfato reducida

NADP: Nicotinamida adenina dinucleótido fosfato oxidada

NFkβ: Factor nuclear kappa β

NO_2^-: Nitrito

NO_3^-: Nitrato

NOS: Óxido nítrico sintasa

NOX: NADPH oxidasa

O_2: Oxígeno

$^1O_2$: Oxígeno singlete

$^{1/2}O_2$: 0,5 mol de oxígeno

$O_2^{\cdot-}$: Anión superóxido

O_3: Ozono

·OOCR: Alquiperoxil radical

$OH^\cdot$: Radical hidroxilo

OH^-: Anión hidróxido

$ON^\cdot$: Óxido nítrico

$ONOO^-$: Peroxinitrito

pH: Coeficiente de acidez o basicidad de una solución acuosa.

PGC1-a: Activador del proliferador de peroxisoma activados de receptor gamma

PGs: Protaglandinas

$RO^\cdot$: Radical alcoxilo

$RO_2^\cdot$: Radical peroxilo

ROH: Producto hidroxilado

ROOH: Hidroperóxido orgánico

R-X: Xenobiótico

R-SG: Molécula Conjugada

SMAD: *"Mothers Against Decantaplegic"*

SOD: Superóxido dismutasa

TRx: Tioredoxina

VEGF: Factor de crecimiento endotelial vascular

VNO: Volumen de óxido nítrico

VO_2 (max): Consumo de oxígeno (máximo)

XOD: Xantina oxidasa deshidragenasa

1. Introducción General

1.1 Resumen

El desequilibrio entre la producción de pro-oxidantes y la capacidad antioxidante en favor de los primeros es conocido como estrés oxidativo [1]. Los primeros estudios sobre estrés oxidativo inducido por ejercicio se llevaron a cabo durante la década de los 70, en éstos se demostró que era posible encontrar efectos nocivos sobre el organismo con la producción elevada de pro-oxidantes [2–4]. Un ambiente oxidativo es capaz de dañar proteínas, carbohidratos, lípidos y el ADN celular, alterando algunas funciones biológicas. Sin embargo, en la actualidad es reconocido que niveles controlados de estrés oxidativo son capaces de gatillar respuestas adaptativas protectoras, impulsadas a partir de procesos de señalización celular, los cuales han sido observados también durante entrenamiento con ejercicio físico. Por ejemplo, se ha demostrado que la producción de pro-oxidantes durante el ejercicio aeróbico provoca un incremento en las concentraciones celulares del activador del proliferador de peroxisoma activados de receptor gamma (PGC1-a), factores de crecimiento endotelial vascular (VEGF) y de las proteínas quinasas activadas por mitogenes (MAPK), impulsando la biogénesis mitocondrial, angiogénesis y consumo de glucosa muscular respectivamente [5]. Lo anterior favorece la distribución del oxígeno y producción de energía en la musculatura durante el ejercicio, mejorando a largo plazo el rendimiento en el esfuerzo físico ejecutado. Algunos pro-oxidantes, de forma específica, también controlarían la adaptación biotipológica de la fibra muscular producida por el entrenamiento [6]. Para lograr el nivel de estímulo adecuado, será necesaria la acción de los antioxidantes, sustancias encargadas de reducir el nivel de pro-oxidantes presentes, las cuales ayudarán en la instauración de un ambiente redox tal, que evite las consecuencias nocivas del estrés oxidativo, y que favorezcan la adaptación. En este contexto, se ha demostrado que la práctica rutinaria de ejercicio físico incrementa la formación de nuevos elementos antioxidantes [7].

Ahora bien, es posible que algunos órganos puedan ser mayormente perjudicados durante el ejercicio, el pulmón es uno de ellos, ya que éste se puede ver expuesto a una elevada exigencia ventilatoria, aire contaminado (material particulado, óxidos de nitrógeno, etc), aire frío-seco o al ambiente hipóxico en altura. Por lo tanto, la capacidad antioxidante puede verse muchas veces sobrepasada.

Otro mecanismo fisiológico de respuesta que se observa en conjunto con la oxidación durante el ejercicio, es la inflamación [8, 9], la cual puede ser impulsada por el daño

mecánico sobre algunas estructuras, tales como el tejido conectivo, músculo, tendón y hueso, o bien, sobre elementos no estructurales como eritrocitos, endotelios y epitelios corporales [10–12]. En el pulmón, la condición oxidativa o inflamatoria inducida por ejercicio ha sido ampliamente estudiada. El efecto de la intensidad del esfuerzo, el ambiente clorado en nadadores de piscina, polución del aire en carreras, ejercicio en sujetos con patologías, ambientes extremos, entre otros, han sido relacionados con el aumento de células inflamatorias, cambios en el pH e incremento de citoquinas inflamatorias en diversas muestras pulmonares (línea de fluido epitelial, esputo inducido, aire exhalado, etc) [13]. A pesar de esto, los resultados aún son controversiales. Existe una clara tendencia de que una mayor intensidad y duración del ejercicio favorecería la producción de pro-oxidantes y la instauración de un ambiente inflamatorio en el pulmón [14], sin embargo aún no se ha establecido con exactitud cuánto es lo suficientemente intenso o duradero para que estos efectos se manifiesten. Lo anterior podría tener una enorme relevancia científica, ya sea en la creación de modelos de investigación en oxidación e inflamación pulmonar inducidos por ejercicio, en el conocimiento del uso de sustancias antioxidantes y des-inflamatorias, para observar la exacerbación oxidativa/inflamatoria por condiciones ambientales extremas (altura) o tóxico-nocivas (contaminación), para la mejora del desempeño deportivo, entre otras.

Entre las técnicas de muestreo pulmonar mas utilizadas para estudiar los efectos oxidativos e inflamatorios pulmonares producidos por condiciones respiratorias patológicas (p.e. Asma, EPOC, etc) y no patológicas (p.e. en ejercicio, aire contaminado, ambiente clorado de piscinas, etc) se encuentran el esputo inducido (EI) [15], aire exhalado (AE) [16] y el aire exhalado condensado (AEC) [16, 17]. La popularidad de estas técnicas radica en que son escasamente (EI) o nulamente invasivas (AE y AEC). Con el AEC ha sido posible analizar una serie de biomarcadores (p.e. H_2O_2, NO_2^-, NO_3^-, ATP, adenosina, SOD, pH, etc), capaces de analizar ambas respuestas, no así en el exhalado directo, el cual ha permitido medición de un número menor de estos (p.e. óxido nítrico). Por otro lado, el esputo inducido es una maniobra descrita como semi-invasiva (se inhala una solución salina hipertónica) y exigente para el sistema respiratorio (tos voluntaria), por lo que es posible que durante su ejecución estemos favoresciendo la aparición de mayores efectos oxidativos e inflamatorios. Es por esto, que en esta tesis se ha utilizado el AEC como técnica de muestreo, la cual nos permitirá dar un paso importante en el estudio y estandarización del efecto oxidativo e inflamatorio del ejercicio sobre el pulmón, sin ser invasivos.

1.2 Pro-oxidantes

Los pro-oxidantes son átomos o moléculas que tienen la capacidad de quitar uno o más electrones desde un elemento biológico (oxidándolo). Los pro-oxidantes pueden ser clasificados como radicales libres o no radicales (tabla 1). Los radicales libres también son conocidos como especies reactivas derivadas de oxígeno o nitrógeno, con las siglas ERO o ERN respectivamente, los cuales presentan una reactividad química pronunciada [18].

Tabla 1. Pro-oxidantes radicales y no radicales

Pro-oxidantes radicales		**Pro-oxidantes no radicales**	
Nombre	**Sigla**	**Nombre**	**Sigla**
Oxígeno singulete	$^1O_2$	Peróxido de hidrógeno	H_2O_2
Anión superóxido	$O_2^{\cdot-}$	Ozono	O^3
Radical hidroxilo	$OH^{\cdot}$	Peroxinitrito	$ONOO^-$
Radical peroxilo	$RO_2^{\cdot}$	Ácido hipocloroso	HOCL
Radical alcoxilo	$RO^{\cdot}$	Óxido Nítrico	$ON^{\cdot}$
Hidroperoxilo	$HO_2^{\cdot}$		

Entre los pro-oxidantes de importancia biológica se encuentran el anión superóxido ($O_2^{\cdot-}$), ión radical hidroxilo ($OH^{\cdot}$), peróxido de hidrógeno (H_2O_2) y el óxido nítrico ($ON^{\bullet}$), además son los más estudiados en el pulmón en condiciones patológicas (EPOC, Asma, etc) así como fisiológicas, tales como el ejercicio e hipoxia por altura [13, 19]. A continuación se destacan sus características.

- **Anión Superóxido:** La formación del $O_2^{\cdot-}$ implica la aceptación de 1 electrón por parte de una molécula de oxígeno (O_2), tal como se aprecia en la reacción 1. De todos las ERO, el $O_2^{\cdot-}$ ha sido el más estudiado. Este pro-oxidante puede ser formado durante el metabolismo oxidativo celular en la cadena transportadora de electrones presente en la mitocondria, aquí aproximadamente entre el 2 - 4% del oxígeno consumido *in vivo* se transforma anión superóxido [20].

$$O_2 + e^- \rightarrow O_2^{\cdot-} \text{ (Reacción 1)}$$

- **Ion Hidroxilo:** Tiene un elevado potencial oxidante y es reconocido como la ERO más dañina. Puede producirse en la reacción de Fenton (Rección 2) o Haber-Weiss

(Reacción 3). Este elemento es altamente reactivo y suele atacar moléculas inmediatamente después de su producción, además es capaz de producir un importante daño celular, ya que induce la peroxidación lipídica [18]. Es difícil de cuantificar sus concentraciones en el organismo vivo.

1) $Fe^{3+} + O_2^{\cdot-} \rightarrow Fe^{2+} + O_2$

2) $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH\cdot$ (Reacción 2)

$H_2O_2 + O_2^{\cdot-} \rightarrow OH^- + O_2 + OH\cdot$ (Reacción 3)

- **Peróxido de Hidrógeno:** Este pro-oxidante no radical induce la formación de nuevos radicales, tales como el ion hidroxilo (Reacción 2 y 3). Estas reacciones en cadena pueden formar gran cantidad de OH·, transformando el lugar rápidamente en un ambiente dañino para membranas celulares, atacando también a proteínas y colesterol. También puede ser formado por dismutación del $O_2^{\cdot-}$ (Reacción 4) por la acción de la enzima antioxidante superóxido dismutasa (SOD).

$$\xrightarrow{SOD}$$

$O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2$ (Reacción 4)

- **Óxido Nítrico:** Molécula sintetizada por la enzima Óxido Nítrico Sintasa (ONS) a partir de L-arginina utilizando NADPH y O_2. (Reacción 5). Se ha demostrado que el ejercicio físico induce la activación de la ONS que se encuentra en las membranas, mitocondria, células endoteliales, miocitos cardiacos, células inmunes (macrófagos), musculatura lisa, entre otras [21, 22]. Algunas respuestas fisiológicas relevantes durante el ejercicio incluyen la vasodilatación y un incremento de la actividad inmune. En un ambiente oxidativo, el ON reaccionará uniéndose con el $O_2^{\cdot-}$ para formar peroxinitrito ($ONOO^-$), un producto altamente oxidante; reacción liderada por la enzima mieloperoxidasa. En algunas células (macrófagos, células endoteliales), el ON· es un intermediario de la vía de la arginina en su transformación a nitrito (NO_2^-) y nitrato (NO_3^-), tal como se aprecia en la reacción 6.

$$\xrightarrow{NOS}$$

L-arginina + NADPH + O_2 → $ON\cdot$ + L-citrulina (Reacción 5)

$ON\cdot \leftrightarrow NO_2^- \leftrightarrow NO_3^-$ (Reacción 6)

1.3 Antioxidantes

Ahora bien, las concentraciones de pro-oxidantes presentes en el organismo, o específicamente en algún órgano, dependerán de su producción y remoción. La efectividad para prevenir su producción o favorecer su remoción se encuentra determinada por la capacidad de acción de los antioxidantes, moléculas preparadas para retardar o prevenir la oxidación de otras moléculas. Estos pueden ser clasificados según su naturaleza (origen) en enzimáticos o no enzimáticos. Los principales antioxidantes enzimáticos pulmonares de nuestro organismo, implicados con el ejercicio, son la SOD, la catalasa (CAT), glutatión peroxidasa (GSH-Px) y la Tioredoxina (TRx) [23]. Entre los elementos antioxidantes no enzimáticos se encuentran la mucina, urato, glutatión (GSH), ascorbato, ceruloplasmina, transferrina, vitamin E, ferritina y moléculas pequeñas tales como la bilirrubina [23–26]. En el pulmón, específicamente en la línea de fluido epitelial existen una gran cantidad de elementos antioxidantes enzimáticos y no enzimáticos [23, 27], de estos últimos, los más activos en la defensa reductora son la mucina, urato, ascorbato y el GSH, y de los enzimáticos son la SOD, CAT y GSH-Px [27].

1.3.1 Antioxidantes enzimáticos

- **Superóxido dismutasa:** Participa en la conversión de O_2^{-} en H_2O_2 y O_2 (Reacción 4). En la actualidad se conocen 3 isoformas, la SOD cobre-zinc que se aloja en el núcleo y peroxisomas, la SOD manganeso que se encuentra en la mitocondria y la SOD extracelular que se encuentra fuera de la membrana plasmática [23].

- **Catalasa:** Es posible encontrar esta enzima principalmente en el citosol (retículo endoplasmático), mitocondrias y peroxisomas, catalizando la reacción que transforma el H_2O_2 en agua y O_2 (Reacción 7).

$$\xrightarrow{CAT}$$

$2H_2O_2 \rightarrow 2H_2O + {}^1/_2O_2$ (Reacción 7)

- **Glutatión peroxidasa:** La GSH-Px tiene la capacidad de remover peróxidos orgánicos utilizando GSH, el cual actúa como reductor (Reacciones 8 y 9). Como producto obtendremos glutatión oxidado (GSSG) y agua. El glutatión GSSG será reducido a GSH por la glutatión reductasa (Reacción 10), utilizando NADPH. Distintos tipos de GSH-Px las podemos encontrar en fosfolípidos de membrana [28], gastrointestinal y extracelular [29]. En el pulmón es producida y secretada por las células epiteliales alveolares y macrófagos [30].

GSH-Px

$H_2O_2 + 2GSH \rightarrow GSSG + 2H_2O$ (Reacción 8)

GSH-Px

$ROOH + 2GSH \rightarrow ROH + H_2O + GSSG$ (Reacción 9)

GR

$NADPH + H+ + GSSG \rightarrow NADP+ + 2GSH$ (Reacción 10)

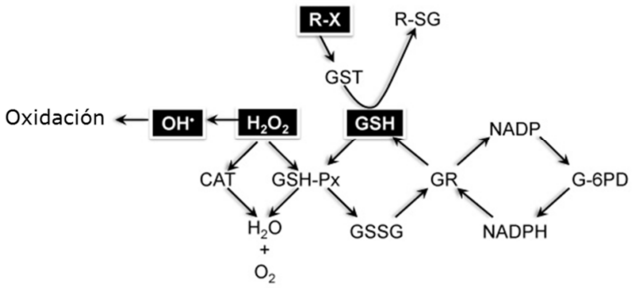
1.3.2 Antioxidantes no enzimáticos

- **Mucina:** Este antioxidante regula la viscosidad del mucus respiratorio. Combate oxidantes ambientales tales como el ozono, óxidos de nitrógeno y el humo del tabaco. Se encuentra elevada en condiciones patológicas inflamatorias crónicas, como por ejemplo fibrosis quística [31].

- **Urato:** Este antioxidante se secreta en la vía respiratoria en conjunto con la mucina, son esenciales en la defensa de algunos elementos oxidantes como el ozono [31].

- **Glutatión:** El glutatión es un antioxidante abundante en las células epiteliales pulmonares y en la línea de fluido epitelial [27, 32], es la clave en la modulación del desarrollo inflamatorio-oxidativo en la lesión pulmonar. Como se observa en la figura 1, el glutatión colabora en la reducción del peróxido de hidrógeno para formar agua y oxígeno utilizando glutatión reducido (GSH), convirtiéndolo en glutatión oxidado (GSSG). Este último es convertido nuevamente en GSH por la glutatión reductasa (GR), utilizando NADPH.

Figura 1. Actividad antioxidante del glutatión

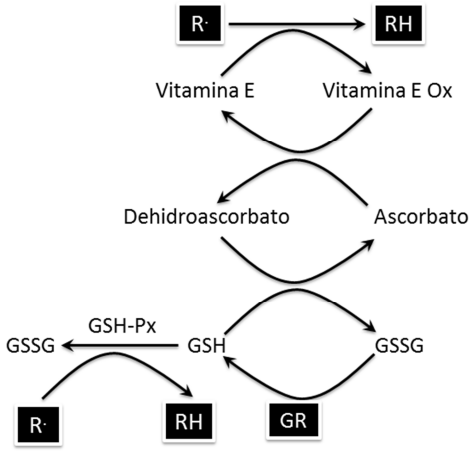


CAT: Catalasa; H_2O_2: Peróxido de hidrógeno; GR: Glutatión reductasa; GSH: Glutatión reducido; GSH-Px: Glutatión peroxidasa; GSSG: Glutatión oxidado; GST: Glutatión s-transferasa; G-6PD: Glucógeno 6 fosfato deshidrogenasa; NADP: Nicotinamida adenina dinucleótido fosfato oxidada; NADPH: Nicotinamida adenina dinucleótido fosfato reducida; OH·: Radical hidroxilo; R-X: Xenobiótico; R-SG: Molécula Conjugada.

- **Ascorbato:** El ascorbato es capaz de donar electrones a pro-oxidantes radicales y no radicales (H_2O_2, $OH^{\cdot}$, NO_2^{-}, entre otros). Además ayuda a regenerar Vitamina E reducida, utilizando GSH (Figura 2).

- **Vitamina E:** Esta vitamina soluble en grasas aporta electrones a un radical de la lipoperoxidación, formando peróxidos estables, previniendo su avance (Figura 2).

Figura 2. Actividad antioxidante del ascorbato y vitamina E



GSH: Glutatión reducido; GSH-Px: Glutatión peroxidasa; GSSG: Glutatión oxidado; GR: Glutatión reductasa; R·: Radical oxidado; RH: Radical reducido

1.4 Daño oxidativo sobre las biomoléculas y efectos del ejercicio

Como se comentó con anterioridad, los pro-oxidantes pueden atacar algunas biomoléculas del organismo, estas son los lípidos, proteínas, carbohidratos y el ADN. El estudio del daño oxidativo inducido por ejercicio ha sido interpretado principalmente a través de los subproductos de la oxidación de lípidos, proteínas y ADN. A continuación se describen estos mecanismos.

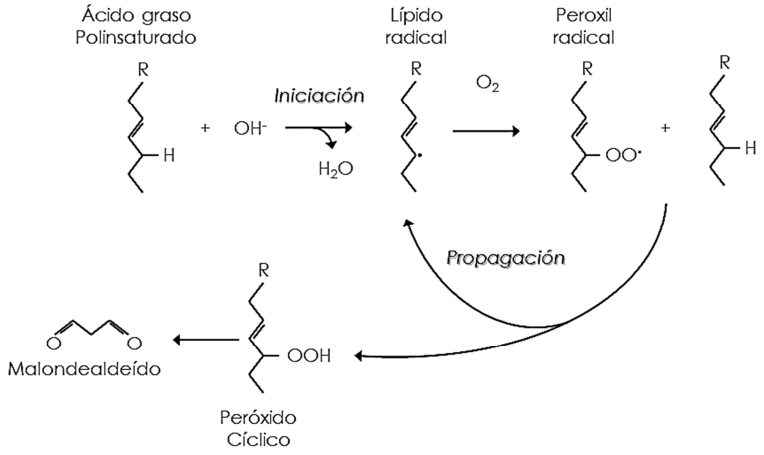
- **Lipoperoxidación:** Daño oxidativo de lípidos que tiene como principales moléculas diana los ácidos grasos poli-insaturados (AGPI). Los principales pro-oxidantes responsables de la iniciación del proceso de lipoperoxidación son el $O_2^{\cdot-}$, $OH^{\cdot}$ y el alquilperoxil radical (·OOCR) [33]. En efecto, los AGPI se encuentran en gran cantidad en las membranas celulares, por lo que el daño oxidativo de estas moléculas lideran las alteraciones en la propiedades biológicas de estas membranas (fluidez, función enzimática, intercambio iónico, etcétera). La iniciación del proceso de lipoperoxidación ocurre usualmente cuando un pro-

oxidante radical es agregada a un AGPI, o bien cuando el radical sustrae un hidrógeno desde su grupo metileno (-CH_2-) de este lípido [18], formando un lípido radical (figura 3). Este último es muy inestable y se unirá rápidamente a una molécula de oxígeno, formando un peroxil radical. Este puede reaccionar con otro ácido graso, produciendo por un lado un nuevo radical carbono, el cual junto al oxígeno formará nuevamente un peroxil radical que continuará la reacción en cadena de la lipoperoxidación (figura 3), y por otro lado peróxido cíclico capaz de formar aldehídos estables tales como el malondealdeído (MDA), 4-hidroxi-2-nonenal [34, 35], 2-propenal (acroleína) y los isoprostanos [36, 37] (figura 3). En efecto, el MDA e isoprostanos han sido utilizados para observar si ha existido daño oxidativo en los tejidos producido por ejercicio, y el pulmón no ha sido la excepción [38, 39]. Araneda et al. (2005) y DDD et al. (2016) mostraron que la lipoperoxidación en el AEC fue inducida por la hiperventilación inducida por ejercicio más altitud o ambiente clorado (piscina) respectivamente. Esto hace pensar que factores extras al ejercicio fuerzan el desarrollo de lipoperoxidación en el aire exalado de sujetos luego de realizar ejercicio [38, 39].

Bajo ambiente acidótico y temperatura elevada, las muestras biológicas reaccionan con ácido tiobarbitúrico formando productos de color rosa, los cuales pueden ser medidos por colorimetría o fluorometría para deteminar los niveles de MDA. La terminación es el proceso de la lipoperoxidación donde se juntan dos radicales y forman un no radical.

Cabe destacar lo dañino de este proceso, ya que el peroxil radical acumulado durante la lipoperoxidación no sólo es capaz de dañar nuevamente a un AGPI, sino que además a ácidos nucleicos y amino ácidos. El proceso de terminación ocurre cuando dos radicales reaccionan y producen una especie no radical, normalmente esto acontece a concentraciones de elevadas de radicales.

Figura 3. Lipoperoxidación



Etapas de iniciación y propagación del proceso oxidativo de lipoperoxidación

- **Oxidación de proteínas:** El elevado número de proteínas presentes en nuestro organismo las hace susteptibles de daño oxidativo cuando existe una elevación de los niveles de pro-oxidantes radicales de oxígeno y/o nitrógeno [40] o por la interacción de la proteína con un producto radical durante la oxidación lipídica o de glúcidos [17]. El ataque oxidativo puede ocurrir sobre el esqueleto de la proteína o las cadenas laterales de sus residuos de aminoácidos [41]. Las consecuencias de la oxidación pueden llevar a la pérdida de la función enzimática, contráctil o estructural de las proteínas afectadas, haciéndolas más susceptibles a la degradación proteolítica.

  El pro-oxidante principal en la oxidación de las proteínas es el $OH\cdot$ [42]. La oxidación del esqueleto es provocada por el $OH\cdot$ sobre el grupo a-carbono luego de extraer un hidrógeno, formando un radical centrado en carbono relativamente estable [41]. Este pro-oxidante puede ser obtenido desde la radiación ionizante del agua o por la reacción con metales del H_2O_2 (por ejemplo en la rección de Fenton; ver reacción 2), mediado por el radical hidroperoxilo ($HO_2\cdot$) [41]. Con la presencia de oxígeno se producirá un radical alquilperoxilo, seguido por la formación de un peróxido alquilo, el cual se convertirá en un alcoxilo que puede ser convertido a un derivado proteico hidroxilo [42]. Todos estos intermedios oxidativos proteicos pueden atacar a otros residuos de aminoácidos de otra

proteína o en la misma, produciendo un nuevo radical centrado en carbono. Además, el radical alcoxilo determina la etapa para la escisión del enlace peptídico por la diamida o a-amidación, favoreciendo el proceso de fragmentación. Sin oxígeno el radical centrado en carbono puede reaccionar con otro carbono y formar un derivado proteína-proteína [41].

La extracción de un hidrógeno desde los residuos de cadenas laterales por de cualquier aminoácido puede ser llevada a cabo por el radical $OH\cdot$. Sin embargo, se ha observado que los pro-oxidantes radicales derivados del oxígeno en general tienen preferencia por los aminoácidos aromáticos (fenilalanina, tirosina y triptófano) [41]. Ahora bien, algunos aminoácidos al oxidarse dan lugar a grupoas carbonilos los cuales se pueden emplear como indicador de daño oxidativo de proteínas. Los aminoácidos que pueden dar lugar a estos productos son la lisina, prolina y arginina [41, 43]. Estos productos son los más utilizados para evaluar el daño oxidativo proteico, y el ejercicio no ha sido la excepción. Sin embargo, los resultados son algo controversiales. Esta diferencias han sido atribuidas a al nivel de entrenamiento de los sujetos y al tiempo de medición posterior al esfuerzo ejecutado [44–47].

Por otro lado, los residuos de metionina y cisteína son particularmente susceptibles a la oxidación por casi todas las formas de pro-oxidantes derivados del oxígeno [41], sin embargo no forman rgrupos carbonilos [41].

El peroxinitrito también puede inducir la oxidación de residuos de aminoácidos, por ejemplo tirosina, triptófano, cisteína y metionina [41]. Para poder actuar sobre el residuo de tirosina, el $ONOO^-$ produce especies pro-oxidantes ($OH\cdot$) y nitrantes (NO_2^-), los cuales pueden extraer el hidrógeno desde el grupo hidroxilo del residuo de tirosina, alterando irreversiblemente la estructura y función de la proteína [42]. Una variación alternativa de la nitración sobre los residuos de tirosina es la reacción de un radical tirosilo con $NO\cdot$ para formar 3-nitrosotirosina, el cual puede impulsar o inhibir la activación proteica mediante la fosforilación o desfosforilación, regulando algunas funciones celulares [42].

- **Oxidación del ADN:** Es un proceso lento y muy dañino [48]. No todos los pro-oxidantes atacan al ADN, el principal es el $OH\cdot$. Este pro-oxidante puede atacar a la guanina en su posición C-8 para producir 8-OHdG [49, 50], sin embargo otras posiciones pueden ser atacadas. Además, el $OH\cdot$ puede atacar otras bases, por ejemplo la adenina, produciendo 8 (o 4-, 5-)-hidroxiadenina. También existen

productos oxidativos en la interacción con las pirimidinas, produciendo peróxido de timina, glicoles de timina, 5-(hidroximetil), uracilo y otros productos con estas características. El daño directo del $O_{2}^{\cdot -}$ y H_2O_2 no es significativo, sin embargo ambos sirven como fuentes para otros intermediarios reactivos que pueden causar más daño, por ejemplo al producir $OH^{\cdot}$ a través de la reacción de Haber-Weiss (reacción 3). Asimismo, el $ON^{\cdot}$ y el $O_{2}^{\cdot -}$ pueden conducir a la formación de $ONOO^{-}$, el cual puede causar daño al ADN similar al obtenido cuando están involucrados radicales hidroxilo.

El estudio de la producción de 8-hidroxi-2-desoxiguanosina (8-OHdG) en ejercicio se ha extendido significativamente para observar la oxidación del ADN, sin embargo la mayoría de los estudios no han reportado ningún cambio después de una variedad de protocolos de ejercicio [51–57]. Las diferencias en los hallazgos tienen explicación en la duración moderada y/o la intensidad del ejercicio aeróbico, las cuales deben ser suficientes para provocar el incremento de las concentraciones de 8-OHdG. Cabe considerar también la capacidad rápida de reparación que tiene el ADN después de la oxidación.

1.5 Caracteristicas de la inflamación pulmonar inducida por ejercicio

La inflamación es otra respuesta fisiológica que puede ser observada durante el ejercicio. Al igual que el estrés oxidativo, la inflamación puede provocar efectos dañinos sobre el organismo, tal es el caso de estímulos inadecuados u organismos desadaptados para el nivel de exigencia producido por el ejercicio. Los elementos más importantes relacionados con la inflamación inducida por ejercicio, especialmente en el pulmón, son los neutrófilos, leucocitos, citoquinas pro-inflamatorias (IL-1, IL-6 y TNF-a), derivados del ácido araquidónico (LTs y PGs) y pH [13]. Este último, ha sido utilizado como un marcador indirecto de inflamación aguda, sobre todo a nivel pulmonar [58]. La modificación en la actividad de células inmunes y concentraciones de sustancias relacionadas con la inflamación ha sido observada principalmente en ejercicio agudo extenuante [13]. Esto nos orienta que la duración e intensidad del ejercicio deben ser determinantes en instaurar un ambiente inflamatorio/oxidativo.

- **Citoquinas:** Son proteínas de bajo peso molecular presentes durante la respuesta inmune. Son señalizadoras y coordinadoras en la función de las células inmunes.

Éstas se generan en varios tipos celulares, sin embargo la mayor cantidad se producen en las células inmunes, principalmente en macrófagos y linfocitos T CD_4^+. Los macrófagos pulmonares son las células con mayor actividad fagocítica, la mayoría se encuentra en los alvéolos, un menor número lo podemos encontrar en los bronquios y tejido pulmonar intersticial.

- **pH:** En el pulmón, el descenso del pH se encuentra relacionado con patologías inflamatorias (acidosis). Algunos estudios han sido claros en demostrar respuestas del pH referente a la severidad y tratamiento en una patología [59]. Por ejemplo, se ha observado un descenso del pH en fibrosis quística [60], enfermedad pulmonar obstructiva crónica [59] y lesión aguda pulmonar [61]. En un modelo de ejercicio en humanos se obsevó un descenso del pH luego de la realización de ejercicio [62].

- **Células Inmunes:** Involucradas en la defensa inmune, se ha podido observar un incremento de la actividad y presencia de estas células en el pulmón producto de la realización de ejercicio físico. En relación con las respuestas respiratorias, es reconocido que la hiperventilación inducida por ejercicio puede favorecer la deshidratación del ambiente respiratorio, favoresciendo el ingreso de altos volúmenes aire frío y seco capaz de dañar el epitelio. Por otro lado, es posible que estos grandes volúmenes de aire inspirado contengan elementos nocivos que desencadenen, o bien exacerben la respuesta inmune pulmonar, por ejemplo corredores de larga distancia expuestos a alérgenos y partículas de polución [63] o irritantes derivados del cloro en nadadores en piscina [64, 65].

- **Moléculas derivadas del ácido araquidónico:** Las prostaglandinas, tromboxanos y leucotrienos son las principales moléculas derivadas del ácido araquidónico (eicosanoides) con funciones biológicas relevantes en el control de la respuesta inflamatoria e inmune. Algunas de sus funciones son la producción de fiebre, dolor, vasodilatación, vasocontricción y control de la acción de plaquetas y la trombosis. Se han observado incrementos de estos elementos en el esputo inducido de corredores de larga distancia [66] luego de una careera de intensidad moderada y en el AEC de competidores de judo luego de una prueba de ejercicio incremental maximal [67].

1.6 Mecanismos de producción de pro-oxidantes y sus efectos oxidativos inducidos por ejercicio

- **Mitoncodria:** El ejercicio físico incrementa la necesidad de resíntesis de energía en forma de adenosín trifosfato (ATP) en la mitocondria. Este organelo celular utiliza el oxígeno como aceptor final de electrones en la cadena que transporta de electrones (CTE) ubicada en su membrana interna. Este hecho trabaja de forma acoplada con la fosforilación oxidativa para la producción de energía. Aproximadamente entre un 2 – 4% del oxígeno que ingresa a la mitocondria para ser utilizado en la producción de energía, no logra formar agua junto al hidrógeno y un electrón, y es transformado en pro-oxidantes [20]. Los pro-oxidantes principales derivados del oxígeno son el ion superóxido ($O_2^{\cdot-}$) y peróxido de hidrógeno (H_2O_2) [68] y son producidos en los complejos proteicos I (NADH deshidrogenasa), II (Succinato dehidrogenasa) y III (Coenzima Q y citocromo C oxidasa/reductasa) de la CTE [69–71]. Entonces, un aumento en la actividad de la mitocondria durante el ejercicio favorecerá la producción de pro-oxidantes [72, 73]. Boveris et al. (2008), utilizando un modelo en ratas de ejercicio moderado observó un incremento en la producción de pro-oxidantes mitocondriales [73]. Sin embargo, no es la única fuente durante el ejercicio de alta intensidad, ya que se le ha entregado una mayor relevancia al incremento de la actividad de la xantino oxidasa-deshidrogenasa [74].

- **Xantino oxidasa-deshidrogenasa (XOD):** Este mecanismo participa en la reacción que cataliza la formación de xantina desde hipoxantina, y luego a ácido úrico. Es considerada como una fuente importante de pro-oxidantes durante los procesos de isquemia y reperfusión [75, 76]. Asimismo, ha sido demostrado un incremento de su actividad durante el ejercicio en modelos animales [77] y humanos [7, 78]. Utilizando un modelo de contracción muscular en ratas Gómez-Cabrera et al. (2010) observaron un incremento de $O_2^{\cdot-}$, el cual fue atribuido a la activación de la XOD [79].

- **NADPH oxidasas (NOXs):** son 7 tipos de enzimas flavoproteicas asociadas a membranas celulares que actúan como donador de electrones en la reacción que reduce el oxígeno a ion superóxido. El $O_2^{\cdot-}$ producido, al entrar con la enzima antioxidante superóxido dismutasa (SOD), incrementará la producción de H_2O_2.

También es posible que disminuyan las concentraciones de $ON\cdot$ al unirse con el O_2^{-}, para incrementar los niveles de $ONOO^{-}$. El estudio sobre la estimulación en la actividad de estos complejos proteicos inducida por ejercicio, se ha centrado principalmente en células fagocitarias, musculares lisas de vasos sanguíneos, fibroblastos, miocitos cardiacos y esqueléticos. En este último, el Ca^{+2} y ejercicio incrementa la actividad de la NOX, elevando la producción de pro-oxidantes [4, 80]. Asimismo, el daño producido por ejercicio de alta intensidad favorece la estimulación de la NOX por la activación de la fosfolipasa A_2, incrementando la producción de pro-oxidantes [80].

- **Ácido araquidónico:** Este elemento también se encuentra relacionado con la oxidación, ya que durante su paso por la via de la ciclo-oxigensa o lipo-oxigenasa para la formación de prostaglandina y leucotrienos favorece el incremento en la formación de pro-oxidantes ERO [81].
- **Miostatina:** Bloqueador de la diferenciación celular, ha sido identificada también como un inductor de la producción de pro-oxidantes radicales de oxígeno. La miostatina es capaz de incrementar la producción de pro-oxidantes ERO via canonical Smad3, NFkB y factor de necrosis tumoral alfa (TNF-a) [82]. También la miostatina puede inducir la producción de ERO a través de las vias de las proteínas kinasas activadas por mitogenes (MAPK) mediadas por TNF- a, IL-6, NOX y XOD [83].

1.7 Efectos del ejercicio sobre el estado redox e inflamatorio pulmonar

Tal como se mencionó anteriormente, el ejercicio puede favorecer el incremento de los pro-oxidantes en el organismo. El pulmón, es uno de los órganos que puede salir más afectado, ya que un incremento en la ventilación minuto durante un ejercicio intenso y prolongado causará estrés mecánico epitelial [84] y favorecerá el ingreso de aire seco y frío [85, 86], deshidratando las vías aéreas [87]. Asimismo, el mayor flujo de aire favorece el contacto con sustancias ambientales irritativas, tales como el ozono, material particulado y los óxidos de nitrógeno y azufre, instaurándose un ambiente oxidativo e inflamatorio [88–90]. A pesar del enorme respaldo científico-teórico, los resultados entre algunos grupos de investigación han sido controversialesm[38, 62, 91–99]. Las diferencias en los resultados de estos estudios dependerán del tipo de ejercicio (laboratorio o ambiente, cicloergómetro o trotadora), los parámetros (intensidad y duración), nivel deportivo de la muestra (elite o

recreativo), técnicas de análisis (EI, AEC, lavado bronquioalveolar), entre otros. Novak et al. [93] y Araneda et al. [38] no lograron encontrar un incremento en la concentración de H_2O_2 en el AEC de humanos luego de ejercicio moderado y máximo a baja altura (670 y 2160 metros sobre el nivel del mar), respectivamente. Marek et al. [96] y [100] tampoco encontró cambios en las concentraciones de H_2O_2 luego de ejercicio submáximo (6 minutos a 60 W + 5 min a 120 W) y máximo (300 W) en cicloergómetro. Sin embargo, un estudio de Araneda et al. [14], observó un incremento del H_2O_2 y nitritos en el AEC de corredores luego de competencias urbanas de 21 y 42.2 kilometros. Matsumoto et al. [101] estudiando el $ON\cdot$ en el aire exhalado, observó un incremento en las concentraciones en un ejercicio incremental hasta la fatiga. Maroun et al. [102] también encontró incrementos en el $ON\cdot$ durante el ejercicio incremental maximal en deportistas, pero no en sujetos no deportistas. En otro estudio, Bonsignore et al. [94] encontraron incrementos en el $ON\cdot$ exhalado luego de un maratón.

Algunos grupos de investigación han estudiado los productos del estrés oxidativo luego de realizar ejercicio, por ejemplo Radak et al. [103] observaron incrementos de grupos carbonilos en pulmones de ratas después de una carrera en trotadora hasta la fatiga [103, 104] y malondealdheido en ratas que nadaron durante 20 minutos [105] (Prigol et al., 2009). Asami et al. [106] encontraron incrementos de 8-OHdG en los pulmones de ratas después de ejercicio forzado en trotadora. Sin embargo, Nowak et al. [93] no observó diferencias en las concentraciones de especies reactivas de ácido tiobarbitúrico (TBARS) luego de ejercicio submáximo sobre un cicloergómetro (120 W x 6 minutos). Recientemente, Araneda et al. [14] no observaron variaciones en las concentraciones de MDA en el AEC de corredores de 10 y 42.2 km.

Respecto a las defensas antioxidantes, la CAT y SOD incrementaron su actividad en el pulmón luego de ejercicio agudo [107]. En ratas, Prigol et al. [105] encontró un incremento en la actividad de la CAT luego de que nadaron durante 20 minutos. Reddy et al. [108] también observaron un incremento en la SOD y glutatión transfersa, sin embargo encontraron una disminución leve en la actividad de la glutatión peroxidasa en ratas que nadaron hasta la fatiga.

La generación de pro-oxidantes y daño oxidativo se encuentran relacionados con el proceso inflamatorio. Como ya se nombró anteriormente, las células inmunes son reconocidas como fuente de pro-oxidantes (Bréchard and Tschirhart et al., 2008). El incremento de sustancias pro-inflamatorias (citoquinas y derivados del ácido araquidónico) y en la activación de células inmunes se encuentra bien documentado [109]. En el contexto inflamatorio, el ejercicio agudo ha demostrado incrementar la

actividad de las células inmunes, sobre todo cuando este resulta extenuante. Bonsignore et al. [94] reportó un nivel más elevado de leucocitos polimorfonucleares en el esputo inducido de corredores luego de una maratón. Además, Pucsok et al. [67] observó incrementos en las concentraciones de protaglandina E_2 y tromboxano B_2 en el AEC de competidores de judo después de ejercicio maximal en trotadora. En carreras de 21 kilómetros se han reportado aumentos de la interlequina-8 en el sobrenadante de muestras de esputo inducido [98].

Por otro lado, se ha observado una inflamación persistente como efecto crónico del ejercicio en algunos grupos de deportistas. De esta forma, estudios han observado un incremento en las concentraciones de células polimorfonucleares, interlequina-8, leucotrienos E_4 e histamina en el sobrenadante de muestras de EI [Denguezli et al. 1998]. Sujetos que han participado en entrenamientos de alto rendimiento obtuvieron valores más bajos de pH en el AEC respecto a grupo control [110]. En biopsias bronquiales de esquiadores, se observó un incremento en la cantidad de neutrófilos, eosinófilos, macrófagos y linfocitos T comparado con control [92]. Existe una estrecha relación en ambas direcciones entre la producción de pro-oxidantes junto con la producción y activación de mediadores, y células inflamatorias [111], y el pulmón no es la excepción.

2. Objetivos

A pesar de toda la evidencia observada, aún es necesario estandarizar las cargas y duración del esfuerzo realizado, las características del muestreo, almacenamiento, técnicas de análisis, entre otras. Para poder resolver algunas de estas dudas y descubrir algo más sobre los efectos oxidantes e inflamatorios del ejercicio sobre el pulmón, se han propuesto los siguientes objetivos:

2.1 Objetivo 1 (Artículo Científico 1)

Dar a conocer toda la información científica actualizada existente sobre la producción de pro-oxidantes e inflamación pulmonar inducida por ejercicio en modelos animales y humanos sanos (deportistas o no deportistas), clasificándolos según las características de ejecución del ejercicio en agudo o crónico (entrenamiento).

2.2 Objetivo 2 (Artículo Científico 2)

Evaluar los efectos de la intensidad y duración de un ejercicio agudo en terreno sobre la producción de pro-oxidantes e inflamación pulmonar de deportistas recreativos sanos, medidos en el aire exhalado condensado.

2.3 Objetivo 3 (Artículo Científico 3)

Evaluar los efectos de la intensidad y duración de un ejercicio agudo en condiciones controladas de laboratorio, sobre la producción de pro-oxidantes e inflamación pulmonar de sujetos sanos activos, medidos en el aire exhalado condensado.

3. Informe del Director sobre los Artículos Científicos Publicados

El Dr. Oscar F. Araneda Valenzuela y la Dra. Teresa Carbonell Camós, como directores de la Tesis Doctoral presentada por Marcelo Rodolfo Tuesta Roa, hacen constar que el doctorando ha participado activamente en los artículos que forman esta memoria, tal y como queda reflejado en el orden y la composición de cada uno de ellos. El doctorando ha tenido un papel fundamental en el diseño experimental, obtención y tratamiento de los datos. También ha sido relevante su papel en el proceso de búsqueda de información, redacción y publicación de los resultados y conclusiones. Ha intervenido, en muchos casos como actor principal, en la redacción de los manuscritos y en el proceso de revisión por pares determinado por las revistas científicas.

Así mismo, los directores hacen constar que ningún coautor ha utilizado o utilizará estos artículos para su tesis doctoral.

Los factores de impacto de las revistas donde se han publicado y aceptado los artículos que conforman esta Tesis Doctoral son los siguientes:

ARTÍCULO CIENTÍFICO 1

Título: **Update on the Mechanisms of Pulmonary Inflammation and Oxidative Imbalance Induced by Exercise**

Autores: O. F. Araneda, T. Carbonell, and M. Tuesta.
Revista: Oxidative Medicine and Cellular Longevity
Volume 2016 (2016), Article ID 4868536, 23 pages
http://dx.doi.org/10.1155/2016/4868536

JCR impact factor (2016):	4.593	
JCR 5 years IF:	4.667	Q2 in Cell Biology

Participación del doctorando: Búsqueda de información bibliográfica. Metaanálisis de los resultados. Redacción del manuscrito y participación en el proceso de revisión.

ARTÍCULO CIENTÍFICO 2

Título: **Increase of pro-oxidants with no evidence of lipid peroxidation in exhaled breath condensate after a 10-km race in non-athletes.**

Autores: O. F. Araneda; R. Urbina-Stagno; M. Tuesta, D. Haichelis ; M. Alvear; M. P. Salazar and C. García.
Revista: Journal of Physiology and Biochemistry
March 2014, Volume 70, Issue 1, pp 107–115
DOI: 10.1007/s13105-013-0285-0

JCR impact factor (2014): 1.969
JCR 5 years IF: 2.000 Q3 in Physiology

Participación del doctorando: Participación en el protocolo de entrenamiento y obtención de las muestras. Responsable de la preparación, procesamiento y análisis de las muestras. Análisis estadístico de los resultados y elaboración de los gráficos. Participación en la discusión de los resultados. Colaboración en la redacción del manuscrito y en el proceso de revisión.

ARTÍCULO CIENTÍFICO 3

Título: **Effect of exercise duration on pro-oxidants and pH in exhaled breath condensate in humans**

Autores: M. Tuesta ; M. Alvear; T. Carbonell; C. García; R. Guzmán-Venegas and O. F. Araneda
Revista: Journal of Physiology and Biochemistry
June 2016, Volume 72, Issue 2, pp 353–360
DOI: 10.1007/s13105-016-0486-4

JCR impact factor (2016): 2.444
JCR 5 years IF: 2.000 Q2 in Physiology

Participación del doctorando: Elaboración del protocolo de entrenamiento. Obtención de las muestras. Responsable del almacenamiento, la preparación, procesamiento y análisis de las muestras. Análisis estadístico de los resultados y elaboración de los gráficos.

Participación en la discusión de los resultados. Colaboración en la redacción del manuscrito y en el proceso de revisión.

Firmado:

Dr. Oscar F. Araneda Valenzuela

Dra. Teresa Carbonell Camós

4. Artículos Científicos

A continuación se exponen 3 artículos científicos derivados de esta tesis:

- El primero es una revisión bibliográfica **(artículo científico 1)**, aquí se han descrito los los efectos y mecanimos implicados en la producción de pro-oxidantes, daño oxidativo e inflamación pulmonar inducidos por ejercicio físico. Los desencadenantes de ambas respuestas fisiopatológicas son el enfriamiento de las vías respiratorias, la evaporación de fluidos de la superficie epitelial, el aumento del contacto con sustancias contaminantes y la activación de la respuesta inflamatoria local y sistémica. Esta revisión contiene el más amplio cuerpo de evidencia científica sobre los efectos del ejercicio en la respuesta oxidativa e inflamatoria en sujetos sanos con distintos niveles de entrenamiento físico, incluyendo diferentes tipos de ejercicio en términos de duración e intensidad, efecto agudo y/o crónico, y la influencia de condiciones ambientales especiales tales como clima frío, altitud y contaminación del aire. Los resultados que fueron recolectados desde los artículos científicos se obtuvieron desde participantes (animales o humanos) que formaron parte del grupo experimental, control o placebo, siempre y cuando cumplieran con la condición de ser normales sanos. Los niveles de pro-oxidantes y antioxidantes, productos del daño oxidativo a las biomoléculas y celularidad, así como los niveles de mediadores solubles de la respuesta inflamatoria y sus efectos sobre los tejidos, se describen en todos los tipos de muestras pulmonares, incluyendo las invasivas como los homogeneizados de tejido pulmonar, líquido de lavado broncoalveolar y biopsias, otra semi-invasiva como el esputo inducido y otras no invasivas derivadas del aire exhalado (AE y AEC). En el documento se destaca la masificación del uso del AEC como una técnica no invasiva y confiable, principalmente en humanos. Por último, se destaca la necesidad de explorar simultáneamente los parámetros oxidativo e inflamatorio para comprender la interrelación entre ellos y el aporte individual al proceso.

- El segundo fue un experimento orientado a observar el efecto de la intensidad de un ejercicio físico sobre la instauración de un proceso oxidativo e inflamatorio en el pulmón de sujetos sanos levemente entrenados **(artículo científico 2)**. Para esto se estudiaron las variables oxidativas e inflamatorias en sujetos que realizaron ejercicio a alta intensidad, esto es una carrera de 10 kilómetros al aire libre. Aquí se compararon los niveles de peróxido, nitrito, malondealdeído y pH antes del ejercicio con los valores obtenidos 20 y 80 minutos luego de su finalización. De esta forma, se describen los efectos de un ejercicio intenso y del tiempo pos-ejercicio. Aquí se observaron los aumentos en los pro-oxidantes pulmonares con la técnica de AEC, pero no en el plasma sanguíneo, los cuales ocurrieron a mayor tiempo pos-ejercicio (80 min). A pesar del aumento de estas especies, no hubo aumento de la lipoperoxidación ni descenso significativo del promedio del pH, sin embargo se observó una

tendencia al descenso de este último a mayor oxidación. Por último, este estudio ayudó a demostrar el efecto localizado (pulmonar) del ejercicio, gatillado al parecer por los efectos de la hiperventilación descritos previamente sobre el epitelio respiratorio.

- El tercero fue un experimento que tenía por objetivo estudiar la oxidación e inflamación pulmonar inducida por ejercicio **(artículo científico 3)**, observando los efectos de la duración (controlando la intensidad). Para esto se compararon los niveles de pro-oxidantes y pH antes y después (80 min) de un ejercicio de baja intensidad (~30% VO_2 max). Aquí se pudo observar que una mayor ventilación y consumo de oxígeno, debido a una mayor duración, se encuentran relacionados con el aumento en la producción de pro-oxidantes, pero no de la inflamación pulmonar. Pareciera que esta última se ecuentra relacionada a otros aspectos del ejercicio, principalmente ambientales y mayor intensidad. Asimismo, el efecto localizado en el pulmón fue confirmado, reafirmando la idea de utilizar el ejercicio como un modelo de oxidación pulmonar, para el estudio entre otros, de mecanismos antioxidantes.

4.1 Artículo Científico 1: Update on the Mechanisms of Pulmonary Inflammation and Oxidative Imbalance Induced by Exercise (Objetivo 1)

Review Article

Update on the Mechanisms of Pulmonary Inflammation and Oxidative Imbalance Induced by Exercise

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Abstract

The mechanisms involved in the generation of oxidative damage and lung inflammation induced by physical exercise are described. Changes in lung function induced by exercise involve cooling of the airways, fluid evaporation of the epithelial surface, increased contact with polluting substances, and activation of the local and systemic inflammatory response. The present work includes evidence obtained from the different types of exercise in terms of duration and intensity, the effect of both acute performance and chronic performance, and the influence of special conditions such as cold weather, high altitude, and polluted environments. Levels of prooxidants, antioxidants, oxidative damage to biomolecules, and cellularity, as well as levels of soluble mediators of the inflammatory response and its effects on tissues, are described in samples of lung origin. These samples include tissue homogenates, induced sputum, bronchoalveolar lavage fluid, biopsies, and exhaled breath condensate obtained in experimental protocols conducted on animal and human models. Finally, the need to simultaneously explore the oxidative/inflammatory parameters to establish the interrelation between them is highlighted.

1. Introduction

When doing physical exercise, the usual levels of organic performance are exceeded. However, we are designed to execute the exercise, depending on its variety, duration, intensity, and the environmental conditions under which it is done. The physiological and pathological processes will be activated, which can lead to the generation of an oxidative imbalance and the establishment of an inflammatory process [1, 2]. The oxidative damage happens as an additional cost of using oxygen to obtain energy and can occur when there is an increase in the formation of prooxidants and/or when the antioxidant defense decreases, causing an alteration of tissue product functionality of the structural damage to all the cellular components that contain lipids, carbohydrates, proteins, and nucleic acids [3]. Another response mechanism to physical stress is inflammation, which is triggered as a reaction to the mechanical damage of structural components (connective tissue; muscle, tendon, and bone) and nonstructural components (erythrocytes, endothelium, and epithelia) of the body [4–8]. As a result, stress hormones are released, such as cortisol and catecholamines, which activates the immune system, causing a particular response profile based on the release of soluble mediators (cytokines) and arachidonic acid derivatives (prostaglandins and leukotrienes). The latter and the stress hormones will cause changes in the number and activation of leukocytes subpopulations to the point that intense exercise of long duration can induce immune suppression (increasing the susceptibility to infection) [9], in contrast to the exercise of moderate intensity, which boosts the immune response. Both the alteration of the redox system and the inflammatory reaction have multiple points of interaction that have been previously evidenced [10–12]. The study of inflammatory/oxidative damage at a pulmonary level has been a topic poorly addressed [13–15], particularly in healthy humans and even more so in athletes. Most of the information in this subject arises from pathophysiology of pulmonary diseases, such as asthma, cystic fibrosis, and chronic obstructive pulmonary disease [16–27]. The lung has the crucial role of gas exchange and experiences great modifications of its activity during the exercise. This mobilizes larger volumes of air and modifies the breathing pattern from nasal to oral, increasing contact with a greater amount of pollutants that may be present in the environment. Also, the lung receives a greater amount of blood flow to increase the exchange in places that are well ventilated, which causes changes in the functioning of the vascular parenchyma [28, 29]. However, the anatomo-functional characteristics of the lungs make it very difficult to obtain information of the redox/inflammatory state in the different sectors of this organ. This work brings together the scientific papers that have

addressed the phenomenon of altered pulmonary redox/inflammation environment induced by acute or chronic exercise, in a hypoxic environment, cold or contaminated, in both animal and human models, by focusing on the protocols and mechanisms that explain the phenomenon, as well as their potential implication on those who exercise.

2. Effects of Exercise on the Respiratory System and Its Relationship with the Generation of Oxidative/Inflammation Damage

When exercising, the mobilized air flow or pulmonary ventilation increases. This is explained by the increase of the respiratory rate, the tidal volume, and the appearance of bronchodilation. In addition to this, the pulmonary vascular bed will vasodilate to receive a greater blood flow. These changes, taken together, aim to increase gas exchange. Large air flows entering the lung during exercise will cause a modification of the breathing pattern towards one predominantly oral, favoring the evaporation of the fluid covering the pulmonary epithelium and the decrease of temperature of the airways. As a result, the pulmonary passages will cool down and the osmolarity of the epithelium will increase [30]. It should be noted that the cooling of the pulmonary passages as a result of the hyperventilation has been observed at comfortable environment temperature (+20°C) [31]. In this way, McFadden Jr. and Pichurko [31] showed a decrease of the tracheal temperature of 34°C at pulmonary ventilation of 15 L/min and of 31°C at 100 L/min. The cooling of the airway by hyperventilation produced by exercise is homologous to breathing cold air at rest. The latter is probably in the absence of air pollutants, the main irritative/proinflammatory factor of this region of our body. In cold environments, there is a greater amount of reports of respiratory symptoms [32] and chronic changes of epithelium similar to those of patients with chronically inflamed airways (e.g., asthmatics). Some authors observed, in humans, that the product of intense exercise appears to have similar symptoms to those observed in infection of upper airways [33–35]. However, with moderate training these symptoms decreased [36, 37]. It is probable that intense exercise of long duration, such as a marathon, will increase the susceptibility to infection of the airway by depression of the immune function, contrary to the effect caused by exercise of moderate intensity. Another factor involved in the oxidative/proinflammatory process of the airway is the greater contact with toxic particles and microorganisms present in the environment due to hyperventilation by exercise [38–40]. For example, the damaging effect on lung tissue of environmental substances such as chlorine, ozone, nitrogen oxides, particulate matter, and pollen is recognized [14, 41–43]. The entry of these substances by the

pulmonary route can potentially generate systemic inflammation [44, 45] and this will affect the lungs. Finally, another factor of the recognized destabilizing effect of the oxidative balance and in favor of pulmonary inflammation is hypoxia [46, 47]. The general framework for the development of functional changes of the lung by exercise, the activation of the redox imbalance, and the inflammatory system are described in Figure 1.

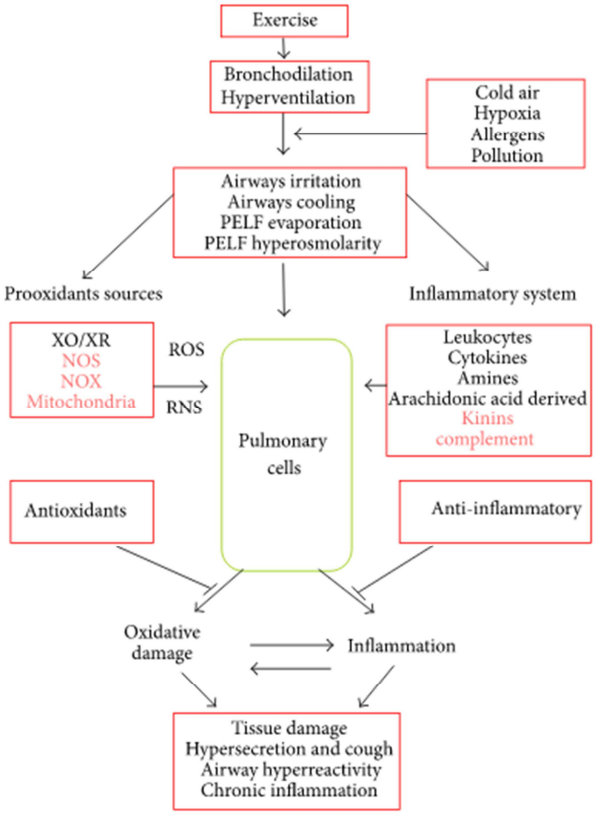

Figure 1. Proposed mechanisms related to the process of oxidative damage and pulmonary inflammation induced by exercise. Once the exercise starts the pulmonary ventilation increases and favors bronchodilation. This cools the airways, and also the part of PELF evaporates with subsequent increase of osmolarity and irritation appears. This activates the generating sources of free radicals and the inflammatory system. As a result of the foregoing, oxidative damage and a concomitant inflammatory process are potentially generated at pulmonary cell level; this may involve tissue damage, the increase of cough and the increased secretion of mucus, and the appearance of bronchoreactive phenomena and in the case that this stimulus is repeated (chronic exercise) to establish a process of chronic inflammation and remodeling of pulmonary tissue, particularly in the airways. This process is exacerbated when the exercise is performed in the presence of environmental conditions such as cold and hypoxia, in environments where pollen is abundant or in presence of contaminants (water/air). In red color the prooxidants sources and the parts of the inflammatory system that have not been studied are both appreciated. XO/XR = xanthine-oxidase/dehydrogenase; NOS = nitric oxide synthase; NOX = NADPH oxidase; PELF = pulmonary epithelial lining fluid.

3. Changes in Pulmonary Redox State and Exercise-Induced Inflammation

As mentioned previously, physical exercise induces changes in the redox/inflammatory state of the organism, at both systemic level and the different organs. In this regard, lung is one of the less studied organs in this context. In the following paragraphs, the most relevant results regarding pulmonary oxidative damage and inflammation caused by exercise are summarized. In this review, the work carried out in healthy subjects was privileged. Regarding the special conditions, hypoxia, water contaminants (chlorine), and cold have been included, leaving aside air pollutants, because there are several reviews regarding this subject [48, 49]. The details of the studies included in terms of goals, characteristics of the sample, the protocol used, and the results related to the pulmonary oxidative/inflammation damage by exercise are summarized in Tables 1 and 2 for human and animals, respectively.

4. Pulmonary Redox Balance and Acute Exercise

A direct relationship has also been reported during exercise, between the acute exercise intensity and the volume of exhaled nitric oxide (VNO), namely, volume minute (VE) multiplied by exhaled nitric oxide (eNO), for sedentary healthy [50, 60, 68, 69, 71, 82, 85–87, 90] and trained subjects [75, 89]. During exercise, eNO have been reported to be decreased when increasing VO_2 [59, 75] and VE [75] in sedentary and active subjects [51, 60, 68, 69, 75, 82, 85, 86, 92]. In athletes, unlike Maroun et al. [75], Kippelen et al. [68] showed changes in eNO during exercise. In animal model, while exercising healthy horses, Mills et al. [112] observed a linear increase of the VNO as the oxygen consumption increased. After exercise, nitric oxide concentrations have shown controversial results. In swimmers, Bonsignore et al. [57] reported a decreased eNO after 5 km (~179 min) in slightly chlorinated pool; when performing the same test at the sea no changes were observed in this pair but the same distance was maintained at the sea. In other studies, also a decreased eNO after exercise has been observed in healthy subjects [64, 70, 88, 91]. However, in youngsters not trained in swimming, Carbonnelle et al. [58] found increases of eNO after swimming 2 sessions of ~1300 m in 45 min in a pool sanitized with electrical process (nonchlorinated water). Also, De Gouw et al. [61] found an increased eNO in healthy subjects after cycling for 6 min using dry air, while ventilation was kept constant in 40–50% of his or her predicted maximal voluntary ventilation (35 × FEV_1). Other studies showed no changes in the eNO after exercise; Font-Ribera et al. [65] found no differences

in eNO concentrations in pool swimmers; the same occurred with eNO in swimmers after an exercise of 45 min [81] and in healthy subjects after either cycloergometer [66, 94] or treadmill incremental exercise test [80].

Through the exhaled breath condensate (EBC) analysis, to observe the oxidative effects of the moderate acute exercise, Nowak et al. [79] subjected a group of healthy subjects to a submaximal exercise on cycloergometer during ~6 min; they found no changes in H_2O_2 and thiobarbituric acid reactive substances (TBARs). Araneda et al. [46] found no changes of H_2O_2 in EBC after three maximal cycle ergometries of 1 min in elite cyclists carried out at 670 and 2160 masl, but malondialdehyde (MDA) was higher at 2160 meters. Marek et al. [72], in two submaximal cycle ergometries to 60 W (~7 min) and 120 W (~5 min), and later in maximal exercise (~13 min), found no differences in H_2O_2 concentration in EBC [73]; however, in both studies, increases were found in the flow of formed H_2O_2 after exercise. On the same prooxidant, Mercken et al. [76] found an increase after maximal cycle ergometry in healthy subjects, with increments of 10 w/min, but they did not find any differences in subjects with chronic obstructive pulmonary disease after exercise. However, in another study they found no differences in H_2O_2 when healthy subjects performed a cycle ergometry with one leg (40% of maximum power output) during 20 min [77]. Marek et al. [74] found that, after 50 min of high intensity running developed at ~18°C of environmental temperature, the concentration and production rates of H_2O_2 in EBC were higher when the exercise was carried out in a cold environment. Recently an increase in H_2O_2 and nitrite concentrations and correlations between both metabolites in the EBC of 21 and 42.2 km race participants were found. Also in this study, while nitrite increased in EBC, plasmatic nitrite showed no modifications and no correlations between these variables, which suggests a probable localized origin of this process [53].

Table 1: (a) Human studies on lung oxidative stress and inflammation induced by acute exercise. (b) Human studies on lung oxidative stress and inflammation induced by chronic exercise.

(a)

Author, year	Aim	Sample's characteristics	Exercise protocols	Samples obtained	Oxidative or inflammatory main results
Adachi et al. 1997 [50]	eNO and VNO in patients with CHF during exercise	CHF patients and healthy control subjects (C)	Maximal incremental cycloergometer test in CHF patients (10 W/min) and C (25 W/min) until exhaustion	EB	DE: ↑ VNO during exercise peak in C
Agostoni and Bussotti 2003 [51]	Correlation between eNO and lung mechanics during exercise in CHF	CHF patients and healthy control subjects (C)	25-W constant workload exercise cycle-ergometry test	EB	DE: ↓ eNO during 3rd and 5th minutes of exercise in C
Araneda et al. 2005 [46]	Lung oxidative damage from exercise at a medium altitude	Highly trained mountain bikers	Three repetitions of cycle-ergometries of 1 min at maximum intensity in 670 and 2160 MASL with breaks of 1 min	EBC and serum	PE: ↑ [MDA] in EBC, with no changes in serum at 2160 MASL
Araneda et al. 2012 [52]	Duration of a long distance exercise on pulmonary oxidative damage	Amateur runners	Urban 10 km (~53 min), 21 km (~101 min), and 42.2 km races (~246 min)	EBC	PE: ↑ [H_2O_2] and ↑ [] in 21 km and 42.2 km races and no changes in [MDA]; there was a tendency to ↓ of pH
Araneda et al.	Pulmonary oxidative	Healthy active subjects	10 km race in outdoor athletic track	EBC	PE: ↑ [H_2O_2], ↑ with no

2014 [53]	damage in long distance exercise		(~50 min)		changes in the [MDA]; there was a tendency to ↑ of pH
Bikov et al. 2010 [54]	Changes in [Cys-LTs] caused by exercise in asthmatic patients	Nonsmoking asthmatic patients (A) and nonsmoking healthy control subjects (C)	Race on treadmill at a speed and slope maintaining 80–90% (220 − age), which was regulated in 2 min and then maintained during 6 min	EBC	PE: with no changes in [Cys-LTs] in C, but ↑ in A
Bikov et al. 2014 [55]	Changes in during EIB in asthmatic patients	Asthmatics, who reported breathlessness following exercise, and healthy control subjects (C)	Exercise challenge test on a treadmill (details were not described by authors)	EBC and EB	PE: no change of pH in EBC in C
Bonsignore et al. 2001 [56]	Endurance exercise on inflammatory cells in AWs and eNO	Amateur runners	Marathon race (~179 min)	IS and EB	PE: ↑ PMN in IS and ↑ eNO in EB
Bonsignore et al. 2003 [57]	Swimming on inflammatory cells and eNO in the AWs	Swimmers (S) and healthy control subjects (C)	Swimming of 5 km only in the swimmers group, an open pool series (~70 min) and other series in the sea (~54 min)	IS and EB	B: >PMN and <MØ in the IS of S versus C PE: ↑ eosinophils, ↑ lymphocytes, and ↓ MØ in the sea versus swimming pool; eNO was > in the sea in comparison to swimming pool
Carbonnelle et al. 2008 [58]	eNO after swimming sessions	Trained healthy young people, not trained with swimming	Swimming in 2 sessions of 45 min (~1300 m), in a disinfected pool with [NaClO] and another sanitized with electrical process	EB	PE: ↑ eNO only in sanitized pool

Chimenti et al. 2009 [40]	Inflammation of the AWs in urban races in different climatic seasons	Amateur runners	21 km race in autumn (~89.1 min), 12 km race in winter (~46.1 min), and 10 km race in summer (~35.4 min)	IS	B: ↑ PMNs with ↑ [TNF-a] and ↑ [IL-8] PE: PMNs tended to ↑
Chimenti et al. 2010 [5]	Damage and inflammation of the lung epithelium in a long distance exercise	Amateur runners and healthy control subjects	20 km outdoor races (~90 min)	IS and serum	PE: ↑ [IL-8] in IS and ↑ CC16 in serum
Chirpaz-Oddou et al. 1997 [59]	eNO and VNO during exercise	Healthy control and trained subjects	Incremental cycloergometry to exhaustion with 5 min of passive recovery in sedentary subjects (♂ ~30 min and ♀ ~20 min) and trained subjects (~14 min)	EB	DE: ↓ eNO progressive with ↑ exercise intensity from 65% VO_2max and ↑ VNO with the ↑ of the intensity of exercise > 30 W in all subjects
Clini et al. 2000 [60]	To evaluate eNO during exercise in patients with stable COPD	COPD patients and healthy control subjects (C)	Maximal cycle-ergometry test (cadence: 60 cycles/min and load: 10 W/min) until exhaustion	EB	DE: ↓ eNO at peak exercise and ↑ VNO in C
De Gouw et al. 2001 [61]	Role of eNO in the airway response to exercise by using L-NMMA, L-arginine, or placebo as pretreatment to exercise challenge	Asthmatic patients and healthy control subjects (C)	Cycle-ergometry for 6 min using dry air, while ventilation was kept constant in 40–50% of his or her predicted maximal voluntary ventilation (35 × FEV_1)	EB	PE: ↑ eNO 30 min after exercise in C
Denguezli-Bouzgarrou et	Endurance exercise and inflammatory cells	Long-distance runners	Races on treadmill at 80% of MAS (~60 min)	IS	PE: ↑ PMNs, ↓ MØ, and ↑ lymphocytes

al. 2006 [62]	of the AWs				
Denguezli-Bouzgarrou et al. 2007 [63]	Inflammatory mediators, cellular composition in AWs, and acute exercise during a sports season	Long-distance runners	Race at 80% MAS during the basic, precompetitive, and competitive period of a sport season in 1 year (~60 min)	IS	PE: ↑ PMNs in the precompetitive and competitive period. ↑ MØ in the precompetitive period; also, ↑ [histamine], ↑ [IL-8], ↑ [LTB_4], and ↑ [LTE_4] in the competitive phase
Evjenth et al. 2013 [64]	To investigate the effect on of a standardized exercise challenge test on a treadmill	Nonasthmatic children with and without allergic rhinoconjunctivitis (AR) symptoms	Run on treadmill (6 to 8 min); heart rate target during the last 4 min was 95% of predicted maximum heart rate (220 − age)	EB	PE: ↓ eNO in nonasthmatic children without allergic rhinoconjunctivitis
Font-Ribera et al. 2010 [65]	Inflammation and postexercise pulmonary oxidative stress	Healthy subjects	Swimming in a chlorinated indoor-swimming pool (40 min), whose average speed was 22.5 ± 9.7 m/min	EBC and EB	PE: no changes of eNO in EB; [RANTES], [IL-12p70], [IFN-γ], [IL-4], [IL-8], [IL-10], [IFN-γ-induced protein 10], [TNF], [VEGF], and [8-isoprostane] in the EBC were not modified
García-Río et al. 2006 [66]	before and after exercise challenge in patients with asthma and its relationship with airway obstruction	Nonsmoking, steroid-naïve, atopic patients with mild persistent asthma and nonsmoking, nonatopic, healthy subjects (C)	Performing an exercise challenge on a cycloergometer, with monitored ventilation (exercise parameters were not presented)	EB	PE: with no changes in eNO of healthy subjects
Hopkins et al.	Pulmonary capillary	Athletes with signs of	4 km cycling with 12% hill sloping during	BALF	PE: >alveolar MØ, >[LTB_4],

1997 [67]	pressure and function of the alveolar-capillary barrier during intense exercise	hemoptysis by exercise and healthy control subjects	~7 min		and < lymphocytes in athletes versus control subjects
Kippelen et al. 2002 [68]	eNO level in endurance-trained athletes during and after intense exercise	Nine athletes with exercise-induced hypoxaemia (EIH), 12 athletes without EIH, and 10 untrained subjects	15 min intense cycling exercise at 90% VO_2max	EB	DE: ↓ eNO and ↑ VNO (last 3 minutes) in all groups
Larsson et al. 1998 [32]	Cold air and inflammation in the AWs during rest and exercise	Healthy subjects	Race on treadmill at −23°C and +22°C, each with 4 stages with 15 min at moderate intensity and 15 min of recovery	BALF	PE: at −23°C ↑ granulocytes and ↑ MØ; no changes in [IL-8]
Lovell et al. 2000 [69]	eNO and incremental exercise test in chronic congestive cardiac failure	Chronic congestive cardiac failure patients and healthy control subjects (C)	Performing Bruce protocol modified by inclusion of an initial 3 min stage at 5% incline, later performing a constant workload test (6 min at 2.7 km h^{-1} and 5% incline)	EB	DE: ↓ eNO and ↑ VNO during Bruce test in C; ↑ VNO during constant workload test
Mantione et al. 2007 [70]	eNO breath levels just before engaging in their respective activity	Healthy control subjects	Going up and down the stairs on a 20-foot staircase for 2 min	EB	PE: ↓ eNO 1 minute after exercise
Matsumoto et al. 1994 [71]	eNO and VNO during exercise	Healthy subjects	Cycle-ergometry at 100 W and maximum intensity with 5 min of recovery (~13 min)	EB	DE: ↑ VNO at 100 W and at maximum pedaling intensity

Marek et al. 2008 [72]	[L-lactate] and [H_2O_2] during exercise	Healthy subjects	Cycle-ergometer steady-state exercise at 60 W (~7 min) and 120 W (~5 min)	EBC	DE: ↑ [L-lactate] and ↑ [H_2O_2] in 60 W and 120 W
Marek et al. 2009 [73]	Maximal exercise, H_2O_2release rate, and acid-base status	Amateur athletes	Incremental cycloergometry to exhaustion (~13 min)	EBC	PE: ↑ [H_2O_2] with no changes in pH
Marek et al. 2013 [74]	Exercising in cold weather and release of	Healthy subjects	Races on treadmill at 75–80% at ~18°C and ~−15°C (~50 min)	EBC	PE: ↑ [H_2O_2] and ↑ rate of H_2O_2 release in both temperatures
Maroun et al. 1995 [75]	Physical condition and release of eNO during exercise	Healthy sedentary subjects (S), active subjects (Ac), and athletes (A)	Cycle-ergometries in steady-state at 1 and 2 L/min of VO_2only performing an additional one at 4 L/min of VO_2	EB	PE: ↓ eNO at >VO_2 in S and Ac; ↑ lineal of VNO with ↑ VO_2 in A
Mercken et al. 2005 [76]	Exercise-induced oxidative stress in COPD	COPD patients and healthy control subjects (C)	Incremental cycle-ergometry exercise test until exhaustion and submaximal constant work rate exercise test (60% maximal power output)	EBC	PE: ↑ [H_2O_2] in maximal but not in submaximal exercise in C
Mercken et al. 2009 [77]	Pulmonary oxidative stress by endurance exercise in COPD and healthy subjects	COPD patients and healthy control subjects	Cycle-ergometry on one leg at 40% of maximum power output (20 min)	EBC	PE: ↑ [H_2O_2] in COPD patients but not in healthy control subjects
Morici et al. 2004 [78]	VE during exercise and inflammation in the AWs	Young rowers	Maximal run of 1000 m on the rower ergometer (~3 min)	IS	DE: ↑ tendency in epithelial cells at a higher VE PE: ↑ MØ with both ↑ VE/kg

					and ↑ VT/kg
Nowak et al. 2001 [79]	Prooxidants and oxidative damage by moderate exercise	Healthy subjects	Cycle-ergometer exercise test at 120 W during 6 min or until a HR of 120 bpm is reached	EBC	PE: with no changes in [H_2O_2] and [TBARs]
Nadziakiewicz et al. 2006 [80]	Effects of the physical activity on eNO levels in healthy subjects and in CAD patients	CAD patients and healthy control subjects smokers and nonsmokers	Bruce protocol exercise test	EB	PE: without changes in eNO in healthy control subjects nonsmokers
Pedersen et al. 2009 [81]	Inflammation in the AWs after 1-exercise session	High performance swimmers	Swimming in indoor-swimming pool at moderate intensity (45 min) whose average heart rate was 162 bpm	EBC and IS, EB	PE: no changes in the cellular composition in IS, eNO in EB, nor pH in EBC of swimmers
Pogliaghi et al. 1997 [82]	VNO after modifying pulmonary blood flow with head-out water immersion or increased gravity at rest and during exercise	Nonsmokers and healthy subjects who underwent air with normal conditions, water immersion, or increased gravity (1 Gz or 2 Gz)	Incremental cycle-ergometry test, loading was increased progressively by 50 W every 3 min until voluntary exhaustion	EB	DE: ↓ eNO and ↑ VNO in all groups
Pucsok et al. 2007 [83]	Lung PGE_2 and TXB_2 and exercise	Judo competitors	Incremental run on treadmill until VO_2max is reached (run time was not recorded)	EBC	PE: ↑ [PGE_2] and ↑ [TXB_2] in ♂, but not in ♀
Riediker and Danuser 2007 [84]	Low-intensity physical activity and pH	Healthy subjects	Walk on treadmill at 60% maximal predicted heart rate with 1 min pause every 10 min (~30 min)	EBC	PE: ↑ pH

Riley et al. 1997 [85]	NO production in patients with abnormalities of the pulmonary circulation	PPH (primary pulmonary hypertension), PF (pulmonary fibrosis), and normal subjects group	Maximal (20 W/min in the normal subjects and 15 W/min in the PF patients and individual estimated exercise tolerance in PPH patients) and submaximal constant work rate cycle-ergometry exercise test (work rate VO_2 midway between each patient's anaerobic threshold and VO_2max)	EB	DE: ↓ eNO and ↑ VNO in normal subjects at peak exercise in maximal and constant work rate exercise test
Rolla et al. 2003 [86]	Relationship between eNO and exercise tolerance in patients with moderate MS	Patients with moderate MS and healthy control subjects (C)	Symptom-limited incremental exercise test with an upright cycle-ergometer (25 W every 3 min until exhaustion)	EB	DE: ↓ eNO and ↑ VNO in all groups at the end of exercise
Shin et al. 2003 [87]	Relationship between exercise and NO exchange	Nonsmoking healthy adults	High-intensity exercise treadmill test at 90% of the predicted maximum heart rate (220 − age in years) for 20 min	EB	PE: ↑ VNO
St Croix et al. 1999 [88]	Effect of exercise on endogenous NO formation by measuring eNO at a constant airflow rate	Healthy, nonasthmatic, and nonsmoking subjects	3 min of constant-load cycle-ergometry exercise test at three different exercise intensities corresponding to 30%, 60%, and 90% VO_2max	EB	PE: ↓ eNO and ↑ VNO for all intensities of exercise in healthy subjects
Therminarias et al. 1998 [89]	Exercise in cold air on eNO and VNO	Highly trained subjects (cross-country skiers, triathlon, and running)	Incremental cycloergometry to exhaustion in a climate chamber at +22°C and −10°C (~30 min)	EB	DE: ↓ eNO with the ↑ of the intensity >60 W in +22°C and ↑ VNO with the ↑ of the intensity >30 W in both temperatures
Trolin et al. 1994 [90]	eNO and VNO during exercise	Healthy subjects	Moderately heavy exercise on a cycloergometer (♀: 90 W for women and ♂:	EB	DE: ↓ eNO

			150 W for ♂)		
Tufvesson et al. 2013 [91]	Relationship between CC16 levels in plasma and urine after exercise with exhaled breath temperature and eNO	Asthmatic and healthy control subjects	During first six minutes speed and slope were adjusted to maintain the heart rate subject to 90% of their theoretical maximum heart rate (220 − age); the next two minutes were adjusted again to reach maximum effort	EB	PE: ↓ eNO in both groups
Verges et al. 2006 [92]	Effect of prolonged exercise on the NO concentration in the lung	Nonsmokers undertaking a moderate to intense training program participated in the study	100 min exercise test was performed on a cycle-ergometer (5 min of rest, 30 min warm-up at 25% Wmax, 10 min at 60% Wmax, 2 min at 25% Wmax repeated five times (S1 to S5), and 10 min of active recovery at 25% Wmax)	EB	DE: ↓ eNO for all exercise sessions (WU, S1 to S5, and active recovery)
Wetter et al. 2002 [93]	EIAH and pulmonary inflammation	Endurance athletes with EIAH who used anti-inflammatory or placebo	Maximal incremental run on treadmill to exhaustion (~18 min)	IS	PE: with no PMNs, lymphocytes, nor MØ; ↑ [Histamine] in placebo
Yasuda et al. 1997 [94]	To examine the origin and role of eNO during exercise	Healthy control subjects	Two sets of 10 minutes in a cycle-ergometer (5 min without load and 5 minutes with 60 W and 60 RPM) separated, with 15 minutes between them	EB	DE: with no changes in eNO
Zietkowski et al. 2010 [95]	To assess the possible association of EIB with low-grade systemic inflammation in asthmatic patients	Asthmatics (14 with EIB, 10 without EIB) and healthy volunteers	Cycle-ergometer test for 9 min with a fixed workload adjusted to increase the heart rate to 85% of the maximum predicted for the age of each patient	EBC	PE: with no changes in hs-PCR in healthy volunteers

AWs: airways; BALF: bronchoalveolar lavage fluid; CAD: coronary artery disease; CC16: Clara cell secretory protein; CHF: chronic heart failure; COPD: chronic obstructive pulmonary disease; Cys-Lts: cysteinyl leukotrienes; EB: exhaled breath; EBC: exhaled breath condensate; EIAH: exercise-induced arterial hypoxemia; EIB: exercise-induced bronchoconstriction; eNO: exhaled nitric oxide; : fractional exhaled nitric oxide; : bicarbonate; H_2O_2: hydrogen peroxide; HRmax: maximum heart rate; IFN-γ: interferon gamma; IFN-γ-induced protein-10: interferon-gamma-induced protein-10; IL-12p70, IL-4, IL-8, and IL-10: interleukin-12p70, interleukin-4, interleukin-8, and interleukin-10; IS: induced sputum; L-NMMA: N-monomethyl-L-arginine; L-lactate: lactate; LTB_4: leukotriene B_4; LTE_4: leukotriene E_4; MØ: macrophages; MAS: maximal aerobic speed; MS: mitral stenosis; MDA: malondialdehyde; MPO: myeloperoxidase; MASL: meters above sea level; NaCLO: sodium hypochlorite; : nitrite; NO output: nitric oxide output (eNO × VE); PGE_2: prostaglandin E_2; : maximal power output; RANTES: regulated upon activation, normal T-cell expressed, and secreted; TBARs: thiobarbituric acid reactive species; TNF(-α): tumor necrosis factor (alpha); TXB_2: thromboxane B2; Se: selenium; VE: minute ventilation; VEGF: vascular endothelial growth factor; VNO: volume of nitric oxide; VO_2max: oxygen uptake (maximal); VT: tidal volume. In "Oxidative or inflammatory main results," DE: during exercise and PE: postexercise. In "Aim," the effect of exercise was not the primary aim of the study.

(b)

Author, year	Aim	Sample's characteristics	Experimental protocols	Samples obtained	Oxidative or inflammatory main results
Belda et al. 2008 [96]	Type of sport (aquatic or terrestrial) and cell count	Elite healthy athletes and with asthma	Comparison of baseline samples between healthy and asthmatic athletes who practice water sports in pools or terrestrially (T: ~20 h/wk, with the exception of healthy subjects in water with T: ~10 h/wk)	IS	There was a positive correlation between PMNs with training time and water sport in the pool
Carraro et al. 2006 [97]	eNO in regular attendance to swimming pools	Children swimmers attending and control children not attending the swimming pool	Comparison of baseline samples between swimmers who attended a swimming pool (1 h/week/6 months) and control subjects	EB	There were no differences in eNO between both groups
Ferdinands et al. 2008 [98]	Exercise in contaminated environment and inflammation	Cross-country athletes and healthy control subjects	Comparison of baseline samples before and after 10 workouts in 15 d (~1 h/d)	EB	<pH in cross-country athletes compared to their control subjects between their respective sample times
Heinicke et al. 2009 [47]	Pulmonary oxidative damage and prolonged stay in medium height training	Biathletes and sedentary control subjects	Comparison of baseline samples between biathlete (T: ~5 h/wk) and control subjects; both groups were exposed to 2800 MASL during the 6 weeks	EBC	$[H_2O_2]$ and [8-isoprostane PGF_2a] with no differences between groups; by gathering data ↑ $[H_2O_2]$ and tendency to ↑ [8-isoprostane PGF_2a]

Helenius et al. 1998 [99]	AWs inflammation in swimmers	Elite swimmers and nonathletic control subjects	Comparison of baseline samples between swimmers (T: 800–3380 km/year) and control subjects	IS	>Eosinophils, >PMNs, >[EPO], and >[human neutrophil lipocalin] in swimmers in comparison to control subjects
Helenius et al. 2002 [100]	Retirement from swimming in relation to AWs inflammation	High performance swimmers	Comparison of baseline samples between active (T: ~1870 km/year) and inactive swimmers (3 months of inactivity)	IS	>eosinophils and >lymphocytes in active swimmers than inactive swimmers
Karjalainen et al. 2000 [101]	Inflammatory cells in skiers, mild asthmatics, and healthy control subjects	Elite healthy skiers and nonathletic control subjects	Comparison of baseline samples between skiers (T: 200–630 h/year) and control subjects	Endobronchial biopsy	>lymphocytes-T (43 times), >MØ (26 times), >eosinophils (2 times), and >PMNs (2 times) in skiers in comparison to control subjects
Martin et al. 2012 [102]	AWs inflammation and exposure to swimming pool in athletes	Endurance athletes	Comparison of baseline samples of pool based (5 h/wk) and non-pool-based (0.5 h/wk) athletes (T: ~15 h/wk)	EB and IS	PMNs and eosinophils in IS and eNO in EB were not different between groups
Sue-Chu et al. 1999 [103]	AWs inflammation in skiers	Cross-country skiers and nonathletic control subjects	Comparison of baseline samples during the competitive period, in autumn and winter, between skiers (T: 435 h/year) and control subjects	BALF	>total cells, >lymphocytes, and >mast cells in skiers in comparison to control subjects, with no differences in [TNF-a] and [MPO]
Sue-Chu et al. 2000 [104]	Budesonide and AWs inflammation in skiers	Elite cross-country skiers with asthmatic symptoms and budesonide or	Comparison of baseline samples among skiers, after 20 weeks of supplementation with 800 μg/d	BALF and endobronchial biopsy	Lymphocytes, MØ, eosinophils, PMNs, and mast cells were not different

	placebo supplementation	budesonide (T: ~427 h/year) or placebo (T: ~468 h/year)	between groups

AWs: airways; BALF: bronchoalveolar lavage fluid; EB: exhaled breath; EBC: exhaled breath condensate; EPO: eosinophil peroxidase; H_2O_2: hydrogen peroxide; IS: induced sputum; 8-isoprostane PGF2a: 8-isoprostane prostaglandin F_2 alpha; MØ: macrophages; MPO: myeloperoxidase; NO: nitric oxide; PMNs: polymorphonuclear neutrophils; T: training volume; TNF-a: tumor necrosis factor-alpha. In "Aim," the effect of exercise was not the primary aim of the study.

Table 2: (a) Animal studies on lung oxidative stress and inflammation induced by acute exercise. (b) Animal studies on lung oxidative stress and inflammation induced by chronic exercise.

(a)

Author, year	Aim	Sample characteristics	Exercise protocols	Samples obtained	Oxidative or inflammatory main results
Akil et al. 2015 [105]	Se administration affects lipid peroxidation in liver and lung tissues of rats subjected to acute swimming exercise	Sprague-Dawley adult male rats divide into general control, Se-administered, swimming control, and Se-administered swimming groups	Swimming was performed once for 30 minutes	Lung tissue	PE: ↑ MDA and ↑ GSH in swimming control versus general control
Al-Hashem 2012 [106]	VitE and VitC in protection of pulmonary damage induced by exercise in altitude	Wistar rats with 6 months of altitude adaptation	Forced swimming for 2.5 h in glass tank at 600 and 2270 MASL in accordance with altitude adaptation	Lung tissue	PE: ↑ [TBARs], ↓ SOD, and CAT activity at 600 MASL Supplementation with VitE and VitC reversed these results
Caillaud et al. 1999 [107]	Effect of acute exercise on lipid peroxidation in lung compared with locomotor muscles	Wistar rats exercised (E) and control rats (C)	Race on treadmill at 28 m/min and 15% grade (80–85% VO_2max) until exhaustion (~66 min)	Lung tissue	PE: no changes of pulmonary activity of SOD, CAT, and [MDA] of E in comparison to C
Cathcart et al. 2013 [108]	Effects of exercise during different ambient temperatures and humidity on eNO, eCO, and pH	Thoroughbred racehorses	Exercised under saddle on an all-weather 1.6 km track at half-pace canter, full-pace canter, or gallop according to the current training regimen for each horse	EBC and EB	PE: only ↑ pH in EBC

Hatao et al. 2006 [109]	Acute exercise and antioxidant enzyme activation in aged rats	Young rats (YR) or aged rats (AR) exercised (E) or not exercised control (C)	Race on treadmill at 25 m/min for YRE and 18–20 m/min for ARE for 60 min	Lung tissue	PE: ↑ Mn-SOD activity in YRE and ARE in comparison to their control subjects; ↑ CuZn-SOD and CAT activity in YRE and ↓ reactive carbonyls derivative in ARE, in comparison to their control subjects
Huang et al. 2008 [110]	Supplementation with L-Arg on pulmonary inflammation and oxidative damage induced by exercise in aged rats	Sprague-Dawley rats exercised (E) or sedentary (S) with L-Arg (+L-Arg) or without control rats L-Arg (C)	Race on treadmill for groups E at ~70% VO_2max until exhaustion (time for E+L-Arg and EC ~63 and ~51 min, resp.)	Lung tissue	PE: ↑ [XO], ↑ [MPO], and ↑ [MDA] in EC in comparison to SC; with no changes between EC and SC for [SOD], [CAT], [GSH-Px], [GR], and [GSH]
Kirschvink et al. 2002 [13]	Oxidative state, pulmonary function, and airway inflammation in healthy horses and with arcades	Trained healthy horses, affected by arcades or clinical remission	Race on treadmill with 2 min to 8, 9, and 10 m/s and 4% inclination, stages interrupted by 2 jogs of 8 min to 3.5 m/s (10 min of warming up and 10 min of recovery)	BALF	PE: ↑ [UA] in healthy horses
Lin et al. 2005 [111]	Oxidative stress and antioxidant defenses in animals supplemented or not with L-Arg	Sprague-Dawley rats grouped as exercised (E) or sedentary (S) with L-Arg (+L-Arg) or control rats without L-Arg (C)	Race on treadmill for E groups at 20 m/min for 15 min and 25 m/min for 30 min; then they run at 30 m/min and 10% of inclination (70–75% VO_2max) until exhaustion (EC ~81 min and E+L-Arg ~87 min)	Lung tissue	PE: ↑ activity XO and MPO in EC in comparison to SC; ↑ [UA], ↑ [NO], and ↑ [MDA] in EC in comparison to SC; ↑ activity SOD and GR in EC in comparison to SC
Mills et al. 1996 [112]	eNO and VNO during acute exercise	Healthy horses	Maximal incremental race until 9 m/s	EB	DE: positive correlation of eNO and VNO with the race intensity
Radák et al.	Acute anaerobic exercise	Exercised Wistar rats (E) and	Two races on treadmills at 30 m/min	Lung	PE: >pulmonary carbonyls and

1998 [113]	and oxidative modification of pulmonary proteins	sedentary control rats (C)	for 5 min; after 5 min of recovery, a 3rd race to exhaustion was performed	tissue	[glutamine synthetase] in E versus C
Reddy et al. 1998 [114]	Pulmonary oxidative damage by acute strenuous exercise in rats deficient in Se and VitE	Female Wistar albino rats deficient in Se and VitE and control rats	Intense swimming to exhaustion	Lung tissue	PE: >[SOD] and <[GSH-Px] and <[GST] in rats deficient in VitE and in comparison to control rats
Prigol et al. 2009 [115]	Supplementation with $(PhSe)_2$ and pulmonary oxidative damage caused by the exercise	Adult Swiss albino mice supplemented with $(PhSe)_2$ and not supplemented control mice	Swimming exercise (20 min) for both groups after 7 d of supplementation	Lung tissue	PE: ↑ [MDA] and ↑ of CAT activity in mice not supplemented with $(PhSe)_2$
Terblanche 1999 [116]	Exhaustive swimming and CAT activity in the lungs of male and female rats	Sprague-Dawley rats	1 h swimming	Lung tissue	PE: ↑ CAT activity in males and females

BALF: bronchoalveolar lavage fluid; CAT: catalase; $(PhSe)_2$: diphenyl diselenide; GR: glutathione reductase; GSH: glutathione reduced; GSH-Px: glutathione peroxidase; GST: glutathione S-transferase; L-Arg: L-arginine; MASL: meters above sea level; MDA: malondialdehyde; MPO: myeloperoxidase; NO: nitric oxide; Se: selenium; SOD: superoxide dismutase; CuZn-SOD: copper-zinc-superoxide dismutase; Mn-SOD: manganese-superoxide dismutase; TBARs: thiobarbituric acid reactive substances; UA: uric acid; VNO: volume of nitric oxide; XO: xanthine oxidase; VitE: vitamin E; VitC: vitamin C. In "Oxidative or inflammatory main results," DE: during exercise and PE: postexercise. In "Aim," the effect of exercise was not the primary aim of study.

(b)

Author, year	Aim	Sample characteristics	Exercise protocols	Samples obtained	Oxidative or inflammatory main results
Altan et al. 2009 [117]	SOD activity and [TBARs] postadaptation by training in altitude	Wistar albino rats divided into trained in hypobaria (THb) and normobaria (TNb) and nontrained in hypobaria (Hb) and normobaria (Nb)	Comparison of baseline samples between groups trained with swimming (T: 5 at 30 min/day/for 4 days/week for 9 weeks) or nontrained and exposed or not to simulated altitude of 3000 MASL (E: 120 min/day for 4 days/week for 9 weeks)	Lung tissue	PT: >SOD activity in TNb in comparison to Nb; no differences in [TBARS] for the same groups
Asami et al. 1998 [118]	DNA oxidative damage by chronic exercise	Sprague-Dawley rats with spontaneous (S), forced (F) exercise and sedentary control rats (C)	Comparison of baseline samples among rats with spontaneous exercise (wheel), trained on treadmill (T: 30–90 min/day for 25 days), and control rats	Lung tissue	PT: >[8-OH-dG] in F in comparison to S; the DNA oxidative damage was related to the exercise intensity
Aydin et al. 2009 [119]	Long period of dietary restriction and stress produced by high intensity swimming	Sprague-Dawley rats with restricted diet (RD) or ad libitum (AL), grouped in trained (+T), exercised (+E), and sedentary control rats (C)	Comparison of baseline samples of RD and AL in +T (T: 8 weeks of swimming with 2% BW as extra load during ~50–80 min), PE in +E (E: swimming until exhaustion), and baseline C	Lung tissue	PT: <GSH activity and >GSH-Px of AL+T compared to ALC; <LPO, >GSH, and GSH-Px in AL+E that AL+T PE: ↑ [MDA], ↓ [GSH], ↓ GR activity, and ↑ GSH-Px of AL+E compared to ALC (acute effects)
Chimenti et al. 2007 [120]	Epithelial remodeling, inflammatory cells, and apoptosis in the AWs after	Trained Swiss mice (T) and sedentary control mice (C)	Comparison of baseline samples among trained mice (T: 5 d/week for 6 wk at moderate to high	Lung tissue	PT: >apoptosis, >proliferation, >loss of hair cells, and infiltration of leukocytes in the AWs in T versus C

	chronic exercise		intensity)		
da Cunha et al. 2013 [121]	Chronic exercise on oxidative stress and NF-κβ/p65 pulmonary immunocontent of rats with lung injury	Trained Wistar rats (T) and nontrained control rats (C)	Comparison of baseline samples among rats trained on treadmill (T: 20 min at 60% VO_2max during 24 days in 3 months)	BALF and lung tissue	PT: >pulmonary catalase activity in T versus C; there are no changes in [TBARs], carbonyls, dichlorofluorescein, [], and NF-κβ/p65 in the lung
Gündüz et al. 2004 [122]	Oxidant and antioxidant systems in rats organs after a year of training	Wistar albino rats grouped in young control rats (YC), aged control rats (AC), and aged rats-training (AT)	Comparison of baseline samples between AT in swimming (T: 1 h/day for 5 days/week for 1 year) with YC and AC	Lung tissue	PT: >SOD activity and >GSH-Px in AT in comparison to AC; no difference of [TBARs] between the same groups
Lee et al. 2013 [123]	Administration of a ginseng intestinal metabolite (IH901) and exercise-induced oxidative stress in trained rat	Sprague-Dawley rats divided into resting control (RC), training control (EC), resting with IH901 consumption, or exercise with IH901 consumption groups	Training was carried out during 8 weeks on a treadmill; two weeks with 0% inclination and 25 cm/sec; then 2 weeks with 10% and 30 cm/sec; then 4 weeks with 15% and 35 cm/sec	Lung tissue	PT: ↑ TBARs and ↑ protein carbonyls in EC versus RC
Menegali et al. 2009 [124]	Therapeutic effects of physical exercise on histological and oxidative stress markers in animals exposed to cigarette smoke	Old C57BL-6 mice divided into control (C), training (T), cigarette smoke (CS), and cigarette smoke plus training (CS+E) groups	Training groups swam for 10 min/day during one habituation week; then they performed a swimming program 5 days/week for 8 weeks	Lung tissue	PT: ↑ SOD and ↑ CAT activity in E versus C
Olivo et al. 2014 [125]	Moderate aerobic exercise training prior to Streptococcus pneumoniae infection	BALB/c mice divided into sedentary untreated (SU), sedentary infected (SI),	Comparison between SU and ATU during 4 weeks after an individual maximal exercise capacity test	BALF and lung tissue	PT: ↑ CuZn-SOD and ↑ Mn-SOD expression in lung parenchyma of ATU versus SU after an individual

	influences pulmonary inflammatory responses	aerobic trained untreated (ATU), and aerobic trained infected groups (ATI)	was performed (0.1 km/h every 2.5 min, 25% inclination); training was for 60 min/day, 5 days/wk for 4 wk at 50% of the maximal speed		maximal exercise capacity test
Reis Gonçalves et al. 2012 [15]	Chronic aerobic exercise on pulmonary inflammation, cytokine, and antioxidant enzymes in animal model of acute pulmonary damage	Trained BALB/c mice	Comparison of samples before and after a low intensity training on treadmill (T: 50% of MS for 60 min/d, 3 d/week for 5 weeks)	BALF, EB, and lung tissue	PT: with no changes in leukocytes, [IL-6], [IL-10], nor [TNF-α] in BALF; with no changes in [NO] in EB; ↑ expression of IL-6 and Mn-SOD in the lung, but no changes of activity of GSH-Px and GR in the lung
Toledo et al. 2012 [126]	Regular physical exercise in an experimental mouse model exposed to cigarette smoke	C57BL/6 mice divided into control mice (C), trained (T), exposed to cigarette smoke (Sk), and Sk plus T (Sk+T)	Comparison of baseline samples in T at moderate intensity on treadmill (T: 50% MS for 60 min/d, 5 d/week for 24 weeks)	BALF and lung tissue	PT: <[ROS] in BALF of En compared to C; >GSH-Px activity, but not of Mn-SOD nor CuZn-SOD in lungs of T compared to C; with no changes in the expression of IL-1ra, TNF-α, and IL-10 between T and C
Yang 2011 [127]	Chronic exercise and expression of cytokines related to inflammation in the lung tissue	Old male Sprague-Dawley rats, group with trained rats (T) and sedentary control rats (C)	Comparison of baseline samples between rats trained on treadmill (T: 25 m/min for 120 min/day for 1 week) and control rats	Lung tissue	>expression of mRNA for TNF-α and IL-4 and <expression of mRNA for IFN-γ of group T versus C

BALF: bronchoalveolar lavage fluid; BW: body weight; DEP: diesel exhaust particles; DNA: deoxyribonucleic acid; EB: exhaled breath; 8-OH-dG: 8-hydroxydeoxyguanosine; GR: glutathione reductase; GSH: glutathione reduced; GSH-Px: glutathione peroxidase; IFN-γ: interferon gamma; IL-1ra, IL-4, IL-6, or IL-10: interleukin-1ra, interleukin-4, interleukin-6, or interleukin-10; LPO: lipid peroxidation; MDA: malondialdehyde; MS: maximal speed; mRNA: messenger RNA; MS: maximal speed; NF-κβ/p65: factor nuclear kappa-β/p65; NO: nitric oxide; : nitrite; ROS: reactive oxygen species; SOD: superoxide dismutase; CuZn-SOD: copper-zinc-superoxide dismutase; Mn-SOD: manganese-superoxide dismutase; TBARs: thiobarbituric acid reactive substances; TNF-α: tumor necrosis factor-alpha. In "Oxidative or inflammatory main results," PE: postexercise and PT: posttraining. In "Aim," the effect of exercise was not the primary object.

Until now, only two studies have determined one of the potential sources of prooxidants; thus, it has been described as an increment of xanthine oxidase activity in the pulmonary homogenate of rats that performed strenuous exercise (~15 min) on a treadmill (20 m/min), besides MDA and NO [111]. Likewise, Huang et al. [110] observed an increase of the activity of xanthine oxidase and lung MDA in older rats after running on a treadmill until fatigue, during ~63 min at 70% of VO_2 max. Prigol et al. [115] and Akil et al. [105] found increases in TBARs in rats that swam for 20 min and 30 min, respectively, while Reddy et al. [114] found increases in MDA in rats with a vitamin E deficient diet that swam until fatigued. Also in rats, increases of TBARs after swimming during ~2.5 h until fatigue were found [106]. The same result was found in pulmonary homogenates of untrained rats which swam until exhaustion [119]. A strenuous exercise protocol of ~66 min (80–85% VO_2 max) showed no changes in TBARs in rats [107].

In healthy horses, no differences were observed in isoprostane 8-epi-PGF2a of supernatant of bronchoalveolar lavage fluid (BALF) after 50 min of running [13]. An increment of carbonyls in the lungs of rats was observed by Radák et al. [113] after an exercise till exhaustion on the treadmill. However, after an hour of a moderate intensity run in young and old rats, no changes were observed in the lung carbonyls [109].

With regard to the pulmonary antioxidant enzymes, after an hour of acute moderate exercise protocols on treadmills, young rats' lungs showed an increase in the activity of enzymes superoxide dismutase (SOD) of the type CuZn-SOD, Mn-SOD, of the catalase (CAT), without changes in the glutathione peroxidase (GSH-Px). The mRNA expression for these enzymes did not show differences [109]. Lin et al. [111] found an increase in SOD and glutathione reductase (GR) activity with no changes in CAT and GSH-Px activity in rats that ran at 30 m/min and 10% slope until fatigued. Finally, acute and prolonged exercise (more than an hour) at 80–85% VO_2 max showed no changes in the activity of GSH-Px and SOD [107]. In acute exercise protocols, using swimming, Reddy et al. [114] found an increase in SOD and glutathione transferase (GST), while mild decreases in GSH-Px activity were observed in rats that swam until fatigued. Prigol et al. [115] found increase in CAT activity in rats that swam for 20 min. In rats that exercise for an hour, Terblanche [116] found increased CAT activity without differences between males and females. In rats 18 months old, Huang et al. [110] described an increase of SOD activity and the maintenance of levels of CAT, GSH-Px, and GR after 51 min on treadmill at 70% of VO_2 max. Strenuous exercise increased the activity of GSH-Px, with no changes in GR [119]. In a report of Al-Hashem et al. [106], rats that exercised until fatigue decreased the activity of SOD and CAT.

Acute exercise has also altered the levels of nonenzymatic antioxidants; an increase of uric acid has been described, with no changes in total glutathione, in GSH, and in GSSG in BALF, after 50 min of incremental exercise in healthy horses [13]. In a study of rats that ran during ~81 min at 70–75% VO_2 max until fatigue, no variations were found in the homogenized lung GSH [111]. In rats that swam until fatigue (~2.5 h), no differences were found at 600 m of altitude, but there was a decrease of GSH levels at 2270 meters [106]; in this same report, it was found that supplementation with nonenzymatic antioxidants such as VitC (20 mg/kg) and VitE (20 mg/kg), a single dose one hour before starting the exercise, decreases pulmonary lipid peroxidation and SOD and CAT activities increases, in both altitudes. Additionally, supplementation shows higher levels of GSH compared to animals not treated in altitude [106].

Thus, the increase in lung prooxidants and its consequences (lipid peroxidation) due to acute exercise appear to be related to the high intensity and duration of the effort, in terms of either minute ventilation or oxygen consumption, and are enhanced by a hostile environment (hypoxia, pollution, cold, etc.). However, a mainly enzymatic antioxidant adaptive response is still controversial. In contrast, the use of vitamin reducers (C and E) allows the antioxidant capacity to be increased and oxidative damage to be controlled (see Tables 1(a) and 1(b)).

5. Pulmonary Redox Balance and Chronic Exercise

In a first study of pulmonary prooxidants and chronic exercise, Carraro et al. [97] found no differences in eNO of child swimmers (trained 1 h/week during 6 months). Martin et al. [102] observed no differences in eNO of athletes based in pool and not based in pool exposed to pool environment during 5 and 0.5 h/week, respectively. For oxidative damage, Heinicke et al. [47] found a tendency towards increase of 8-isoprostanes in the EBC of biathletes who trained at 2800 meters during 6 weeks (4–6 h/d with 1 d/weeks of rest), which included extensive cross-country skiing, strength training, and shooting technique training.

In a model of physical training of rats, which jogged in 3 months a total of 24 sessions of 20 min/d at 60% of VO_2 max, no differences were found in pulmonary carbonyls, nitrite, or TBARs [121]. After 24 weeks of training at 50% of maximal speed for 60 min/d for 5 d/week, ROS decreased in BALF and no changes of increase were found in pulmonary 8-isoprostanes in trained mice [126]. Using the same load and frequency as before, the levels of eNO and MDA were not altered in lung homogenates of rats trained during 5 weeks [15]. However, during the 8 weeks of training in rats that swam with a 2% of additional body

weight during ~50–80 min, an increment of pulmonary carbonyls and MDA was observed [119]. Gündüz et al. [122] found increases of TBARs in older rats (21 months) versus young rats (9 months), without any variations between old rats which were either trained or untrained in swimming during 12 months 1 h/d for 5 d/week. Altan et al. [117] found increases in MDA in rats trained at 3000 meters of altitude (120 min/d for 4 d/week during 9 weeks) compared to sedentary control rats and the ones not trained maintained at sea or height level. In Sprague-Dawley rat that was trained during 8 weeks on a treadmill, an increase in pulmonary TBARs and protein carbonyls was observed [123]. Regarding oxidative stress on nucleic acids, Asami et al. [118] found increases in 8-hydroxydeoxyguanosine in rats after a forced race on treadmill for five weeks in daily sessions with a gradual increase in the time of 30–90 min.

The chronic exercising has also had as a subject of study the potential changes of the expression/activity of the enzymes and nonenzymes pulmonary antioxidant. Likewise, Reis Gonçalves et al. [15] found an increase in the lung Mn-SOD expression of mice subjected to five weeks of training at moderate intensity (60 min/d in 3 d/wk); however, no changes were observed in the GSH-Px, GR, GST, and CAT activities. In another study, Olivo et al. [125] observed an increased expression in pulmonary CuZn-SOD and Mn-SOD postmaximal exercise test of trained mice during 4 weeks at 50% of the maximal speed on treadmill. Altan et al. [117] found increases of SOD activity after nine weeks of progressive training in a normobaric environment (5 to 30 min/d for 4 d/week), with no differences with a trained group at 3000 meters of altitude. da Cunha et al. [121] observed a higher pulmonary CAT activity in the ones trained on a treadmill during 12 weeks at 60% of VO_2 max (20 min/d), compared to control rats. In another study, Menegali et al. [124] found an increase of the CAT and SOD activity in lung of trained rat in swimming during 8 weeks. In mice trained on a treadmill for 24 weeks at 50% of maximal speed (60 min/d and 5 d/week) increases of GSH-Px were observed without changes of expression of CuZn-SOD, Mn-SOD, and Ec-SOD, studied in sections of pulmonary tissue [126]. In another study, older animals of 21 months that were trained for a year (1 h/d and 5 d/week) had a greater amount of SOD in comparison to control rats of their same age and to young rats. No differences were found in CAT activities, while GSH-Px had a greater activity than a group of their same age [122]. Finally, Aydin et al. [119] observed a decrease in the concentrations of GSH and an increase of GSH-Px activity in pulmonary homogenates of rats, after eight weeks of swimming with overload and progressive weekly time increment (50–80 min).

This reflects the fact that oxidative stress induced by chronic pulmonary exercise in animals is closely associated with high-intensity protocols, but not with those of moderate intensity

(see Table 1(b)). However, when moderate chronic exercise was executed while at high altitude, both human and animals presented pulmonary oxidative damage (see Tables 1(b) and 2(b)). In contrast, antioxidant adaptation seems to be more closely related to the animal training time, with an increase in the activity of SOD and CAT in the medium term and the expression of SOD in the short term (see Table 2(b)).

6. Acute Exercise-Induced Lung Inflammation

In horses, Kirschvink et al. [13] found no cellular count variation in BALF after 50 minutes of exercise. In runners' sputum of 10 km (~35.4 min), 12 km (~46.1 min), and 21 km (~89.1 min) a trend of increasing polymorphonuclear neutrophils (PMNs) in samples of induced sputum was found [40]. In the same direction, Bonsignore et al. [56] reported a higher percentage of PMNs in induced sputum, compared to values previous to exercise and an increase in these cells after the marathon (~179 min). Also in induced sputum of runners, Denguezli-Bouzgarrou et al. observed in 2006 [62] and 2007 [63] an increase of PMNs after 60 minutes of moderate racing. In the latter study, higher concentrations of histamine, interleukin-8 (IL-8), LTB_4, and LTE4 were also detected, subsequent to acute exercise during the precompetitive phase versus the competitive phase [63]. Chimenti et al. [5], in a 20-kilometer race (~90 min), reported an increase in IL-8 in the supernatant. Races in smaller time frames (~18 min) showed no changes in the amount of PMNs in induced sputum [93]. In rowers, after a short test of high intensity (1000 m in ~3 min), there was a trend towards an increase of epithelial cells and a positive association between the pulmonary ventilation/body weight (L/kg) and macrophages in induced sputum [78]. In swimmers, increases in lymphocytes and eosinophils and a decrease in macrophages were observed in induced sputum, after a 5 km race in the ocean (hypertonic environment) in relation to the same test performed in an open pool with low concentration of chlorine. However, there is no evidence of the increase in inflammatory cell activation [57]. In a chlorinated pool, in high performance swimmers, no changes were observed in the cellular composition of the induced sputum and the pH in EBC after 45 min at moderate intensity [81]. Larsson et al. [32] found an increase of granulocytes and macrophages in subjects that performed one hour of exercise, on a treadmill, at −23°C, without IL-8 changes in BALF samples. Derivatives of arachidonic acid have been studied in three works; thus, in a maximum acute exercise of approximately 12 min, increases in E_2 prostaglandin and B_2 thromboxane in EBC after exercise were found in men [83]. The leukotrienes in EBC were studied by Bikov et al. [54]; thus, after an eight-minute test on a treadmill no differences in

the concentration of cysteinyl leukotrienes were found in normal people. In a test of 4 km of cycling with a 12% hill sloping during ~7 min, an increase of leukotriene B4 in BALF of athletes was found in comparison to the control subjects [67]. Also in EBC, Zietkowski et al. [95] found no changes in high sensitive C-reactive protein after 9 minutes of cycle-ergometry at 85% of maximal predicted heart rate in healthy subjects.

The pH in EBC is a potential marker of pulmonary inflammation that has been used in pathologies that have this condition. In acute exercise, the results have been variable; thus, Marek et al. [73] did not find differences after an exercise until fatigue (~13 min) in of amateur athletes. Bikov et al. [55] did not observe changes in the of healthy subjects after exercise, while there are other reports that show increases in pH after outdoor exercise [128] and after low-intensity (60% of maximal predicted heart rate) exercise (~30 min) in nonathlete healthy subjects [84]. In races up to 10 km, no changes have been reported up to 80 min after the race, in both amateur runners [52] and physically active runners [53]. However, there are inverse correlations between changes in prooxidants and changes of [53]. In distances that exceed 21 and 42 km, ~101 min and ~246 min, respectively, an acute decreasing trend of was observed [52]. However, in an animal study conducted in horses, the group of Cathcart et al. [108] found an increase in after running 1.6 km.

In summary, the majority of published papers demonstrate the infiltration of inflammatory cells (macrophages or granulocytes) after acute exercise in humans. A factor that probably influences this is the duration of the exercise, as the increase in PMNs was found only in protocols involving longer periods (see Table 1(a)). Cellular infiltration was found to be due to cold or chlorine. The role of exercise training is difficult to assess, given that the studies were conducted almost exclusively in trained subjects. We must add to this the reported changes in soluble inflammatory mediators. As a whole, these could be an expression of an asymptomatic acute inflammatory process similar to that observed in other tissues (muscle tissue). This would happen in a self-limiting way whenever the necessary conditions of time, environmental factors, and intensity are encountered.

7. Chronic Exercise-Induced Lung Inflammation

Studies in animals have shown that training during 120 min/d for a week on treadmill at 25 m/min increases the expression of mRNA to tumor necrosis factor-alpha (TNF-a) together with promoting a decrease of interferon gamma in pulmonary tissue samples [127]. Chimenti et al. [120] trained mice at moderate intensity for 6 weeks (5 d/week), showing leukocyte infiltration in the airway. At this level of epithelia, an increase of apoptosis and a

decrease of the ciliated cells were also observed. In mice that trained 60 min/d to 50% of maximal speed for 24 weeks (5 d/week), no variation was observed in the number of macrophages in BALF, but it was possible to see a decrease of the capacity of these cells to form free radicals [126]. However, it is possible that the elaboration of training programs at moderate intensity (66% of VO_2 max) generates a reduction of the inflammatory response after the completion of ischemia and pulmonary reperfusion, which was evidenced as a decrease of the release of interleukin 1β and tumor necrosis factor-alpha (TNF-a) at plasmatic level in a model performed in rats [129]. An analogous result was described by Toledo et al. [126], who did not find differences in TNF-a, interleukin 10, monocyte chemotactic protein, and interleukin 1 receptor antagonist, quantified in lung sections of mice, after training to 50% for 1 h/day, 5 days per week, for 24 weeks.

In studies conducted in humans, it has been reported that the participation in a long distance race training program over the course of a year generates a persistent inflammatory process with no apparent clinical repercussion and an increase in PMNs and in IL-8 concentrations, leukotriene E_4, and histamine in the supernatant of induced sputum samples [130]. Subjects who participated in high performance athletic training in sessions of 1 h/day for 10 days, interspersed with rest 5 days, had lower pH values in EBC compared to healthy control subjects [98]. The same result in this parameter was reported in runners by Greenwald et al. [128]. In the same direction, in amateur runners (~50 km/week) low levels of pH were reported compared to values of healthy control subjects [52]. High performance pool swimmers showed no differences in basal inflammatory parameters when compared with non-pool-based athletes; however, the analysis of the subgroup of athletes that had a positive result in the voluntary hyperventilation test (exercise-induced bronchial hyperreactivity indicator) presented a higher concentration of eNO and a higher count of eosinophils and of epithelial cells when compared to the group that had negative results on this test [102]; among other factors, this could be related to the number of years of practice of pool swimming, since no differences in eNO, in EBC pH, and in cellularity of induced sputum in adolescents were found when compared to normal subjects [131]. Elite swimmers, who trained between 800 and 3380 km/year, had more eosinophils and PMNs in induced sputum compared to nonathlete control subjects [99]. The cessation of the training for 3 months of swimmers decreases eosinophils and lymphocytes in induced sputum compared to active swimmers (~1870 km/year) [100]. The comparison between healthy athletes who are swimmers and others who are engaged in land exercise has shown an increased number of PMNs in induced sputum samples [96]; the same comparison showed no differences in PMNs and eosinophils in induced sputum [102].

Chronic inflammation can be associated with pulmonary epithelial damage; thus, increases of clear cell protein (CC16) in plasma of swimmers who trained during 20 weeks in a chlorinated pool have been reported [132].

In skiers, who trained 435 h/year, increase of lymphocytes and mast cells has been found, with no differences in the concentration of TNF-a and myeloperoxidase in BALF compared to nonathlete control subjects [103]. Karjalainen et al. [101] reported, through the study of bronchial biopsies, an increase in neutrophils, eosinophils, macrophages, and T lymphocytes in elite skiers (435 h/year) compared to healthy control subjects, along with air tract remodeling indicators as an increase in collagen I and collagen III deposits in the submucosa, a hyperplasia of racket cells, and a higher expression of type 5 mucin. The use of anti-inflammatories (800 micrograms/day of budesonide) by cross-country elite skiers (~427 h/year) during 20 weeks did not generate differences regarding the placebo (~468 h/year) in the cellularity (PMNs, macrophages, lymphocytes, eosinophils, and mast cells), studied in BALF and in endobronchial biopsy [104].

In summary, animal models of physical training show increases of soluble inflammatory mediators, which include TNF-a. Human studies have focused on subjects who have greater contact with irritants in the airway due to the specificity of their sport, whether runners (large ventilation volumes), skiers (cold), or swimmers (chlorine gas in the pool room). In these subjects, permanent tissue infiltration of granulocytes, macrophages, and lymphocytes has been observed. Evidence of these changes has been found in both noninvasive samples, such as induced sputum, and in biopsies in the bronchial region. At the same time, an increased presence of soluble proinflammatory substances has been reported. Overall, this suggests that these athletes in particular may suffer from persistent changes in tissue (chronic inflammation and airway remodeling) that have been associated with pulmonary symptoms and functional changes (see the bottom of Figure 1).

8. Oxidative Damage and Inflammation, Relations, and Potential Effects

The generation of prooxidant substances and the establishment of tissue oxidative damage are closely associated with inflammatory processes; thus, inflammatory cells are a known source of prooxidants derived from both oxygen and nitrogen [133]. At the same time, the increase of prooxidants has been involved in the intracellular signaling which leads to inflammatory cell activation, increased secretion of soluble mediators of inflammation [134], endothelial activation, and also increased expression of adhesion molecules and endothelial permeability [135]. This relation implies that, in many situations,

the increase of prooxidants participates in the activation of inflammation and vice versa, demonstrating the close relationship between both phenomena [134]. The establishment of both oxidative damage and inflammation in the lungs has been involved in the origin/evolution of various pathological states; for example, both phenomena are a fundamental part of adult respiratory distress [136], asthma [137], chronic obstructive pulmonary disease [138], pulmonary hypertension [139], and viral infectious processes [140]. In the lungs, the relationship between oxidative changes and inflammation has rarely been studied as a main goal, but it is presumed that, in view of the studies conducted in other organs, it must be closely related. This is particularly important in subjects practicing sport, as both inflammation damage and oxidative damage have been implicated in the pathogenesis of phenomena of high prevalence in athletes such as rhinitis, bronchial hyperreactivity, asthma, and airway remodeling [27, 141]; so, most respiratory symptoms (coughing, wheezing, breathlessness, and chest tightness) in endurance athletes such as cross-country skiers are known [142]. In addition, cross-country skiers show a presence of PMNs and lymphocytes infiltration in the airways [101]. This phenomenon can also be extrapolated to other endurance athletes [143] such as marathon runners, cyclists, and swimmers, the latter of which are also exposed to the chlorine in swimming pools, which could be one of the main factors inducing increased eosinophils and leukocytes in the sputum.

9. Methods for the Study of Lung Inflammation/Oxidative Damage by Exercise

The study of the oxidative/inflammatory damage in the lungs is challenging due to both anatomic functional limitations and the limitations of currently applied techniques. Current evidence on this topic focuses primarily on the study of lung diseases, while studies on the effect of exercise as a trigger effect of this phenomenon in healthy people are scarce. Summarizing what is known to date for the species analyzed, the determinations made and the samples obtained are shown in Tables 1 and 2. Lung tissue microenvironment has challenged developers of study methodologies, so, although systemic markers have been proposed (CC16, surfactant proteins A and B, and Krebs von den Lungen-6), they do not yet have sufficient capacity to indicate minor damage, which implies that the processes of the lung itself cannot always be ascertained. For this reason, it is preferable to test samples originated from the lung; those currently under study are exhaled breath (whether direct or condensate), fluids (BALF, induced sputum, and nasal lavage), and cells and portions of whole tissue (biopsies, tissue homogenates, and cut pieces of tissue). Unfortunately, today

there is still much controversy regarding the interpretation of the results obtained with these methods. In relation to oxidative/inflammatory exercise phenomenon, in animals, exhaled breath [112], lung tissue homogenates [113, 114, 117, 118, 120, 121, 127], bronchoalveolar lavage [121, 126], and lung tissue sections [126] have been used. In humans, most methods are focused on noninvasive methods and, among these, the induced sputum is the most widely used [40, 56, 57, 62, 63, 78, 81, 93, 96, 99, 100, 102, 144]. Another sample studied corresponds to exhaled breath, which was analyzed whether directly [56, 57, 59, 65, 71, 75, 81, 89, 97, 102] or after being condensed at low temperature [46, 53, 65, 72–74, 77, 79, 81, 83, 84, 128, 139]. Very few studies have used bronchoalveolar lavage [32, 103, 104] and lung tissue obtained by endobronchial biopsy [101, 104].

10. Discussion

In summary, we found that in acute exercise (see Tables 1(a) and 2(a)) there is more evidence of changes in cellularity (predominantly granulocytes) when it (was) is a prolonged high-intensity exercise. This change was not so evident in animals; however, this should be resolved in further studies because it is a parameter measured recently in this population. Long-term of acute moderate exercise (>60 min) in humans stimulated an increase of pulmonary inflammatory mediators (IL-8, LTB_4, and LTE_4). Now, regarding prooxidants, a systematic increase in humans is observed after more than thirty minutes of exercise. It is noteworthy that, in acute exercise in animals, reports of an increase in lung lipid peroxidation are the majority, while it has not been observed in humans, except for intense exercise at high altitudes. This may be partially explained by the techniques used: while tissue samples were analyzed in animals, EBC samples were analyzed in humans; in another aspect, the change with greater support in relation to the enzymatic activity corresponds to the maintenance or decreased levels of GSH-Px and to the increase in SOD.

With regard to chronic exercise (training) and its effects (see Tables 1(b) and 2(b)), the number of studies is still very small, but there is a tendency observed, seen in humans, towards changes in cellularity compatible with chronic inflammation of the airways, particularly in subjects exposed to cold and chlorine. In animals, changes in pulmonary cellularity (leukocyte infiltration) were observed in only one study [120]. For soluble inflammatory mediators, in animals the scientific evidence has shown an increase in the concentration of these substances (IL4, IL6, and mRNA TNF-a) subsequent to chronic exercise. The oxidative damage was observed in animals following moderate chronic

exercise (>4 sem), specifically in older rats, and cold or altitude environment. In humans, only one study showed oxidative damage by altitude training [45, 47]. With regard to enzymatic antioxidants, a tendency towards higher levels in SOD and GSH-Px is observed in humans. As for nonenzymatic antioxidants, only one study showed a decrease in the concentration of pulmonary GSH in trained rats [119].

The problem requires further study to clarify numerous questions in order to have a more definitive overview; thus, several challenges for researchers in the field have arisen. Likewise, the activity of the sources of production of free radicals in the lung (mitochondria, xanthine oxidase, NADH oxidase, and NOS) should be studied and the knowledge of the status of antioxidant systems, particularly in humans, where there are no records available, should be improved. Regarding inflammatory parameters, the study of soluble mediators of inflammation should be extended; in addition, the effect of both substances with antioxidant and anti-inflammatory effect should be explored. Furthermore, it is necessary to generate research projects which explore the parameters of oxidative/inflammatory mechanisms simultaneously in order to establish the interrelation mechanisms between both processes. It is also necessary to characterize the effect of time and intensity of performed exercise, the role of environmental conditions, and the level of training of the subjects on oxidative damage/lung inflammation by exercise. Finally, to advance the resolution of this problem, it is urgent to improve the technical conditions to allow obtaining representative samples of lung environment in its different compartments, and it is also necessary for these methods to be noninvasive and contribute to monitoring the athletes.

Conflict of Interests

The authors have no conflict of interests to declare.

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4.2 Artículo Científico 2: Increase of pro-oxidants with no evidence of lipid peroxidation in exhaled breath condensate after a 10-km race in non-athletes (Objetivo 2)

Increase of pro-oxidants with no evidence of lipid peroxidation in exhaled breath condensate after a 10-km race in non-athletes

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Abstract

It is a well-established fact that exercise increases pro-oxidants and favors oxidative stress; however, this phenomenon has been poorly studied in human lungs. Pro-oxidative generation (H_2O_2, NO_2^-), lipid peroxidation markers (MDA), and inflammation (pH) in exhaled breath condensate (EBC) have been determined through data from 10 active subjects who ran 10 km; samples were obtained immediately before, at 20, and at 80 min post-exertion. In EBC, the concentration of H_2O_2 at 80 min post-exertion was increased. NO_2^- concentration showed a tendency to increase at 80 min post-exertion, with no variations in MDA and pH. No variations of NO_2^- were found in plasma, while there was an increase of NO_2^- at 80 min post-exertion in the relation between EBC and plasma. NO_2^- in EBC did not correlate to plasmatic NO_2^-, while it did correlate directly with H_2O_2 in EBC, suggesting a localized origin for the exercise-related NO_2^- increase in EBC. MDA in plasma did not increase nor correlate with MDA in EBC. In conclusion, high-intensity exercise increases lung-originated pro-oxidants in non-athlete subjects with no evidence of early lipid peroxidation and changes in the pH value in EBC.

Introduction

It is a well-documented fact that exercise favors the increase of pro-oxidants and that in some situations it produces oxidative stress [21, 30]. A reduced group of studies on animals have been focused on the impact of exercise on pulmonary redox equilibrium state, reporting evidence of oxidative stress [4, 36].

Exercise increases lung ventilation and favors higher contact with cold air, air pollutants, and chlorine in swimming pools [22, 43]; at the same time, it favors immune system activation [28]. The aforementioned may be particularly important in subjects who have regimes of long training hours. Consequently, previous studies in humans have demonstrated inflammation and redox state changes in the lungs of athletes such as swimmers [14], skiers [41], and runners [3, 11].

The study of redox state changes in the lungs resulting from exercise is difficult because sampling involves some risks to the participants; for this reason, the use of exhaled breath condensate (EBC), extensively studied in lung diseases and which has been proposed for the evaluation of various tissue processes (oxidative damage, cancer, remodeling, and inflammation) located in this organ [18, 20], can be a useful tool in the characterization of this phenomenon in athletes. Using EBC samples, no changes in the concentration of H_2O_2($[H_2O_2]_{EBC}$) were found during exercise [32], but changes in their flow [25] were found. It has also been reported that there was an increase in $[H_2O_2]_{EBC}$ on climbers exposed to altitudes of 6,125 m [1] and in biathletes who trained for 6 weeks at an altitude of 2,800 m [17]. In both protocols, an increase and a trend to the increase of lipid peroxidation measured as malondialdehyde (MDA) and 8-isoprostane, respectively, was shown. Recently, our research group compared amateur long distance runners who trained 50 km a week in 10-, 21.1-, and 42.2-km races, reporting increases in $[H_2O_2]_{EBC}$ and NO_2^- concentration in EBC ($[NO_2^-]_{EBC}$) for the 21.1- and 42.2-km races with no modifications in the participants of the 10-km race. In the three evaluated distances, no increase in lipid peroxidation, measured as the MDA concentration in EBC ($[MDA]_{EBC}$), was shown [3]. In this paper, we extend the description of redox state changes which occur in EBC from participants of long distance races to physically active but non-athlete subjects. We hypothesized that, in this group, a 10-km race could generate an increase in pro-oxidants and favor lung lipid peroxidation since they are not chronically exposed to the pulmonary effects (irritation, dryness, inflammation, cell damage) of the distance runners' training regime. A second objective was to advance in the characterization of

EBC markers as originated either locally or from the systemic environment; for this purpose, we compared the concentrations of NO_2^- and MDA in both EBC and plasma.

Materials and methods

Subjects

Ten non-smoking students of Physical Education (see Table 1) with no history of high or low respiratory tract inflammation during the month previous to the study were made subjects of this study. They also had no history of chronic respiratory diseases (asthma or allergic rhinitis) and did not consume nutritional supplements, antioxidants, or anti-inflammatory medicaments. They practiced 9.2 ± 3.3 h/week of moderate to intense exercise. The distribution of total exercise time, expressed in hours per week, is presented as mean ± standard deviation, and the percentage of the total sample performed in this activity is shown in parentheses: running 2.5 ± 0.5 (80 %), swimming 1.2 ± 1.4 (40 %), football 1.5 ± 1.7 (30 %), mountain bike 1.5 ± 6.3 (20 %), tennis 0.9 ± 2.1 (20 %), handball 0.6 ± 1.4(20 %), volleyball 0.5 ± 0.7(20 %), and basketball 0.5 ± 0.7 (20 %). Participants were informed orally and in writing, before signing an informed consent. This study was approved by the Ethics Research Committee of the Universidad de los Andes.

Table 1 General description of participants

	Values
Men/Woman	9/1
Age (years)	20.50 ± 1.60
Weight (kg)	62.64 ± 6.8
Height (cm)	172.4 ± 5.3
VO_2 max (ml kg^{-1} min^{-1})	47.37 ± 6.0
Time of race (min)	50.65 ± 4.63

Values are shown as mean ± SD

Protocol

After being evaluated at rest, they went through a 10-min warm up before running 10 km at maximum effort in an open 330-m racetrack. On each complete turn, the cardiac frequency was determined (Polar, model T31) in order to quantify the intensity of the

exercise. All subjects performed this test simultaneously. EBC samples were taken using the previously described device [1, 2]. Subjects were at rest, wearing a nasal clip, and having previously washed their mouths with distilled water. Sampling time was between approximately 10 to 15 min or until 1.5 mL of EBC was obtained. Also, venous blood was drawn, heparinized, and then centrifuged at 3,000 rpm to obtain plasma. Once samples were obtained, they were stored in liquid nitrogen and later at −80 °C until they were analyzed. EBC or plasma samples were taken before (pre) exercise, 20 min after exercise completion (20-post), and 80 min after exercise completion (80-post).

Malondialdehyde in EBC and plasma

MDA concentration was measured according to Larstad et al. [23]. EBC at 300 µL or 50 µL of plasma was mixed with 100 µL of 25 mM thiobarbituric acid. The mixture was incubated for 1 h at 95 °C. After cooling, first in ice for 5 min and then for 40 min at room temperature, the mixture was submitted for high-performance liquid chromatography (Shimadzu LC10AD, Corporation), where a C-18 column 150-mm long and 4.6-mm I.D. (Supelcosil LC-18, Supelco) was used. The mobile phase (1 mL/min) was a 20:80 (v/v) mixture of acetonitrile in 20 mM potassium phosphate buffer (pH 6.8). Measurements were performed with a fluorescence detector (RF-551, Shimadzu), excitation and emission wavelengths, being at 532 and 553 mm, respectively. Malondialdehyde bis (diethyl acetal) from Merck was applied as standard.

Hydrogen peroxide in EBC

It was measured using FOX2 [31] reagent. This reagent contains Fe^{+2} (250 µM), which in an acidic medium ($HClO_4$, 110 mM), and is oxidized to Fe^{+3} by the presence of H_2O_2. The amount of H_2O_2 is monitored through the reaction between the ferric ion and the xylenol orange indicator (250 µM). Sorbitol (100 mM) was added to the original reagent according to Gay and Gebicki [15]; this method has been previously used by our research group [1, 2, 3]. For measurements, 350 µL of EBC and 150 µL of modified FOX2 were taken, then the sample was incubated for 1 h at room temperature, and absorbance was read at 560 nm (Jenway 6405). Three calibration curves were performed for each group's measurements using H_2O_2 (Merck) as standard.

pH in EBC

It was measured using the protocol of Paget-Brown et al. [33]. EBC at 100 µL was bubbled with argon for 8 min at a flow rate of 350 mL/min, and pH was later measured using a

3 × 38 mm (Diameter × Length) microelectrode (Cole and Palmer) connected to a pH meter (Oakton® Acorn pH 6).

Nitrites in EBC and plasma

Nitrite concentration was measured using spectrophotometric test based on the Griess reaction [16]. Griess reagent at 300 µL (0.1 % naphthylethylenediamine–dihydrochloride, 1 % sulphanilamide, 3 % H_3PO_4) was added to 300 µL of EBC or plasma deproteinized with NaOH/$ZnSO_4$. The mixture was incubated for 10 min, and absorbance was measured at 550 nm. Three calibration curves were performed for each group's measurements using sodium nitrite (Merck) as standard.

Statistics

Using the Shapiro–Wilk normality test, it was observed that the samples did not come from a Gaussian distribution; therefore, non-parametric tests were applied. The Friedman test was used for repeated samples, and Dunn's test was used as a further test for all the measured parameters. Correlations were determined by the Spearman correlation coefficient. The significance level used was of $p < 0.05$. For statistical analysis, GraphPad Prism, USA software was used.

Results

Exercise intensity estimated as the percentage of cardiac reserve was at 91.2 ± 4.7 %. The race time was 50.6 ± 4.6 min. Both variables are expressed as mean and standard deviation. An increase in $[H_2O_2]_{EBC}$ (Fig. 1) as compared with the pre-value at 80-post ($p < 0.05$) was seen. $[NO_2^-]_{EBC}$ showed a trend to significance; it had a value of $p = 0.045$ in the Friedmann test, with no differences between groups in the posteriori test. No changes in nitrites in EBC and plasma ($[NO_2^-]_P$) after the race ($p = 0.97$) were seen. The relation $[NO_2^-]_{EBC}/[NO_2^-]_P$ showed increases (Fig. 2) on the pre-value in the 80-post ($p < 0.05$). No differences in $[MDA]_{EBC}$ ($p = 0.60$), in the values of malondialdehyde in EBC and plasma ($[MDA]_P$; $p = 0.83$), or in the relation between $[MDA]_{EBC}/[MDA]_P$ ($p = 0.60$) as shown in Fig. 3 were observed. The pH in EBC (pH_{EBC}; $p = 0.39$) showed no post-race differences (Fig. 1).

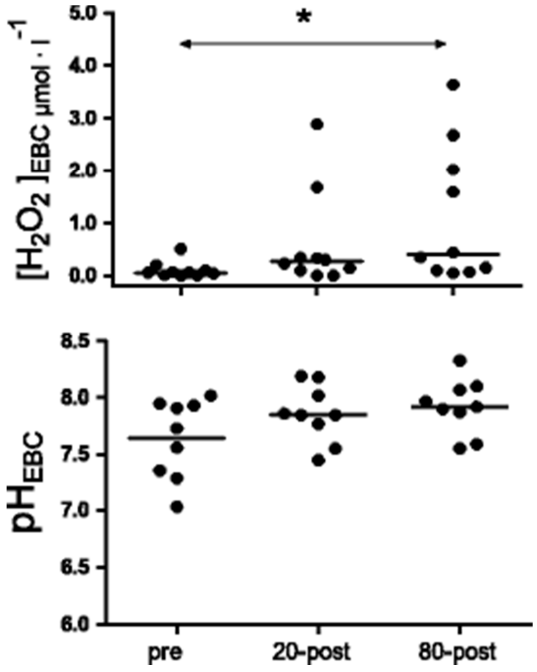

Fig. 1 $[H_2O_2]_{EBC}$ and pH_{EBC} in participants of a 10-km race. The *line* represents the median value. $*p < 0.05$ is different from the pre-value.

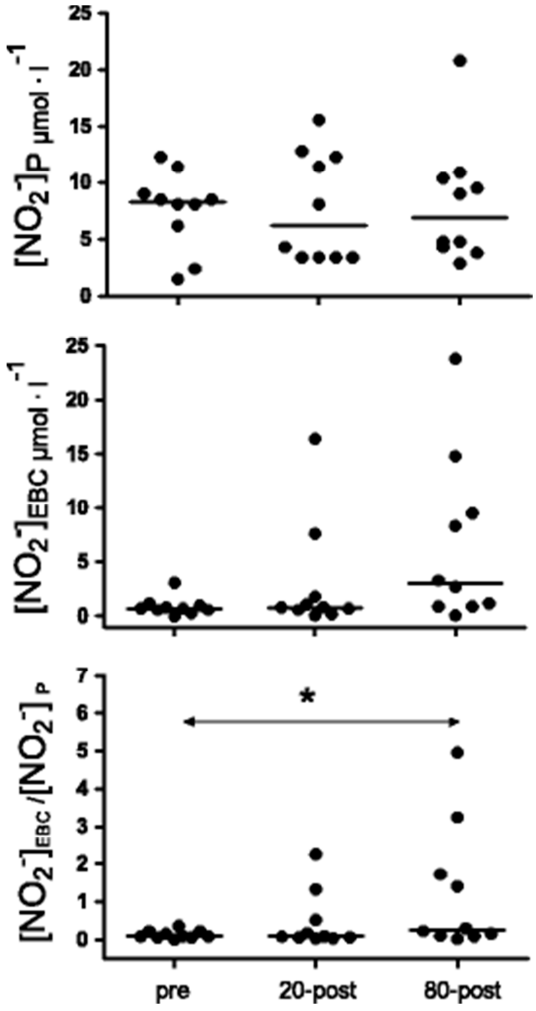

Fig. 2 $[NO_2^-]_P$, $[NO_2^-]_{EBC}$, and $[NO_2^-]_{EBC}/[NO_2^-]_P$ in participants in a 10-km race. The *line* represents the median value.*$p < 0.05$ different from pre-value

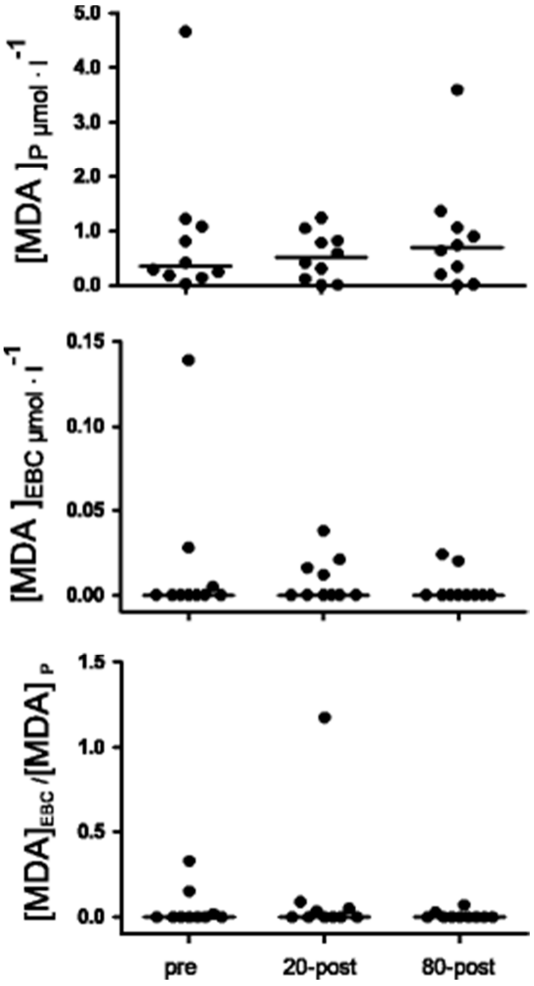

Fig. 3 $[MDA]_P$, $[MDA]_{EBC}$, and $[MDA]_{EBC}/[MDA]_P$ in participants in a 10-km race. The *line* represents the median value

Correlations were made between absolute values and their absolute changes (deltas). A first group of absolute deltas was obtained from the difference between the absolute values of the 20-post-stages minus pre-stages. The second group of deltas was obtained from the differences between the absolute values of the 80-post-stages minus 20-post-stages; in the performed delta correlations, both sets of data were considered together.

Regarding nitrite, no significant correlations between absolute values of $[NO_2^-]_P$ versus $[NO_2^-]_{EBC}$ ($r = 0.21$, $n = 30$, $p = 0.26$) and for absolute changes between these same variables ($r = 0.18$, $n = 20$, $p = 0.46$) were observed. A similar result was found for $[MDA]_P$ versus $[MDA]_{EBC}$ for absolute values ($r = -0.22$, $n = 30$, $p = 0.24$) and between absolute changes ($r = -0.16$, $n = 20$, $p = 0.49$). Both the relation between absolute values

of $[H_2O_2]_{EBC}$ versus $[NO_2^-]_{EBC}$ ($r = 0.69$, $n = 30$, $p < 0.0001$) and absolute changes ($r = 0.73$, $n = 20$, $p < 0.0002$) showed a significant association as shown in Fig. 4. No significant correlations between absolute values of $[H_2O_2]_{EBC}$ and $[NO_2^-]_{EBC}$ with pH_{EBC} were found. Correlations between absolute changes of $[H_2O_2]_{EBC}$ and pH_{EBC} showed a trend to significance ($r = -0.45$, $n = 18$, $p = 0.06$), while absolute changes between $[NO_2^-]_{EBC}$ versus pH_{EBC} were significant ($r = -0.61$, $n = 18$, $p = 0.007$; see Fig. 5). No significant correlations between the race time and intensity (measured as the percentage of cardiac reserve) with the studied variables in EBC and plasma in 20-post and 80-post were found.

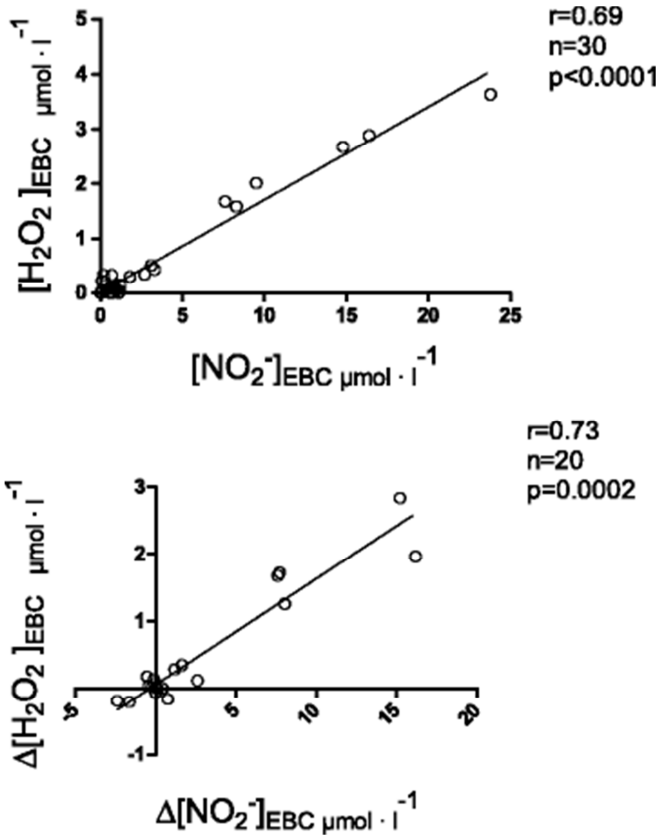

Fig. 4 Relationship between $[NO_2^-]_{EBC}$ versus $[H_2O_2]_{EBC}$ (top) and $\Delta[NO_2^-]_{EBC}$ versus $\Delta[H_2O_2]_{EBC}$ (bottom) in participants of a 10-km race

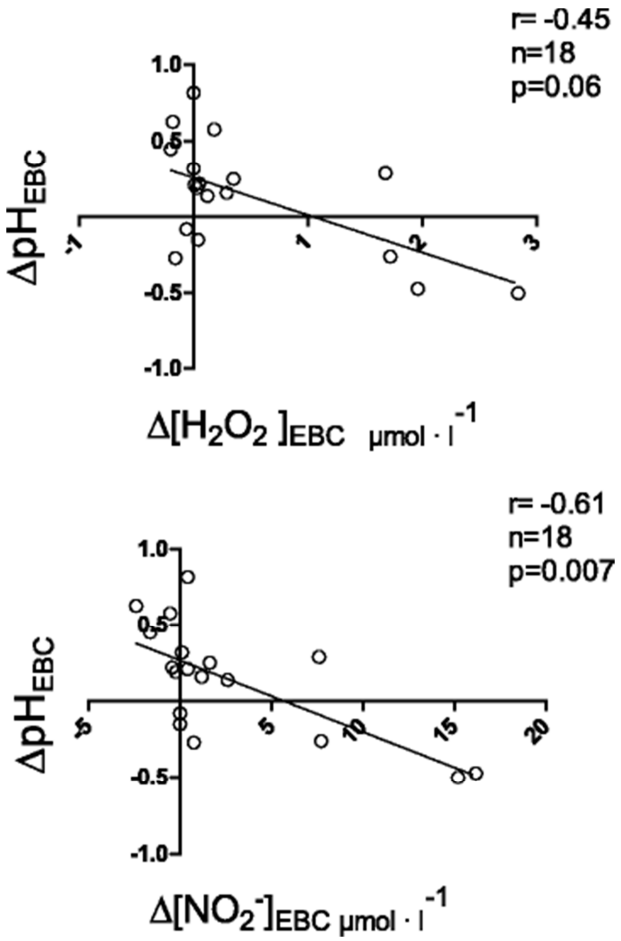

Fig. 5 Relationship between $\Delta[H_2O_2]_{EBC}$ versus $\Delta[pH]_{EBC}$ (top) and $\Delta[NO_2^-]_{EBC}$ versus $\Delta[pH]_{EBC}$ (bottom) in participants of a 10-km race

Discussion

Exercise increases lung ventilation and the speed with which the surrounding air reaches our lungs. High-intensity and prolonged exercising, typical of endurance races, inflame the airways [6, 42] and increase its pro-oxidants [3] as it has been previously described. One of the factors that have an influence on oxidative stress produced by exercise is the fitness degree of the participants; Brooks et al. [7] showed higher NO and superoxide anion formation because of acute exercise in sedentary rats' perfused/infused muscle versus muscles of trained rats. In patients with chronic obstructive pulmonary disease, there was less oxidative stress induced by acute exercise after their participation in a physical training program [34]. In this sense, it is possible that subjects, such as those from the present study, physically active but not subjected to high endurance athletes' regime (long sessions of prolonged aerobic exercise), are more prone to increase in pro-oxidative formation since the intensity of the performed exercise reached 90 % of the cardiac reserve (see the "Results" section). In this study, the main result is the increase in $[H_2O_2]_{EBC}$ and $[NO_2^-]_{EBC}/[NO_2^-]_P$; both parameters show a tendency at 20 min that becomes significant at 80 min post-race. At the same time, despite the increase of these species, there is no lipid peroxidation increase, as it has been previously described in the lungs of animals [38], nor pH_{EBC} decrease as an indicator of tissue inflammation, as it has been described in pathologies such as asthma, bronchiectasis, and adult respiratory distress [20].

Regarding $[H_2O_2]_{EBC}$ results, there are few similar experiences in the literature to the one presented here, especially because of the increased exercise time (over 30 min) of our protocol, which makes it difficult to compare; Nowak et al. [32], Marek et al. [25], and Mercken et al. [26] conducted submaximal and maximal exercises finding no differences in $[H_2O_2]_{EBC}$, but they used a protocol with only 6- and 15-min exercising times, respectively. Data presented herein are comparable to our previous report on amateur long distance runners [3]. In that report, no changes in 10-km runners were observed and absolute changes were minor in this distance in comparison to 21.1- and 42.2-km races. In this report, we found that in a group of non-runner subjects, $[H_2O_2]_{EBC}$ does increase after the race, as measured on the same times as of the aforementioned work. The fact that trained subjects do not show any increase in this pro-oxidant in this distance may be partly explained by the induction of anti-oxidant defenses [37] and by a lower inflammatory response as a result of chronic exercise [47]. This finding shall be later reevaluated in subjects with different levels of training directly compared under the same conditions.

During physical exercise, in the lungs, nitric oxide is involved in both dilatation of airways to increase mobilized airflow and vasodilatation in order to avoid excessive increase in pulmonary artery pressure [45]. In the pathological context, nitric oxide participates in lung redox imbalance that occurred in inflammatory processes [44]. Nitric oxide has a short half-life; therefore, in many experimental models, more stable metabolites such as nitrite and nitrate are determined [46]. Nitric oxide and its related compounds have a complex metabolism; thus, it is not yet fully clarified. This happens, among other aspects, because of its multiple origins; it can be formed from typical lung cells (epithelial cells, endothelial cells, smooth muscle cells) as well as in leukocytes and erythrocytes [8].

Regarding the effect of a long distance race on $[NO_2^-]_{EBC}$, to our knowledge, this parameter has been previously reported only by our research group, and no changes in $[NO_2^-]_{EBC}$ after a 10-km race were observed, while changes in 21.1- and 42.2-km races were observed [11]. Unlike this previous work, we currently report a trend to increased $[NO_2^-]_{EBC}$ and an increase in the relation between $[NO_2^-]_{EBC}/[NO_2^-]_P$, which means an increase in this pro-oxidant in the lungs by exercise in subjects who are not accustomed to this physical effort unlike usual runners. In the particular case of the time in which the increase of the relationship, $[NO_2^-]_{EBC}/[NO_2^-]_P$(80-post-stage) is potentially observed; it can result from nitric oxide increases that occurred during exercising, since NO_2^- may remain without being completely debugged, in the increase of the lung endothelial nitric oxide synthase activity as seen in animal models and/or in the increased activity of this enzyme as described in human leukocytes after exercise [24, 29].

Contrary to the few measurement reports of $[NO_2^-]_{EBC}$, nitrite has been previously measured in plasma during exercise. In this regard, some reports have observed increases in plasma levels of nitrite + nitrate combination after 10 min of exercise at 75 % of maximal oxygen consumption [10]; however, this finding is not systematic for acute exercise. Bloomer et al. [5] found no nitrite increases in plasma after 30 min of treadmill exercise. In both athletes and sedentary people, Poveda et al. [35] found no changes in $[NO_2^-]_P$ after maximum exercise on a treadmill. In our study, $[NO_2^-]_P$ determination was done to evaluate if the eventual $[NO_2^-]_{EBC}$ increase could be explained by simultaneous increases in plasma (something that was not observed). This lack of NO_2^- increase in plasma and the lack of correlation between individual values and $[NO_2^-]_{EBC}$ and $[NO_2^-]_P$ absolute changes indicate that it is likely that the increase of EBC, because of exercise in these species, may be a localized phenomenon. This idea is also supported by the increase in $[NO_2^-]_{EBC}/[NO_2^-]_P$ relation and by the fact that $[NO_2^-]_{EBC}$correlates with another exhaled air marker such as $[H_2O_2]_{EBC}$ (see Fig. 4). The finding of this statistically

significant association is consistent with our previous report on long distance runners [3]. We believe that our collected data, as a whole, strongly supports the idea that intense prolonged exercise in this population—under the described conditions—alters the redox state of the pulmonary microenvironment.

The increase of the described pro-oxidants was not concomitant with $[MDA]_{EBC}$ increases (lipid peroxidation/oxidative damage indicator) and pH_{EBC} decreases (indicator of tissue inflammation) that were expected to occur. Regarding pH, Riediker and Danuser [39] reported a pH_{EBC} increase immediately and until 60 min post-exercise (30 min of fast walking at 60 % of maximal cardiac frequency).

A later report by Marek et al. did not report any changes in pH_{EBC} that was measured immediately after performing a maximal exercise to exhaustion in cycling (time is not reported) [25]. Ferdinands et al. [13] reported the absence of pH_{EBC} changes after acute exercise; however, they found lower pH_{EBC} values in regular runners. In a recent report on racehorses, Cathcart et al. found increased pH_{EBC} 20 to 30 min after running 1.6 km at a moderate to high intensity [9]. The tendency to maintain pH_{EBC} values after exercise, herein reported or alkalinization reported by other groups, has no explanation yet; however, some authors have suggested the hypothesis that this phenomenon is due to the increase in ammonium secretion (buffer) of the airway epithelium in response to exercise [9]. In the pathological context, subjects with chronically inflamed airways, like asthmatic patients, have lower ammonium levels [19]. Similarly, Mickleborough et al. [27] did not find any changes in the pH_{EBC} in asthmatic subjects after hyperventilation at 85 % of maximal voluntary ventilation for 6 min. These patients decreased their levels of airway inflammation after ingesting a preparation rich in omega three fatty acids for 3 weeks. So, a decrease of exhaled nitric oxide and an increase in the basal value of pH_{EBC} was evidenced, and in contrast to that previously observed in the first hyperventilation test, an alkalinization of pH_{EBC} after the said test was evidenced, which can be interpreted as a better response to acidosis of the airway [27].

In another aspect, our previous data obtained on amateur runners showed the same trend to the increase (proved herein) of pH value in the 10-km runners' group, while there is a trend to a decrease in the groups of 21.1- and 42.2-km races [3]. The difference in the pH_{EBC} response between 10-km races and longer distances may be related to the greater intensity of the inflammatory response against the increased stimulus (distance of the race) and the time necessary to establish an inflammatory process in the tissue; thus, the time in a 10-km race is about 1 h, while a marathon of amateurs takes about 4 h. In this regard, it will be a great contribution, in the future, to extend the follow-up time of this

parameter after the race and to include specific markers of inflammation such as cytokines. Furthermore, the different acids and bases found in the EBC samples should be more specifically analyzed.

Although there was no decrease in pH_{EBC} or inverse correlations between the absolute values of the pro-oxidants as we found in our report [3], in this work, we found inverse associations between the absolute changes of pro-oxidants (trend to H_2O_2 and significance for NO_2^-) studied in EBC and the absolute changes of pH_{EBC} (see Fig. 5), which supports the hypothesis that pro-oxidant changes are related to inflammation at this level.

Regarding $[MDA]_{EBC}$, previous results showed no differences at low heights (670 m) after high-intensity cycloergometric exercise [1]. Similar results were found in a 120-W cycloergometric protocol [32]; both findings are equivalent to those reported here but with protocols dissimilar to ours. Failure to find an expected relation between the increase in pro-oxidants and the increase in lipid peroxidation may take place because changes in this parameter occur at a later time as compared to our measuring. Senturk et al. [40] found MDA increases in plasma 12 h after 10 min of extenuating exercise; Fatouros et al. [12] found the highest $[MDA]_P$ at 24 h after a soccer match. Another possibility is that this pro-oxidative increase may be part of a physiological process in the described groups and conditions and is not associated to tissue damage in this organ. In this work, $[MDA]_P$ is also determined (see Fig. 3) in order to advance in the elucidation of the localized or systemic origin of this marker in EBC. In this respect, similar to that observed in $[MDA]_{EBC,}$ we did not find any changes in neither $[MDA]_P$ nor changes in the relation between $[MDA]_{EBC}/[MDA]_P$ (see Fig. 3), so the interpretation of our findings becomes difficult. However, the lack of correlation between absolute values as well as between their absolute changes support the hypothesis that $[MDA]_{EBC}$ is not related to $[MDA]_P$; this was also observed in a previous work in which cyclists performed a maximal exercise at 2,160 m of altitude, showing increases in $[MDA]_{EBC}$ with no changes in MDA measured in serum. In the aforementioned work, no significant correlations between the said parameters were found [1].

In conclusion, unlike the previous results obtained in amateur runners, in physically active subjects, 50 min of high-intensity race (10 km) produces an increase in oxygen- and nitrogen-derived pro-oxidative species. Probably, this could be related to a stronger reaction response regarding the formation of pro-oxidant/inflammatory factors which are common in subjects less adapted to high-intensity and prolonged exercise. Despite the increase of pro-oxidants, we did not find any early modifications in lung lipid peroxidation

and pH value in EBC. Nitrites in EBC most likely originated from a localized process in lungs.

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4.3 Artículo Científico 3: Effect of exercise duration on pro-oxidants and pH in exhaled breath condensate in humans (Objetivo 3)

Effect of exercise duration on pro-oxidants and pH in exhaled breath condensate in humans

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Abstract

Exercise promotes pulmonary oxidative imbalance. In this regard, some evidence has been obtained from the study of exhaled breath condensate (EBC) during urban races, in which the factors involved in the occurrence of this process are still not characterized. In this paper, under laboratory conditions, both the role of time of exercise on the generation of pro-oxidants (H_2O_2, NO_2^-) and pH have been assessed in EBC of 16 under-trained subjects who completed three tests of cycloergometric exercise at low intensity (30 % of VO_2 max) with a duration of 10, 30, and 90 min. Samples were obtained as follows: immediately before and at 80 min post exertion in each test. In the 90-min test, an increase in H_2O_2, NO_2^- concentration in EBC at 80 min post exertion with no changes in the pH was observed. Total O_2 consumption and total ventilation weakly correlated with the changes in H_2O_2 and NO_2^-. In conclusion, the concentration of pro-oxidants in the EBC depends on the duration of the exercise when it is performed at low intensity under laboratory conditions.

Introduction

Physical exercise is a recognized trigger of changes in the redox state, particularly when this activity takes place under special environmental conditions (cold, altitude, and pollution) and when the intensity is high or the exercise is performed for a prolonged period of time [2, 23]. So, redox state changes have been previously described, with the muscle tissue being the main focus of study, in view of its major functional changes during exercise. Thus, free radicals have been involved in the contractile activity, cell damage, inflammation, and fatigue [25, 26, 29]. Another organ that undergoes great changes in its activity due to exercise is the lung; hence, the flow of mobilized air increases, the temperature of the airways decreases [19, 31], the contact with environmental pollutants increased [10], and blood flow is also increased [9]. More details of the mechanisms were recently extensively reviewed by our group [1]. Although some of these changes have been suggested as destabilizers of the redox state, few works have ventured to study this issue, and those are mostly in animal models [27]. In humans, the difficulty in obtaining samples, on the one hand, has limited the study of the lung and fostered the development of non-invasive methods (induced sputum, exhaled air, exhaled breath condensate) to study the lung tissue microenvironment. In exercise, there are previous reports regarding exhaled breath condensate (EBC) to study changes in the redox state of both athletes and physically active subjects [6]. Thus, EBC analysis has yielded an increment of H_2O_2 and malondialdehyde on climbers [2] and subjects training at medium altitude [15], showing that exercise in hypobaric conditions implies oxidative lung damage [5]. In a subsequent study, an increase in the concentration of pro-oxidants and a tendency towards airway acidification (a phenomenon associated with lung inflammation) were found in the EBC of runners of 21.1- and 42.2-km urban races [3]. In the same report, direct correlations between the running time and the absolute changes in the concentration of nitrite and hydrogen peroxide in EBC are found. At the same time, there was an inverse correlation between the race time and the absolute changes of pH in EBC [3]. The relations described were found in a field study, under different environmental conditions, in different subjects, and the exercise was performed with varying intensity, which could have affected the results. Consequently, this study aimed to measure EBC samples, pro-oxidants, and pH of subjects who exercised under controlled laboratory conditions, and the main focus was how exercise duration affected changes in these parameters.

Methods

Subjects

Sixteen male, healthy, active (see Table 1), non-smoker subjects with no history of rhinitis or asthma, without respiratory infection during the last month and who had not participated in any scheduled aerobic physical activity such as urban races, swimming, or cycling. They also did not consume anti-inflammatories, antioxidants, or any other nutritional supplements. Participants were informed orally and in writing, before signing an informed consent. This study was approved by the Ethics Research Committee of the University of Los Andes.

Table 1 General description of participants

	Values
Age (years)	22.3 ± 4.2
Weight (kg)	73.5 ± 8.4
Height (cm)	1.75 ± 0.1
VO_2 max (ml kg^{-1} min^{-1})	46.0 ± 8.2
BMI (kg m^{-2})	24 ± 2.2

Values are shown as mean ± SD

Protocol

Evaluations were performed as described as follows: (1) Survey of habits and anthropometric assessment (Anthropometric Gaucho Kit, Rosscraft™, USA); (2) Determination of maximum oxygen uptake (VO_2max) on a cycle ergometer (VIAsprint™ 150/200p, Viasys™, USA) using exhaled gases analysis (Oxycon mobile, Jaeger™, Germany); (3) In the following three visits, exercise was performed on a cycle ergometer at a stable load equal to 30 % of VO_2 max for 10, 30, and 90 min, in which ventilation, VO_2, heart rate, perceived exertion, and pedaling cadence (60 rpm) were controlled. Participants appeared between 8:00 and 12:00 a.m., at least 1 h after a light breakfast and they hydrated only with the same isotonic electrolyte replacement drink, free of stimulating substances, antioxidants and/or anti-inflammatories, after exercise was completed. All the described physical tests were performed at a temperature between 18 and 22 °C and humidity between 60 and 70 %. To obtain EBC samples, exhaled air was cooled and condensed through an instrument designed and previously validated by our group [2, 4].

Subjects were at rest, wearing a nasal clip and having previously washed their mouths with distilled water. Then, they were asked to breathe at tidal volume for approximately 15 min or until 1.5 ml was obtained. The team had a saliva trap to avoid contamination with some mediators that occur in the mouth. Once samples were obtained they were stored in liquid nitrogen and later at −80 °C up until their analysis. In all three protocols, EBC samples were taken before (pre) exercise and 80 min after exercise completion (80-post), given that previous studies conducted by our group have typically shown changes in this time of sampling [3, 6].

Hydrogen peroxide

The hydrogen peroxide in EBC was measured using a FOX2 reagent [21]. This reagent contains Fe^{+2} (250 μM), which, in an acidic medium ($HClO_4$, 110 mM), is oxidized to Fe^{+3} by the presence of H_2O_2. The amount of H_2O_2 is monitored through the reaction between the ferric ion and the xylenol orange indicator (250 μM). Sorbitol (100 mM) was added to the original reagent according to Gay and Gebicki [12]; this method has been previously used by our research group [3, 6]. For measurements, 350 μL of EBC and 150 μL of modified FOX2 were taken, then the sample was incubated for one hour at room temperature and absorbance was read at 560 nm on a microplate spectrophotometer (EPOCH™, BioTek Instruments, USA). Three calibration curves were performed for each measurements' group by using H_2O_2 (Merck) as standard.

pH

The pH was measured using Paget-Brown et al. protocol [24]. One hundred microliters of EBC were bubbled with Argon for 8 min at a flow rate of 350 mL/min, and pH was later measured using a 3 × 38 mm (diameter × length) microelectrode (Cole and Palmer) connected to a pH meter (Oakton™ Acorn pH 6).

Nitrites (NO_2^-)

Nitrite concentration was measured using the spectrophotometric test based on the Griess reaction [13]. Three hundred microliters of Griess reagent (0.1 % naphthylethylenediamine-dihydrochloride, 1 % sulphanilamide, 3 % H_3PO_4) were added to 300 μL of EBC. The mixture was incubated for 10 min, and absorbance was measured at 550 nm on a microplate spectrophotometer. Three curves were made for each measurement, with sodium nitrite as standard.

Statistics

Once individual values were tabulated, the Shapiro-Wilk test was applied to evaluate the distribution of the samples. When a normal distribution was obtained, a Student's *t* test for paired samples was applied to the mean values; otherwise, the Wilcoxon test was applied. The absolute changes were compared using ANOVA or the Friedman test. Correlations were determined using the Spearman correlation coefficient or the Pearson test according to the distribution. For the parameters measured in the EBC, the average and range of the intra-day coefficients of variation were obtained from the pre-exercise values of the three assessments for the same subjects. The significance level used was of $p < 0.05$. For statistical analysis, GraphPad Prism 6.0, USA software was used.

Results

With regard to the parameters pertaining to physical exercise (see Table 2), no differences in mean heart rate ($p = 0.24$), external load ($p = 0.69$), or pedaling cadence ($p = 0.47$) were observed. Regarding the minute ventilation and relative oxygen consumption, a higher average value was observed in the 90-min test, when compared to the 10-min test. The perceived effort had a greater value for the 90-min test than in the 10- and 30-min tests. For both total mobilized air and total oxygen consumed, a smaller value was observed for the 10-min test in comparison to the 30- and 90-min tests. Also, the 90-min test showed higher values in both parameters when compared to the 30-min test.

With respect to the markers analyzed in EBC, no differences were observed in the pre-exercise values of the three tests $[H_2O_2]_{EBC}$ ($p = 0.15$), $[NO_2^-]_{EBC}$ ($p = 0.44$), and pH_{EBC} ($p = 0.12$). From these values, the intra-day coefficient of variation was 51 % (0.65–115) for $[H_2O_2]_{EBC}$, 47 % (12–92) for $[NO_2^-]_{EBC}$, and 1.58 % (0.9–2.8) for pH_{EBC}. An increase in $[H_2O_2]_{EBC}$ at 80-post ($p = 0.0007$) was found in the 90-min protocol, with no differences in the 10-min ($p = 0.47$) and 30 min ($p = 0.23$) protocols, respectively (see Fig. 1). A similar result was found in $[NO_2^-]_{EBC}$; thus, no differences in the 10 min ($p = 0.14$) and 30 min ($p = 0.60$) tests were found, showing increases of this species in the 90 min ($p = 0.047$) protocol, as shown in Fig. 1. The pH_{EBC}values showed no differences when comparing pre-values versus 80-post values in the 30-min ($p = 0.35$) and 90-min ($p = 0.34$) tests, while there is a tendency to increase in the 10 min ($p = 0.051$) test as shown in Fig. 2. Absolute changes (Δ), calculated as the difference between 80-post and pre-exercise, showed a higher value for nitrite between the 90-min versus 10-min protocol. $\Delta[H_2O_2]_{EBC}$ showed differences between the 30-min versus 90-min protocol (see Table 3). Finally, no

differences between the Δ pH_{EBC} of the three protocols ($p = 0.21$) were observed (see Table 3). As for correlations, a significant correlation between $\Delta[NO_2^-]_{EBC}$ versus $\Delta[H_2O_2]_{EBC}$ ($r = 0.32$, $n = 16$, $p = 0.023$) was observed; no significant correlations between ΔpH_{EBC} and $\Delta[H_2O_2]_{EBC}$ or $\Delta[NO_2^-]_{EBC}$ were found. Minute ventilation showed no significant correlations with pro-oxidants and pH_{EBC}. Total ventilation correlated with $\Delta[H_2O_2]_{EBC}$ ($r = 0.30$, $n = 48$, $p = 0.041$) and $\Delta[NO_2^-]_{EBC}$ ($r = 0.38$, $n = 48$, $p = 0.007$).

No significant correlation between this parameter versus the changes in pH was observed. The total oxygen consumption during the test correlated with $\Delta[NO_2^-]_{EBC}$ ($r = 0.33$, $n = 48$, $p = 0.02$) and showed a tendency to show significance with $\Delta[H_2O_2]_{EBC}$ ($r = 0.26$, $n = 48$, $p = 0.06$) minute-relative oxygen consumption did not correlate with pro-oxidants nor with pH_{EBC}.

Table 2 Workload and the physiological response in three different exercise protocols duration

	10 min	**30 min**	**90 min**
Load (W)	59.93 ± 5.1	59.94 ± 5.1	59.80 ± 6.2
Cadence (rpm)	59.69 ± 0.7	59.95 ± 0.2	59.86 ± 0.8
RPE	1.96 ± 1.1	2.25 ± 1.0	5.72 ± 1.6*, **
HR (beats min^{-1})	103.2 ± 8.6	105.1 ± 8.1	106.7 ± 11.5
VE (L min^{-1})	26.18 ± 2.6	27.21 ± 1.7	28.51 ± 1.8*
Total ventilation (L)	261.6 ± 25.96	816.3 ± 53.26*	2568 ± 168.30*, **
VO_2 (mL kg^{-1} min^{-1})	12.66 ± 2.5	13.79 ± 2.4	14.43 ± 2.2*
Total VO_2 (mL kg)	126.5 ± 25.57	413.80 ± 73.50*	1297.0 ± 200.0*, **

Values are expressed as mean ± SD

*$p < 0.05$ difference from the 10-min test; **$p < 0.05$ difference from the 30-min test

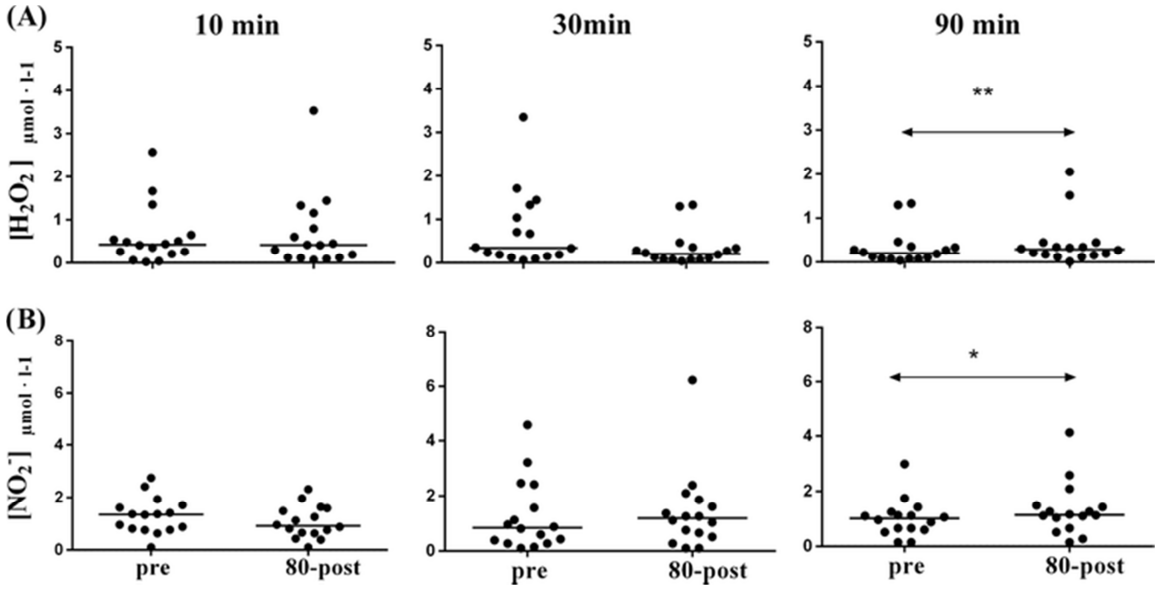

Fig. 1 $[H_2O_2]_{EBC}$ (**A**) and $[NO_2^-]_{EBC}$ (**B**) in participants of a cycloergometric exercise of 10, 30, and 90 min. Values are expressed as median, *$p < 0.05$ different from pre-value

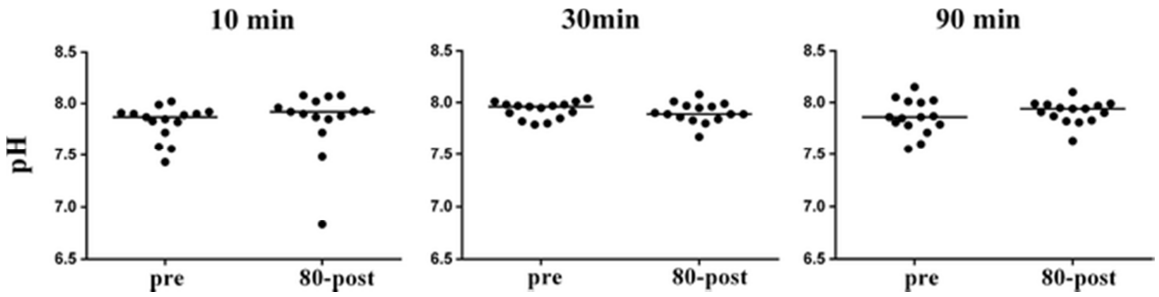

Fig. 2 pH_{EBC} in participants of a cycloergometric exercise of 10, 30, and 90 min. Values are expressed as median

Table 3
Effect of exercise duration on the absolute changes in pro-oxidants and pH in exhaled breath condensate

	10 min	30 min	90 min
Δ [H_2O_2] (μmol L^{-1})	−0.03	−0.03	0.06 *
	(−0.18, 0.07)	(−0.12,0.025)	(0.03,0.12)
Δ[NO_2^-] (μmol L^{-1})	−0.26	0.12	0.21 **
	(−0.59, 0.0)	(−0.48, 0.61)	(0.0, 0.85)
Δ pH	0.10	−0.02	0.00
	(−0.02, 0.19)	(−0.12, 0.04)	(−0.08, 0.23)

Values are expressed as median with interquartile range in parentheses

*$p < 0.05$ difference from the 30-min test; **$p < 0.05$ difference from the 10-min test

Discussion

Exercise requires more body oxygen consumption; therefore, physiological modifications necessary to increase the provision of this element are brought up. Thus, an increase in ventilatory activity is generated by increasing both depth of inspiration/exhalation and respiratory rate. Under exercise conditions, our group has previously reported increases in the pro-oxidants generation measured in EBC and correlations between the time of outdoor exercise and the production of these species in different groups of subjects [3, 6]. In this report, we created a protocol that involved three tests of different duration in which the subjects performed exercise where the temperature, moisture and contaminants from the ambient air were controlled. Moreover, unlike the tests performed in the field in the current experimental set, it was possible to continuously measure relevant physiological parameters and to contrast them against pro-oxidants. Thus, we find that in front of intensity close to 30 % of VO_2 max, a cycloergometric protocol, developed at a fixed external load, produced increases in minute ventilation, minute-relative VO_2, and perceived effort which are probably associated to the fatigue showed in the last part of 90 min test. Although we cannot rule out that these changes are involved in the phenomenon under study, we note that the big difference of stimulus to our subjects was the increase in total ventilation and total relative VO_2 to which they were exposed to; so, in both parameters, nearly 10-fold differences between the 10 and 90 min stages were observed. The large increase in the total described ventilation is of

particular interest, since this implies the possibility of lowering the temperature of the airway, favoring mechanical damage and promoting evaporation of fluid from the epithelial surface; it has been suggested that these factors in exercise are involved in the irritation and inflammation of the airway [1, 11, 19]. As for total relative VO_2 during the test, it is relevant since an association between the highest oxygen consumed and the increase of reactive oxygen and nitrogen species has been described [18].

In the lungs, both normal cells and inflammatory-type cells can form pro-oxidants derived from oxygen and nitrogen. H_2O_2, which is one of the reactive oxygen derivatives, has been determined in EBC samples, in both patients with COPD or asthma [20, 35] and in subjects who perform physical exercise [2, 6, 17, 22]. Although its origin is not clear, there is a history that links its concentration in EBC with both blood phagocytes [32] and inflammatory cells in induced sputum samples [16], which are active producers of pro-oxidants and characteristic of defensive processes. In the present study, an increase in $[H_2O_2]_{EBC}$ in the 90-min test was observed. Furthermore, the $\Delta[H_2O_2]_{EBC}$ was higher in the 90-min test, when compared to the 30-min test. Taken together, these data suggest that exercise time determines the $[H_2O_2]_{EBC}$. The influence of time on exercise has not been the focus of a study previously; however, there are reports of brief protocols of exercises; in this sense, Nowak et al. [22] in a 6-min protocol at 120 W no change was found. Similarly, in samples obtained during the performance of a submaximal exercise (60 and 120 W) lower than 10 min, increases in $[H_2O_2]_{EBC}$ [17] were not found, either. In prolonged exercise, our group has previously reported increases in $[H_2O_2]_{EBC}$, 80 min post exercise in runners who exercise between 1 (10 km) and 4 h (42.2 km). Specifically, we can compare the current 90-min protocol with a 21.2-km race, so while in the first mentioned example, increases of +40 % in 21.1 km (about 100 min) are found, runners showed changes from +200 % to the 80-post [3] at similar baseline of H_2O_2. The foregoing suggests the probable influence of exercise intensity on the generation of H_2O_2 at this level. In this direction, Mercken et al. [20] reported increases in H_2O_2 production, measured in the EBC, in healthy people, after about 12 min when the exercise was maximal, while no changes were observed during the same time of exercise performed at 40 % of maximum output (80 W approx.).

Nitrite is another chemical of great importance in the study of redox state; this substance is part of nitric oxide metabolism, a chemical species with multiple physiological and pathological functions. This substance has also been observed in the EBC from asthmatics [28] and healthy people who exercise [3, 6]. In this report, $[NO_2^-]_{EBC}$ increased in the 90-min protocol. This same exercise time showed higher $\Delta[NO_2^-]_{EBC}$ values than in

the 10-min protocol. According to our judgment, these results support the idea that the increase of time doing exercise, at low intensity, increases $[NO_2^-]_{EBC}$. This parameter has also been previously reported by our group and increases in $[NO_2^-]_{EBC}$ in exercise for more than 1 h duration have been described in both poorly trained people who ran 10 km [6] and runners of 21.2- and 42.2-km races [3]. In this last work, correlations between time of exercise and $\Delta[NO_2^-]_{EBC}$ have been found, which somehow led to specifically study the effect of exercise time. As in the case of H_2O_2, it is seen that the magnitude of changes observed for $[NO_2^-]_{EBC}$ for 90 min of exercise, under controlled conditions, is 13 %, while for the 21.2-km race, increases of 90 % are found. This reinforces the idea that exercise intensity (after total ventilation) is involved in generating this difference.

Airway acidity has been studied by determining the pH_{EBC}, which is lower in the case of pulmonary inflammatory processes [7, 28]. It has a high rate of reproducibility [34], which we found in our sample, too. In this report, a tendency to increase after 10 min is observed, with no changes at a longer exercise time; deltas showed no differences, either. The reported tendency to increase, in the current work, is similar to that found in by another authors [8, 14, 30]. For example, Riediker et al. [30] in an exercise on a treadmill at a slight higher intensity to the one presented here (calculated as 60 % of maximum heart rate), where an increase in pH_{EBC} was found, measured 1 h after exercise.

Along the same lines, we have also found a tendency for pH_{EBC} to increase in participants of 10 km race in both untrained [6] and runners [3]; the last two races were performed at maximum effort. Contrary to what we expected, the result of low pH_{EBC} by prolonged exercise was not reproduced, as it was previously found in runners [3]. In part, this can probably be explained due to the low intensity of our current protocol.

In the search for the mechanisms of the observed variations in pro-oxidants and pH in the EBC as described above, we correlate their absolute changes versus some measured relevant physiological parameters. In first place, it was of particular interest to assess the minute-relative oxygen consumption and total consumption in these samples during exercise, as well as the known relationship between oxygen consumption and formation of pro-oxidants [18]. In this regard, we found a significant but weak correlation between $\Delta[NO_2^-]_{EBC}$ and total relative VO_2 in addition to a tendency to significance for $\Delta[H_2O_2]_{EBC}$; according to this, it is likely that, at least in part, the increased consumption of O_2 may explain the increase in the pro-oxidants in this organ. Regarding ventilatory changes, as mentioned in the first paragraph, these would be the primary source of changes that

occur in the lung microenvironment measured in the EBC. In this way, while minute ventilation did not correlate with any of the markers measured, we did find significant correlations, albeit weak, between the total ventilation and changes in the studied pro-oxidants. From our point of view, this helps to support the idea that the total amount of mobilized air inflames and generates oxidative changes on airway epithelium, but it probably only constitutes one of the factors involved in the phenomenon described. In another aspect, it is likely that the low correlations are also influenced by the reproducibility found (similar to other authors [33]) for $[NO_2^-]_{EBC}$ and $[H_2O_2]_{EBC}$. In this regard, an advance in the search of strategies (improvement of sampling devices, protocols, obtention of fractions) should be made, in order to use these samples in the non-invasive monitoring of athletes in the future.

In conclusion, a low-intensity cycloergometric exercise, performed under laboratory conditions, the concentration of pro-oxidants in EBC depends on the exercise time. Moreover, the increase of the observed pro-oxidants, depends, in part, on the total oxygen consumed and the total air mobilized through the airway during exercise.

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Conflict of interest

The authors declare that they have no conflict of interest.

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5. Discusión general

A pesar de la gran cantidad de literatura científica que existe sobre estudio de los efectos oxidativos (producción de pro-oxidantes; estrés o daño oxidativo) e inflamatorios (actividad de células inmunes, biomarcadores inflamatorios, pH) pulmonares inducidos por ejercicio, la mayoría de estos evidencian cada proceso de forma independiente (artículo científico 1). Es reconocida la estrecha relación que existe entre la inflamación y estrés oxidativo, sobretodo en procesos patológicos. Aquellos estudios que han utilizado marcadores para ambas respuestas, desmostraron que en el pulmón esta relación se mantiene cuando el estímulo es el ejercicio [14, 99, 112]. Durante la actividad física, el incremento de la ventilación favorecerá la deshidratación y daño sobre el epitelio respiratorio, propiciando la inflamación, la cual puede verse exacerbada por el ingreso de contaminantes [113] o aire frío [85]. Todos estos factores nocivos activarán la respuesta defensiva de los macrófagos alveolares con la concomitante activación de la NOX, así como otros mecanismos de producción de pro-oxidantes (ver apartado 1.2), incrementando los niveles de $O_2^{\cdot-}$, H_2O_2 y $ON^{\cdot}$ (o derivados metabólicos, por ejemplo el NO_2^-), instaurando un ambiente oxidativo. Éste último, puede ser producido en las células epiteliales (intracelular) o endoteliales del sistema respiratorio (intravascular). Junto con el proceso oxidativo, se activará una respuesta inflamatoria, incrementando la presencia de células inmunes polimorfonucleares (también con capacidad oxidativa), interleuquinas (IL-8, TNF-a, PGs) y otros marcadores inflamatorios (histamina, pH ácido). Un aspecto a destacar en la revisión bibliográfica (artículo científico 1) fue que la mayoría de los estudios en humanos utilizaron muestras obtenidas con técnicas no invasivas tales como el AE o AEC. Otro método es el esputo inducido, técnica definida como semi-invasiva, ésta es una maniobra muy utilizada para la cuantificación de células inmunes presentes en el sistema respiratorio. Las técnicas invasivas utilizadas para el estudio de la inflamación y estrés oxidativo pulmonar en humanos son la obtención fluido de lavado bronquio-alveolar, broncoscopia y biopsias bronquiales, las cuales son cada vez menos requeridas debido a la popularidad que han alcanzado el AE y AEC. Los principales factores son su alto costo, necesidad de un mayor despliegue técnico y equipamiento, pueden producir daño al epitelio respiratorio durante su ejecución, tienen mayor riesgo de infección y necesitan que el paciente se encuentre sedado [114].

El AEC es una técnica que: 1) no genera incomodidad en el sujeto (se realiza en normoventilación); 2) no provoca (sanos) o agrava (enfermos respiratorios) la inflamación de las vías aéreas; 3) alta portatilidad; 4) tiene una menor dificultad de ejecución para el evaluador; 5) es posible almacenar las muestras para futuros análisis; 6) permite estudiar el

fenómeno oxidativo e inflamatorio en conjunto; y 7) es de bajo costo. Estas características han ampliado su uso, siendo utilizado en situaciones tan complejas como sujetos expuestos a ambientes hipobáricos a grandes alturas [112] o aquellos que se ecuentran hospitalizados en unidades cuidados intensivos [115].

Un aspecto innovador en el estudio del AEC es que hoy en día es posible fraccionarlo, esto ha permitido a investigadores estudiar de forma aislada la respuesta inflamatoria y oxidativa en dos segmentos pulmonares, el alvéolo y las vías aéreas. Lo anterior, permitirá disminuir la variabilidad de los resultados de los biomarcadores, ya que el muestreo no controlado de EBC de ambas regiones puede causar una dilución indefinida del biomarcador en la región afectada por la muestra en la región no afectada. Otra característica que posee el AEC es que permite el estudio longitudonal del paciente, pudiendo evaluar los resultados de la terapias farmacológicas, lo cual no puede ser realizado por la medición del $ON\cdot$ en el AE. A pesar de que este último permite detectar el nivel de inflamación pulmonar de manera rápida, válida y eficaz, su utilidad ha sido limitada a pacientes con asma, y su rol en el manejo de otras patologías respiratorias aún no es conocido [116]. Sin embargo, el AEC también tiene algunas desventajas, por ejemplo existen dudas razonables sobre el origen de las sustancias presentes en el condensado, ya que aunque la mayoría de la muestra proviene de las microgotitas producidas en la interface de fluidos que cubren las vías respiratorias y los alvéolos, no se descarta que pueda estar contaminada la zona por sustancias procedentes de la boca, la orofaringe o incluso el tracto digestivo superior [117].

Desde los resultados descritos en la tabla 1.A en la revisión bibliográfica (artículo científico 1) se puede desprender que el ejercicio físico agudo maximal en humanos sanos (entrenados, activos sanos) provoca un incremento de los pro-oxidantes pulmonares [100, 101, 118–123]. Estos efectos fueron exclusivamente observados para el H_2O_2 y NO_2^-, ya que el VNO también incrementó en intensidades bajas [124]. Asimismo, algunos autores detectaron un incremento en el daño oxidativo (MDA) en altura [38] y en la concentración de pro-oxidantes (VNO y H_2O_2) luego de ejercicio de intervalos de alta intensidad [85, 96, 125] y corta duración (<30 min) [96, 126]. Asimismo, cuando el ejercicio fue submáximo (75 – 80% de la frecuencia cardiaca máxima), pero de mayor duración (~50 min), el H_2O_2 también incrementó [85]. Junto con esto, ejercicios de intensidades bajas no provocaron cambios en el H_2O_2 o MDA en sujetos sanos [93]. Ahora bien, sólo algunos han relacionado estos efectos oxidativos con biomarcadores inflamatorios (células de inmunes, citoquinas inflamatorias, acidosis) en ejercicio agudo, por ejemplo Araneda OF et al. en [38] y [14] analizaron H_2O_2, MDA, NO_2^- y pH en AEC (tabla 1.A del

review). Este autor coincide en que una mayor duración del ejercicio a intensidad moderada (>30 min) provoca un incremento de los pro-oxidantes pulmonares con una tendencia hacia la inflamación.

La inflamación pulmonar inducida por ejercicio agudo en humanos ha demostrado ser significativamente incrementada con ejercicio agudo de alta intensidad y prolongado (~30 a 60 min) [67], principalmente de los polimorfonucleares [66, 127]. Además, un incremento de los biomarcadores inflamatorios (leucotrienos e interlequina-8) pulmonares fue observado cuando el ejercicio ejecutado fue de larga duración (>60 min) e intensidad moderada [66, 90, 128], sin embargo en algunos caso el pH no disminuyó significativamente [62].

Ahora bien, en animales las respuestas oxidativa e inflamatoria inducidas por ejercicio agudo no coinciden en su totalidad con humanos, ya que en animales sí se ha observado lipoperoxidación (↑ MDA) a nivel del mar [104, 129]. Cabe destacar que en los animales se utilizaron muestras de tejido pulmonar y en la mayoría de los estudios con humanos se utilizó el AEC, este último puede ser debido a una mayor dilución de los productos de la liperoxidación, siendo más sensible la biopsia. Respecto a la actividad enzimática antioxidante en animales destaca el incremento de la SOD.

El entrenamiento con ejercicio de larga duración provoca un incremento de la inflamación crónica sin efectos clínicos detectables, esto se exacerba cuando en el ambiente hay presencia de gases del cloro (piscinas), aire frío o cuando los volúmenes ventilatorios son muy elevados (ejercicio de larga duración y elevada intensidad; corredores recreativos) (artículo científico 1). En el caso de los humanos, la infiltración de elementos celulares (PMNs, macrófagos y linfocitos) fue lo más significativo. Para los estudios en animales, los biomarcadores solubles fueron claves en demostrar la inflamación pulmonar, destacando el TNF-a. Sin duda, el problema requiere estudios adicionales para aclarar numerosas preguntas que nos permitan tener una visión más definitiva. Uno de los principales desafíos en este campo consiste en mejorar las condiciones técnicas que permiten obtener muestras representativas del entorno pulmonar en sus diferentes compartimientos (aire exhalado condensado fraccionado). También es necesario caracterizar el efecto del tiempo y la intensidad del ejercicio realizado (artículos científicos 2 y 3), así como el papel de las condiciones ambientales y el nivel de entrenamiento de los sujetos sobre el daño oxidativo/inflamación pulmonar por ejercicio. De igual modo, debe estudiarse la actividad de las fuentes de producción de radicales libres en el pulmón (mitocondria, xantina oxidasa, NOX y NOS) y conocer el estado de los sistemas antioxidantes, particularmente en humanos, ya que no hay registros

disponibles. En cuanto a los parámetros inflamatorios, el estudio de los mediadores solubles de la inflamación debe extenderse; además, el efecto de sustancias con efecto antioxidante y anti-inflamatorio debe ser explorado. Finalmente, se requiere generar investigación que examine los parámetros de los mecanismos oxidativo/inflamatorio simultáneamente, para establecer la interrelación que existe entre ambos procesos (artículos científicos 2 y 3), identificando el nivel de compromiso de cada una por sí sola y en conjunto.

El primer experimento de esta tesis (artículo científico 2), llevado a cabo en un grupo de sujetos sanos levemente entrenados (9,2 ± 3,3 hr/sem) que realizaron una carrera de 10 kilómetros al aire libre, demostró que existe una tendencia hacia una respuesta pulmonar oxidativa/inflamatoria luego de un ejercicio de moderada duración (~50,6 ± 4,6 min) e intensidad alta. Las muestras de AEC y plasma antes del ejercicio fueron comparadas con las medidas 20 y 80 minutos de después del ejercicio. Las respuestas oxidativas e inflamatorias observadas independientemente en las investigaciones previas revisadas en esta tesis (artículo científico 1), fueron en parte confirmadas en este experimento (artículo científico 2) para ambos procesos (oxidación e inflamación pulmonar), ya que se observó que un ejercicio de alta intensidad al aire libre aumenta los pro-oxidantes originados en el pulmón en sujetos no atletas sin evidencia de peroxidación lipídica temprana, además hubo asociación alta, donde un menor pH se relacionó con un mayor nivel de proxidantes (H_2O_2 y NO_2^-) en el AEC. Uno de los aspectos que influyó en la respuesta oxidativa de los participantes fue su bajo a moderado nivel de entrenamiento para esta disciplina (VO_2max promedio= 47,4 ± 6,0 ml/kg/min). Esto podría tener una repercusión negativa en áreas donde el ejercicio es utilizado como una herramienta terapéutica, por ejemplo en rehabilitación cardiorrespiratoria (EPOC, Insuficiencia Cardiaca, etc). Será necesario entonces considerar que una capacidad cardiorrespiratoria reducida, puede favorecer a bajas intensidades de esfuerzo y elevada duración, un mayor efecto oxidativo/inflamatorio pulmonar, limitando o complicando el proceso de rehabilitación en estos pacientes, ya que como es reconocido, el ejercicio terapéutico, incluso en estos pacientes, puede disminuir el estrés oxidativo luego de un adecuado entrenamiento físico [130]. Ahora bien, en sujetos entrenados no se han observado estos cambios, este hecho puede ser explicado por un incremento adaptativo de las defensas antioxidantes [131] y por una menor respuesta inflamatoria como resultado del ejercicio crónico [132]. Por lo tanto, será recomendable a futuro reevaluar sujetos con diferentes niveles de entrenamiento y compararlos en las mismas condiciones.

Durante el ejercicio físico, el incremento del ON· en los pulmones se encuentra implicado en la dilatación de las vías respiratorias y vasos sanguíneos [133]. Sin embargo, también el ON· se encuentra relacionado al desbalance redox en condiciones patológicas, tal es el caso de algunos procesos inflamatorios respiratorio como el asma bronquial y la EPOC [134]. La vida corta de este elemento hace que sea necesario determinar metabolitos más estables, estos son el NO_2^- (artículo científico 2 y 3) y NO_3^- [135], los cuales pueden formarse a partir de células pulmonares típicas (células epiteliales, células endoteliales, células del músculo liso), así como en leucocitos y eritrocitos [136]. En esta tesis (artículo científico 2), se observó una tendencia al aumento de [NO_2^-] del AEC y de la relación entre [NO_2^-]-AEC/[NO_2^-]-plasma después de 80 minutos de finalizado el ejercicio en sujetos que no están acostumbrados a este esfuerzo extenuante, a diferencia de lo que se ha observado en corredores habituales. Es posible que hayan ocurrido aumentos de ON· durante el ejercicio, ya que el NO_2^- puede permanecer sin estar completamente depurado en el aumento de la actividad endotelial de la óxido nítrico sintasa tal como se observa en modelos animales y/o en la actividad aumentada de esta enzima como se ha descrito en los leucocitos humanos después del ejercicio [137, 138]. Al igual que que el estudio llevado a cabo por Araneda et al. [14], intensidad del ejercicio debe significar un esfuerzo mayor en duración para activar considerablemente el incremento del nitrito, más aún si los sujetos son entrenados, ya que este estudio no observó cambios en la [NO_2^-] del AEC después de una carrera de 10 kilómetros, mientras que sí se observaron cambios en carreras de 21.1 y 42.2 kilómetros. Esto confirma lo obtenido en esta tesis (artículo científico 3), ya que en un ejercicio de baja intensidad (30% del VO_2max), llevado a cabo en sujetos levemente entrenados, se observaron cambios significativos en la concentración de NO_2^- luego de 90 minutos de ejercicio, no así en 10 y 30 minutos. Lo mismo ocurrió con el H_2O_2, ambos en AEC pero no en plasma. La muestras fueron obtenidas 80 minutos después de finalizado el ejercicio. Creemos que los datos recolectados, como un todo, apoyan firmemente la idea de que el ejercicio prolongado intenso en esta población, en las condiciones descritas, altera el estado redox del microambiente pulmonar.

La diferencia en la respuesta pH en el AEC entre carreras de 10 kilómetrosm y distancias más largas puede estar relacionada con la mayor intensidad de la respuesta inflamatoria frente al aumento del estímulo (distancia de la carrera) y el tiempo necesario para establecer un proceso inflamatorio en el tejido; esto se ve reflejado al comparar nuestro sresultados de una carrera de 10 kilómetros, donde no observamos modificaciones es de aproximadamente 1 hora (artículo científico 2), con los resutados

de una maratón de aficionados que tomó alrededor de 4 horas, donde Araneda et al. [14] sí observaron cambios en el pH. En este sentido, será una gran contribución para el futuro, extender el tiempo de seguimiento de este parámetro después de la carrera (horas o días posejercicio) e incluir marcadores específicos de inflamación tales como citoquinas. Respecto a MDA en el AEC, no se observaron cambios (artículo científico 2) igual que en estudios previos en muestras obtenidas a pocas horas después del ejercicio [14].

Un grupo de sujetos sanos físicamente activos que realizaron 90 minutos de cicloergometría a baja intensidad (30% del VO_2max), a diferencia de aquellos que hicieron 10 y 30 minutos a la misma intensidad, produjo un incremento en la ventilación minuto, el consumo de oxígeno relativo a los minutos de ejercicio y la percepción de esfuerzo los cuales probablemente están asociados a la fatiga evidenciada en la ultima parte de los 90 min (artículo científico 3). Aunque no podemos descartar que esos cambios están involucrados en este fenómeno de estudio, el gran incremento observado en la ventilación es de particular interés, ya que implica la posibilidad de descender la temperatura de la vía aérea, favoresciendo el daño mecánico, y promoviendo al deshidratatción del epitelio. Ha sido sugerido que estos factores se ecuentran involucrados en la irritación e inflamación de la vía aérea [13, 139, 140]. Asimismo, el VO_2 total relativo durante el ejercicio, es relevante porque se asocia posible incremento de la formación de especies reactivas de oxígeno y nitrógeno, tal como fue descrito previamente [102].

Ahora bien, es necesario proyectar los siguientes objetivos de estudio en el ámbito de la oxidación e inflamación pulmonar inducida por ejercicio:

1. Profundizar en la caracterización de los efectos oxidativo e inflamatorio pulmonar inducido por ejercicio en humanos sanos entrenados y no entrenados, destacando los efectos de la intensidad, duración y tipo de ejercicio, así como el tiempo inmediatamente después de finalizado el ejercicio. Lo anterior permitirá crear un modelo de estudio confiable para este ámbito.

2. Profundizar en el conocimiento sobre los efectos del uso de agentes antioxidantes y desinflamatorios, a través de modelos de estudio sobre oxidación e inflamación pulmonar inducida por ejercicio en sujetos sanos.

3. Estudiar el comportamiento del efecto oxidativo e inflamatorio en días posteriores al momento de evaluación (por ejemplo a las 24, 48 y 72 horas) con ejercicio.

4. Profundizar en el conocimiento de los efectos provocados por diversos factores ambientales (aire frío y seco, contaminación, altura, entre otros) durante la ejecución del ejercicio, destacando la integración de sus efectos.

Extender el estudio de las respuestas oxidativas e inflamatorias pulmonares en la poblaciones que utilizan el ejercicio como herramienta terapéutica (rehabilitación cardiorrespiratoria o metabólica), ya que en la mayoría de los casos, el ejercicio utilizado en rehabilitación tiene una duración entre 30 min a 1 hora de ejercico continuo de moderada intensidad o en intervalos de alta intensidad (según las guías internacionales), y aún se desconocen algunos sus efectos.

6. Conclusiones generales

Las principales conclusiones en esta tesis son las siguientes:

1. El mecanismo de muestreo de Aire Exhalado Condensado (AEC) permite medir un gran número de biomarcadores capaces de caracterizar en conjunto el fenómeno oxidativo e inflamatorio pulmonar inducido por ejercicio, sin agravar estos efectos debido a sus características de no invasividad.

2. Una mayor intensidad de carreras en humanos levemente entrenados, más aún si es maximal, incrementa la producción de pro-oxidantes pulmonares (H_2O_2 y NO_2^-) e induce una tendencia hacia la inflamación, sin producir daño oxidativo.

3. Una mayor duración de cicloergometría (90 minutos) en humanos activos no entrenados, incrementa la producción de pro-oxidantes pulmonares (H_2O_2 y NO_2^-) y provoca una tendencia hacia la inflamación, sin producir daño oxidativo.

4. Los resultados obtenidos en los experimentos (artículos científicos 2 y 3) confirman que estas respuestas tienen un efecto local (pulmonar), sin generar cambios a nivel sistémico.

7. Bibliografía

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8. Apéndices

8.1 Apéndice 1

8.1.1 Artículo científico 1 publicado

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Review Article

Update on the Mechanisms of Pulmonary Inflammation and Oxidative Imbalance Induced by Exercise

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The mechanisms involved in the generation of oxidative damage and lung inflammation induced by physical exercise are described. Changes in lung function induced by exercise involve cooling of the airways, fluid evaporation of the epithelial surface, increased contact with polluting substances, and activation of the local and systemic inflammatory response. The present work includes evidence obtained from the different types of exercise in terms of duration and intensity, the effect of both acute performance and chronic performance, and the influence of special conditions such as cold weather, high altitude, and polluted environments. Levels of prooxidants, antioxidants, oxidative damage to biomolecules, and cellularity, as well as levels of soluble mediators of the inflammatory response and its effects on tissues, are described in samples of lung origin. These samples include tissue homogenates, induced sputum, bronchoalveolar lavage fluid, biopsies, and exhaled breath condensate obtained in experimental protocols conducted on animal and human models. Finally, the need to simultaneously explore the oxidative/inflammatory parameters to establish the interrelation between them is highlighted.

1. Introduction

When doing physical exercise, the usual levels of organic performance are exceeded. However, we are designed to execute the exercise, depending on its variety, duration, intensity, and the environmental conditions under which it is done. The physiological and pathological processes will be activated, which can lead to the generation of an oxidative imbalance and the establishment of an inflammatory process [1, 2]. The oxidative damage happens as an additional cost of using oxygen to obtain energy and can occur when there is an increase in the formation of prooxidants and/or when the antioxidant defense decreases, causing an alteration of tissue product functionality of the structural damage to all the cellular components that contain lipids, carbohydrates, proteins, and nucleic acids [3]. Another response mechanism to physical stress is inflammation, which is triggered as a reaction to the mechanical damage of structural components (connective tissue; muscle, tendon, and bone) and nonstructural components (erythrocytes, endothelium, and epithelia) of the body [4–8]. As a result, stress hormones are released, such as cortisol and catecholamines, which activates the immune system, causing a particular response profile based on the release of soluble mediators (cytokines) and arachidonic acid derivatives (prostaglandins and leukotrienes). The latter and the stress hormones will cause changes in the number and activation of leukocytes subpopulations to the point that intense exercise of long duration can induce immune suppression (increasing the susceptibility to infection) [9], in contrast to the exercise of moderate intensity, which boosts the immune response. Both the alteration of the redox system and the inflammatory reaction have multiple

points of interaction that have been previously evidenced [10–12]. The study of inflammatory/oxidative damage at a pulmonary level has been a topic poorly addressed [13–15], particularly in healthy humans and even more so in athletes. Most of the information in this subject arises from pathophysiology of pulmonary diseases, such as asthma, cystic fibrosis, and chronic obstructive pulmonary disease [16–27]. The lung has the crucial role of gas exchange and experiences great modifications of its activity during the exercise. This mobilizes larger volumes of air and modifies the breathing pattern from nasal to oral, increasing contact with a greater amount of pollutants that may be present in the environment. Also, the lung receives a greater amount of blood flow to increase the exchange in places that are well ventilated, which causes changes in the functioning of the vascular parenchyma [28, 29]. However, the anatomofunctional characteristics of the lungs make it very difficult to obtain information of the redox/inflammatory state in the different sectors of this organ. This work brings together the scientific papers that have addressed the phenomenon of altered pulmonary redox/inflammation environment induced by acute or chronic exercise, in a hypoxic environment, cold or contaminated, in both animal and human models, by focusing on the protocols and mechanisms that explain the phenomenon, as well as their potential implication on those who exercise.

2. Effects of Exercise on the Respiratory System and Its Relationship with the Generation of Oxidative/Inflammation Damage

When exercising, the mobilized air flow or pulmonary ventilation increases. This is explained by the increase of the respiratory rate, the tidal volume, and the appearance of bronchodilation. In addition to this, the pulmonary vascular bed will vasodilate to receive a greater blood flow. These changes, taken together, aim to increase gas exchange. Large air flows entering the lung during exercise will cause a modification of the breathing pattern towards one predominantly oral, favoring the evaporation of the fluid covering the pulmonary epithelium and the decrease of temperature of the airways. As a result, the pulmonary passages will cool down and the osmolarity of the epithelium will increase [30]. It should be noted that the cooling of the pulmonary passages as a result of the hyperventilation has been observed at comfortable environment temperature (+20°C) [31]. In this way, McFadden Jr. and Pichurko [31] showed a decrease of the tracheal temperature of 34°C at pulmonary ventilation of 15 L/min and of 31°C at 100 L/min. The cooling of the airway by hyperventilation produced by exercise is homologous to breathing cold air at rest. The latter is probably in the absence of air pollutants, the main irritative/proinflammatory factor of this region of our body. In cold environments, there is a greater amount of reports of respiratory symptoms [32] and chronic changes of epithelium similar to those of patients with chronically inflamed airways (e.g., asthmatics). Some authors observed, in humans, that the product of intense exercise appears to have similar symptoms to those observed in infection of upper airways [33–35]. However, with moderate training these symptoms decreased [36, 37]. It is probable that intense exercise of long duration, such as a marathon, will increase the susceptibility to infection of the airway by depression of the immune function, contrary to the effect caused by exercise of moderate intensity. Another factor involved in the oxidative/proinflammatory process of the airway is the greater contact with toxic particles and microorganisms present in the environment due to hyperventilation by exercise [38–40]. For example, the damaging effect on lung tissue of environmental substances such as chlorine, ozone, nitrogen oxides, particulate matter, and pollen is recognized [14, 41–43]. The entry of these substances by the pulmonary route can potentially generate systemic inflammation [44, 45] and this will affect the lungs. Finally, another factor of the recognized destabilizing effect of the oxidative balance and in favor of pulmonary inflammation is hypoxia [46, 47]. The general framework for the development of functional changes of the lung by exercise, the activation of the redox imbalance, and the inflammatory system are described in Figure 1.

3. Changes in Pulmonary Redox State and Exercise-Induced Inflammation

As mentioned previously, physical exercise induces changes in the redox/inflammatory state of the organism, at both systemic level and the different organs. In this regard, lung is one of the less studied organs in this context. In the following paragraphs, the most relevant results regarding pulmonary oxidative damage and inflammation caused by exercise are summarized. In this review, the work carried out in healthy subjects was privileged. Regarding the special conditions, hypoxia, water contaminants (chlorine), and cold have been included, leaving aside air pollutants, because there are several reviews regarding this subject [48, 49]. The details of the studies included in terms of goals, characteristics of the sample, the protocol used, and the results related to the pulmonary oxidative/inflammation damage by exercise are summarized in Tables 1 and 2 for human and animals, respectively.

4. Pulmonary Redox Balance and Acute Exercise

A direct relationship has also been reported during exercise, between the acute exercise intensity and the volume of exhaled nitric oxide (VNO), namely, volume minute (VE) multiplied by exhaled nitric oxide (eNO), for sedentary healthy [50, 60, 68, 69, 71, 82, 85–87, 90] and trained subjects [75, 89]. During exercise, eNO have been reported to be decreased when increasing VO_2 [59, 75] and VE [75] in sedentary and active subjects [51, 60, 68, 69, 75, 82, 85, 86, 92]. In athletes, unlike Maroun et al. [75], Kippelen et al. [68] showed changes in eNO during exercise. In animal model, while exercising healthy horses, Mills et al. [112] observed a linear increase of the VNO as the oxygen consumption increased. After exercise, nitric oxide concentrations have

Table 1: (a) Human studies on lung oxidative stress and inflammation induced by acute exercise. (b) Human studies on lung oxidative stress and inflammation induced by chronic exercise.

(a)

Author, year	Aim	Sample characteristics	Exercise protocols	Samples obtained	Oxidative or inflammatory main results
			Maximal incremental		
Adachi et al. 1997 [50]	eNO and VNO in patients with CHF during exercise'	CHF patients and healthy control subjects (C)	cycle ergometer test in CHF patients (10 W/min) and C (25 W/min) until exhaustion	EB	DE: ↑ VNO during exercise peak in C
			Constant workload		
Agostoni and Bussotti 2003 [51]	Correlation between eNO and lung mechanics during exercise in CHF	CHF patients and healthy control subjects (C)	25-W constant workload exercise cycle-ergometry test	EB	DE: ↓ eNO during 3rd and 5th minutes of exercise in C
			Three repetitions of		
Aranda et al. 2005 [44]	Lung oxidative damage from exercise at a medium altitude'	High-trained mountain bikers	cycle-ergometries of 1 min at maximum intensity in 670 and 2160 MASL with breaks of 1 min	HBC and serum	PE: ↑ [MDA] in HBC, with no changes in serum at 2160 MASL
			Urban 10 km (~53 min), 21 km (~101 min), and 42.2 km races (~246 min)	EBC	PE: ↑ $[H_2O_2]$ and ↑ $[NO_2^-]$ in 21 km and 42.2 km races and no changes in [MDA]; there was a tendency to ↓ of pH
Aranda et al. 2012 [52]	Duration of a lung distance exercise on pulmonary oxidative damage	Amateur runners			
			10 km race in outdoor athletic track (~50 min)	EBC	PE: ↑ $[H_2O_2]$, ↑ $[NO_2^-]$, ↑ $[NO_3^-]_{plasma}$ with no changes in the [MDA]; there was a tendency to ↓ of pH
Aranda et al. 2004 [53]	Pulmonary oxidative damage in long distance exercise	Healthy active subjects			
			Ease on treadmill at a speed adapt to maintaining 80–90% HR_{max} (220 − age), which was registered in 2 min and then maintained during 6 min		
Bikov et al. 2010 [54]	Changes in [Cys-LTs] caused by exercise in asthmatic patients	Nonsmoking asthmatic patients (A) and nonsmoking healthy control subjects (C)		EBC	PE: with no changes in [Cys-LTs] in C, but ↑ in A
			Exercise challenge tests on treadmill (details were not described by authors)	EBC and EB	PE: no change of pH in EBC in C
Bikov et al. 2014 [55]	Changes in EBC_{pH} during EIB in asthmatic patients'	Asthmatics, who reported breathlessness following exercise, and healthy control subjects (C)			
			Marathon race (~179 min)	IS and EB	PE: ↑ PMN in IS and ↑ eNO in EB
Bonsignore et al. 2001 [56]	Endurance exercise on inflammatory cells in AWs and eNO	Amateur runners			
			Swimming of 5 km only in the swimmers group, an open pool series (~70 min) and other series in the sea (~54 min)	IS and EB	E: > PMN and < MO in the IS of S vers us C PE: ↑ eosinophils, ↑ lymphocytes, and ↓ MO in the sea versus swimming pool; eNO was > in the sea in comparison to swimming pool
Bonsignore et al. 2002 [57]	Swimming on inflammatory cells and eNO in the AWs	Swimmers (S) and healthy control subjects (C)			

(a) Continued.

Author, year	Aim	Sampled characteristics	Exercise protocols	Samples obtained	Oxidative or inflammatory main results
Carlsen et al. 2008 [58]	eNO after swimming sessions	Trained healthy young people, not trained with swimming	Swimming in 2 seasons of 45 min (~3100 m) in a disinfected pool with [NaClO] and another sanitised with electrical process	EB	IE: ↑ eNO only in sanitized pool
Chimenti et al. 2009 [40]	Inflammation of the AWs in urban races in different climatic seasons	Amateur runners	21 km race in autumn (~89.1 min), 12 km race in winter (~46.1 min), and 10 km race in summer (~35.4 min)	IS	R: ↑ BM Ns with ↑ [TNF-α] and ↑ [IL-8] IE: PMNs tended to ↑
Chimenti et al. 2010 [5]	Damage and inflammation of the lung epithelium in a long distance exercise	Amateur runners and healthy control subjects	20 km outdoor races (~90 min)	IS and serum	IE: ↑ [IL-8] in IS and ↑ CC16 in serum
Chirpan Oddou et al. 1997 [59]	eNO and VNO during exercise	Healthy control and trained subjects	Incremental cycle-ergometry to exhaustion with 5 min of passive recovery in sedentary subjects (5 × 30 min and 9 × 20 min) and trained subjects (4-14 min)	EB	DE: ↓ eNO progressive with ↑ exercise intensity from 65% VO_2max and ↑ VNO with the ↑ of the intensity of exercise > 30 W in all subjects
Chu et al. 2000 [60]	To evaluate eNO during exercise in patients with stable COPD	COPD patients and healthy control subjects (C)	Maximal cycle-ergometry test (ramp: 10 cycles/min and load: 10 W in 1 min) to exhaustion	EB	DE: ↓ eNO at peak exercise and ↑ VNO in C
De Gouw et al. 2001 [61]	Role of eNO in the airway response to exercise by using L-NMMA, L-arginine, or placebo as pretreatment to exercise challenge	Asthmatic patients and healthy control subjects (C)	Cycle-ergometry for 6 min using dry air, while ventilation was kept constant in 40–50% of his or her predicted maximal voluntary ventilation (35 × FEV_1)	EB	IE: ↑ eNO 30 min after exercise in C
Dengozali Bougarnou et al. 2006 [62]	Endurance exercise and inflammatory cells of the AWs	Long-distance runners	Races on treadmill at 80% of MAS (~60 min)	IS	DE: ↑ PMNs, ↓ MO, and ↑ lymphocytes
Dengozali Bougarnou et al. 2007 [63]	Inflammatory mediators, cellular composition in AWs, and acute exercise during a sports season	Long-distance runners	Race at 80% MAS during the basic, precompetitive, and competitive period of a sport season in 1 year (~60 min)	IS	DE: ↑ PMNs in the precompetitive and competitive period. ↑ MO in the precompetitive period, also, ↑ [histamine], ↑ [IL-8], ↑ $[LTB_4]$, and ↑ $[LTE_4]$ in the competitive phase
Evjenth et al. 2013 [64]	To investigate the effect on FE_{NO} of a standardized exercise challenge test on a treadmill	Nonasthmatic children with and without allergic rhinoconjunctivitis (AR)	Run on treadmill 16 to 8 min/k heart rate target during the last 4 min was 95% of predicted maximum heart rate (220 − age)	EB	IE: ↓ eNO in nonasthmatic children without allergic rhinoconjunctivitis

(a) Continued.

Author, year	Aim	Sample characteristics	Exercise protocols	Samples obtained	Oxidative or inflammatory main results
Ferk-Ribera et al. 2010 [65]	Inflammation and postexercise pulmonary oxidative stress'	Healthy subjects	Swimming in a chlorinated indoor-swimming pool (40 min), whose average speed was 225 ± 9.7 m/min	EBC and EB	EB: no changes of eNO in EB; [RANTES], [IL-12p70], [IFN-γ], [IL-4], [IL-8], [IL-8)], [IFN-γ-induced protein 10], [TNF], [VEGF], and [8-isoprostane] in the EBC were not modified
García-Río et al. 2006 [66]	FE_{NO} before and after exercise challenge in patients with asthma and its relationship with airway obstruction'	Nonsmoking, steroid-naive, atopic patients with mild persistent asthma and nonsmoking, nonatopic healthy subjects (C)	Performing an exercise challenge on a cycle ergometer, with monitored ventilation (exercise parameters were not presented)	EB	EB: with no changes in eNO of healthy subjects
Hopkins et al. 1997 [67]	Pulmonary capillary pressure and function of the alveolar-capillary barrier during intense exercise'	Athletes with signs of hemoptysis by exercise and healthy control subjects	4 km cycling with 12% hill slope during ~7 min	BALF	BE: > alveolar MO_2 > [LTB$_4$], and < lymphocytes in athletes versus control subjects
Kippelen et al. 2002 [68]	eNO level in endurance-trained athletes during and after intense exercise'	Nine athletes with exercise-induced hypoxaemia (EIH), 12 athletes without EIH, and 10 untrained subjects	15 min intense cycling exercise at 90% VO_2 max	EB	DE: ↓ eNO and ↑ VNO (last 3 minutes) in all groups
Larsson et al. 1998 [32]	Cold air and inflammation in the AW during rest and exercise'	Healthy subjects	Rest on treadmill at −25°C and +22°C, each with 4 stages with 15 min at moderate intensity and 15 min of recovery	BALF	BE: at −25°C ↑ granulocytes and ↑ MO, no change in [IL-8]
Lovell et al. 2000 [69]	eNO and incremental exercise test in chronic congestive cardiac failure'	Chronic congestive cardiac failure patients and healthy control subjects (C)	Performing Bruce protocol modified by inclusion of an initial 3 min stage at 5% incline, later performing a constant workload test (6 min at 2.7 km h^{-1} and 5% incline)	EB	DE: ↓ eNO and ↑ VNO during Bruce test in C; ↑ VNO during constant workload test
Martioli et al. 2007 [70]	eNO levels in relevant before engaging in their respective activity	Healthy control subjects	Going up and down the stairs on a 20-foot stair case for 2 min	EB	BE: ↓ eNO 1 minute after exercise
Matsumoto et al. 1994 [71]	eNO and VNO during exercise	Healthy subjects	Cycle ergometry at 100 W and maximum intensity with 5 min of recovery (~12 min)	EB	DE: ↑ VNO at 100 W and at maximum pedaling intensity
Marek et al. 2008 [72]	[L-lactate] and [H_2O_2] during exercise'	Healthy subjects	Cycle ergometer steady-state exercise at 60 W (~7 min) and 120 W (~6 min)	EBC	DE: ↑ [L-lactate] and ↑ [H_2O_2] in 60 W and 120 W
Marek et al. 2009 [73]	Maximal exercise, H_2O_2 release rate, and acid-base status	Amateur athletes	Incremental cycle ergometry to exhaustion (~13 min)	EBC	BE: ↑ [H_2O_2] with no changes in pH nor [HCO_3^-]

(a) Continued.

Author, year	Aim	Sample characteristics	Exercise protocols	Samples obtained	Oxidative or inflammatory main results
Marek et al. 2013 [31]	Exercising in cold weather and released H_2O_2?	Healthy subjects	Race on treadmill at 75–80% HR_{max} at −18°C and −5°C (~20 min)	EBC	↑ $[H_2O_2]$ and ↑ rate of H_2O_2 release in both temperatures
Marcum et al. 1995 [75]	Physical condition and release of eNO during exercise	Healthy sedentary subjects (S), active subjects (Ac), and athletes (A)	Cycle ergometer in steady-state at 1 and 2 L/min of VO_2 only performing an additional one at 4 L/min of VO_2	EB	↓ eNO at > VO_2 in S and Ac; ↑ level of VNO with ↑ VO_2 in A
Mercken et al. 2005 [76]	Exercise-induced oxidative stress in COPD?	COPD patients and healthy control subjects (C)	Incremental cycle-ergometry exercise test until exhaustion and sub-maximal constant work rate exercise test (66% maximal power output)	EBC	↑ $[H_2O_2]$ in maximal but not in sub-maximal exercise in C
Mercken et al. 2009 [77]	Pulmonary oxidative stress by endurance exercise in COPD and healthy subjects?	COPD patients and healthy control subjects	Cycle-ergometry exercise bg at 40% of maximum power output (20 min)	EBC	↑ $[H_2O_2]$ in COPD patients but not in healthy control subjects
Moriá et al. 2004 [78]	VE during exercise and inflammation in the AWs	Young rowers	Maximal run of 1000 m on the *rower ergometer* (~3 min)	IS	DE ↑ tendency in epithelial cells at a higher VE IE: ↑ MO with both ↑ VE/bg and ↑ VT/bg
Nowak et al. 2001 [79]	Prooxidants and oxidative damage by moderate exercise	Healthy subjects	Cycle ergometer exercise test at 120 W during 6 min or until a HR of 120 bpm is reached	EBC	IE: with no changes in $[H_2O_2]$ and [TBARs]
Nakhaikiewicz et al. 2006 [80]	Effect of the physical activity on eNO levels in healthy subjects and in CAD patients?	CAD patients and healthy control subjects smokers and nonsmokers	Bruce protocol exercise test	EB	IE: without changes in eNO in healthy control subjects nonsmokers
Pedersen et al. 2009 [81]	Inflammation in the AWs after 1-exercise session	High performance swimmers	Swimming in indoor-swimming pool at moderate intensity (45 min) where average heart rate was 162 bpm	EBC and IS; EB	IE: no changes in the cellular composition in IS; eNO in EB, nor pH in EBC of swimmers
Paglialo et al. 1997 [82]	VNO after modifying pulmonary blood flow with head-out water immersion or increased gravity at rest and during exercise?	Normotensive and healthy subjects who underwent air with normal conditions, water immersion, or increased gravity (1 Gz or 2 Gz)	Incremental cycle-ergometry test, loadings on increased progressively by 50 W every 3 min until voluntary exhaustion	EB	DE: ↓ eNO and ↑ VNO in all groups
Pucsok et al. 2007 [83]	Lung $[PGE_2]$ and $[TXB_2]$ and exercise	Judo competitors	Incremental run on treadmill until VO_2 max is reached (run time was not recorded)	EBC	IE: ↑ $[PGE_2]$ and ↑ $[TXB_2]$ in ♂, but not in ♀
Rinkke and Danner 2007 [84]	Low-intensity physical activity and pH	Healthy subjects	Walk on treadmill at 60% HR_{max} predicted with 1 min pause every 10 min (~30 min)	EBC	IE: ↑ pH

(a) Continued.

Author, year	Aim	Sample characteristics	Exercise protocols	Samples obtained	Oxidative or inflammatory main results
Ribeyet al. 1997 [85]	NO production in patients with abnormalities of the pulmonary circulation*	IPH (primary pulmonary hypertension), PF (pulmonary fibrosis), and normal subjects group	Maximal (20 W/min in the normal subjects and 15 W/min in the PF patients and individual estimated exercise tolerance in PPH patients) and submaximal constant work rate cycle-ergometry exercise test (work rate VO_2 midway between each patient's anaerobic threshold and VO_2 max)	EB	DE: ↓ eNO and ↑ VNO in normal subjects at peak exercise in maximal and constant work rate exercise test
Rolla et al. 2003 [86]	R elationship between eNO and exercise tolerance in patients with moderate MS*	Patients with moderate MS and healthy control subjects (C)	Symptom-limited incremental exercise test with an upright cycle-ergometer (25 W every 3 min until exhaustion)	EB	DE: ↓ eNO and ↑ VNO in all groups at the end of exercise
Shin et al. 2003 [87]	R elationship between exercise and NO exchange	Nonsmoking healthy adults	High-intensity exercise treadmill test at 90% of the predicted maximum heart rate (220 − age in years) for 20 min	EB	PE: ↑ VNO
Sri Cresti et al. 1999 [88]	Effect of exercise on endogenous NO formation by measuring eNO at constant airflow rate	Healthy nonsmokers, and nonsmoking subjects	3 min of constant-load cycle-ergometry exercise test at three different exercise intensities corresponding to 30%, 60%, and 90% VO_2 max	EB	PE: ↓ eNO and ↑ VNO for all intensities of exercise in healthy subjects
Theron Arias et al. 1998 [89]	Exercise in cold air on eNO and VNO*	Highly trained subjects (cross-country skiers, triathlon, and running)	Incremental cycloergometry to exhaustion in a climatic chamber at +22°C and −10°C (~30 min)	EB	DE: ↓ eNO with the ↑ of the intensity > 60 W in +22°C and ↑ VNO with the ↑ of the intensity > 30 W in both temperatures
Todin et al. 1994 [90]	eNO and VNO during exercise	Healthy subjects	Moderately heavy exercise on a cycloergometer (♀: 90 W for woman and ♂: 150 W for ♂)	EB	DE: ↓ eNO
Tufvesson et al. 2013 [91]	R elationship between CO16 levels in plasma and urine after exercise with exhaled breath temperature and eNO*	Asthmatic and healthy control subjects	During first six minutes stepped and slope were adjusted to maintain the heart rate subject to 90% of their theoretical maximum heart rate (220 − age); the next two minutes were adjusted again to reach maximum effort	EB	PE: ↓ eNO in both groups

(a) Continued.

Author, year	Aim	Sample's characteristics	Exercise protocols	Samples obtained	Oxidative or inflammatory main results
Verges et al. 2006 [92]	Effect of prolonged exercise on the NO concentration in the lung	Nonsmokers undertaking a moderate to intense training program participated in the study	100 min exercise test was performed on a cycle-ergometer (5 min of rest, 30 min warm-up at 25% P_{max}, 10 min at 60% P_{max}, 2 min at 25% P_{max} repeated five times (S1 to S5), and 10 min of active recovery at 25% P_{max})	EB	DE: ↓ eNO for all exercise sessions (WU, S1 to S5, and active recovery)
Wetter et al. 2002 [93]	EIAH and pulmonary inflammation[a]	Endurance athletes with EIAH who used anti-inflammatory or placebo	Maximal incremental run on treadmill to exhaustion (~18 min)	IS	PE: with no PMNs, lymphocytes, nor MØ; ↑ [Histamine] in placebo
Yasuda et al. 1997 [94]	To examine the origin and role of eNO during exercise	Healthy control subjects	Two sets of 10 minutes in a cycle-ergometer (5 min without load and 5 minutes with 60 W and 60 RPM) separated, with 15 minutes between them	EB	DE: with no changes in eNO
Zietkowski et al. 2010 [95]	To assess the possible association of EIB with low-grade systemic inflammation in asthmatic patients[a]	Asthmatics (14 with EIB, 10 without EIB) and healthy volunteers	Cycle-ergometer test for 9 min with a fixed work load adjusted to increase the heart rate to 85% of the maximum predicted for the age of each patient	EBC	PE: with no changes in hs-PCR in healthy volunteers

AWs: airways; BALF: bronchoalveolar lavage fluid; CAD: coronary artery disease; CC16: Clara cell secretory protein; CHF: chronic heart failure; COPD: chronic obstructive pulmonary disease; Cys-LTs: cysteinyl leukotrienes; EB: exhaled breath; EBC: exhaled breath condensate; EIAH: exercise-induced arterial hypoxemia; EIB: exercise-induced bronchoconstriction; eNO: exhaled nitric oxide; FE_{NO}: fractional exhaled nitric oxide; HCO_3^-: bicarbonate; H_2O_2: hydrogen peroxide; HRmax: maximum heart rate; IFN-γ: interferon gamma; IFN-γ-induced protein-10: interferon gamma-induced protein-10; IL-12p70, IL-4, IL-8, and IL-18: interleukin-12p70, interleukin-4, interleukin-8, and interleukin-18; IS: induced sputum; L-NMMA: N-monomethyl-L-arginine; L-lactate: lactate; LTB_4: leukotriene B_4; LTE_4: leukotriene E_4; MØ: macrophages; MAS: maximal aerobic speed; MS: mitral stenosis; MDA: malondialdehyde; MPO: myeloperoxidase; MASL: meters above sea level; NaClO: sodium hypochlorite; NO_2^-: nitrite; NO output: nitric oxide output (eNO × VE); PGE_2: prostaglandin E_2; P_{max}: maximal power output; RANTES: regulated upon activation, normal T-cell expressed, and secreted; TBARs: thiobarbituric acid reactive species; TNF(-α): tumor necrosis factor (alpha); TXB_2: thromboxane B2; Se: selenium; VE: minute ventilation; VEGF: vascular endothelial growth factor; VNO: volume of nitric oxide; VO_2 max: oxygen uptake (maximal); VT: tidal volume. In "*Oxidative or inflammatory main results*," DE: during exercise and PE: postexercise. In "*Aim*," [a]the effect of exercise was not the primary aim of the study.

(b)

Author, year	Aim	Sample's characteristics	Experimental protocols	Samples obtained	Oxidative or inflammatory main results
Belda et al. 2008 [96]	Type of sport (aquatic or terrestrial) and cell count[a]	Elite healthy athletes and with asthma	Comparison of baseline samples between healthy and asthmatic athletes who practice water sports in pools or terrestrially (T: ~20 h/wk, with the exception of healthy subjects in water with T: ~10 h/wk)	IS	There was a positive correlation between PMNs with training time and water sport in the pool
Carraro et al. 2006 [97]	eNO in regular attendance to swimming pools[a]	Children swimmers attending and control children not attending the swimming pool	Comparison of baseline samples between swimmers who attended a swimming pool (1 h/week/6 months) and control subjects	EB	There were no differences in eNO between both groups

(b) Continued.

Author, year	Aim	Sample's characteristics	Experimental protocols	Samples obtained	Oxidative or inflammatory main results
Ferdinands et al. 2008 [98]	Exercise induced environment and inflammation	Cross-country athletes and healthy control subjects	Comparison of baseline samples before and after 10 workouts in 15 d (~1 h/d)	EB	↔pH in cross-country athletes compared to their control subjects between their respective sample times
Heinicke et al. 2009 [47]	Pulmonary oxidative damage and prolonged stay in medium height training	Biathletes and sedentary control subjects	Comparison of baseline samples between biathletes (T: ~5 h/wk) and control subjects; both groups were exposed to 2000 MASL during the 6 weeks	EBC	[H_2O_2] and β-isoprostane PGE$_2$ α] with no differences between groups; hypothesing data ↑ [H_2O_2] and tendency to ↑ [8-isoprostane PGE$_2$ α]
Helenius et al. 1998 [99]	AWs inflammation in swimmers	Elite swimmers and nonathletic control subjects	Comparison of baseline samples between swimmers (T: 800–3300 km/year) and control subjects	IS	↑Eosinophils, ↑PMNs, ↑[EPO], and ↑human neutrophil lipocalin in swimmers in comparison to control subjects
Helenius et al. 2002 [100]	Retirement from swimming in relation to AWs inflammation	High performance swimmers	Comparison of baseline samples between active (T: ~1870 km/year) and inactive swimmers (3 months of inactivity)	IS	↑eosinophil and ↑lymphocytes in active swimmers than inactive skiers in comparison to control subjects
Karjalainen et al. 2000 [101]	Inflammatory cells in skiers, mild asthmatics, and healthy control subjects[a]	Elite healthy skiers and nonathletic control subjects	Comparison of baseline samples between skiers (T: 200–650 h/year) and control subjects	Endobronchial biopsy	↑lymphocytes↑ (4.0 times), ↑MAST (2.6 times), ↑eosinophils (2 times), and ↑PMNs (2 times) in skiers in comparison to control subjects
Martin et al. 2002 [102]	AWs inflammation and exposure to swimming pool in athletes[a]	Endurance athletes	Comparison of baseline samples of pool based (5 h/wk) and non-pool based (0.5 h/wk) athletes (T: ~5 h/wk)	EB and IS	PMNs and eosinophils in IS and eNO in EB were not different between groups
Sue-Chu et al. 1999 [103]	AWs inflammation in skiers	Cross-country skiers and nonathletic control subjects	Comparison of baseline samples during the competitive period, in autumn and winter, between skiers (T: 435 h/year) and control subjects	BALF	↑total cells, ↑lymphocytes, and ↑mast cells in skiers in comparison to control subjects, with no differences in [TNF-α] and [MPO]
Sue-Chu et al. 2000 [104]	Budesonide and AWs inflammation in skiers[a]	Elite cross-country skiers with asthmatic symptoms and budesonide or placebo supplementation	Comparison of baseline samples among skiers, after 20 weeks of supplementation with 800 μg/d budesonide (T: ~427 h/year) or placebo (T: ~468 h/year)	BALF and endobronchial biopsy	lymphocytes, MØ, eosinophils, PMNs, and mast cells were not different between groups

AWs: airways; BALF: bronchoalveolar lavage fluid; EB: exhaled breath condensate; EBC: exhaled breath condensate; EPO: eosinophil peroxidase; H_2O_2: hydrogen peroxide; IS: induced sputum; 8-isoprostane PGF2α: 8-isoprostane prostaglandin F2α; MØ: macrophages; MPO: myeloperoxidase; NO: nitric oxide; PMNs: polymorphonuclear neutrophils; T: training volume; TNF-α: tumor necrosis factor alpha. [a]In "Jans," the effect of exercise was not the primary aim of the study.

Table 2: (a) Animal studies on lung oxidative stress and inflammation induced by acute exercise. (b) Animal studies on lung oxidative stress and inflammation induced by chronic exercise.

(a)

Author, year	Aim	Sample characteristics	Exercise protocols	Samples obtained	Oxidative or inflammatory main results
Abd et al., 2015 [105]	Se administration affects lipid peroxidation in liver and lung tissues of rats subjected to acute swimming exercise	Sprague-Dawley adult male rats divided into general control, Se-administered, swimming, control and Se-administered swimming groups	Swimming was performed once for 30 minutes	Lung tissue	PE: ↑ MDA and ↑ GSH in swimming control versus general control
Al-Hashem 2012 [106]	Vit E and VitC in protection of pulmonary damage induced by exercise in altitude	Wistar rats with 6 months of altitude adaptation	Forced swimming for 2.5 h in glass tank at 600 and 2270 MASL in accordance with altitude adaptation	Lung tissue	PE: ↑ [TBARS], ↓ SOD, and CAT activity at 600 MASL Supplementation with VitE and VitC reversed these results
Caillaud et al. 1999 [107]	Effect of acute exercise on lipid peroxidation in lung, compared with locomotor muscles	Wistar rats exercised (E) and control rats (C)	Race on treadmill at 28 m/min and 15% grade (80–85 VO_2max) until exhaustion (~66 min)	Lung tissue	PE: no changes of pulmonary activity of SOD, CAT, and [MDA] of E in comparison to C
Calbert et al. 2013 [108]	Effects of exercise during different ambient temperature and humidity on eNO, eCO_2, and pH	Thoroughbred racehorses	Exercised under saddle on an all weather 1.6 km track at half-pace canter, full pace canter, or gallop according to the current training regimen for each horse	EBC and EB	PE only ↓ pH in EBC
Hatao et al. 2006 [109]	Acute exercise and antioxidant enzyme activation in aged rats	Young rats (YR) or aged rats (AR) exercised (E) or not exercised control (C)	Race on treadmill at 25 m/min for YRE and 18–20 m/min for ARE for 60 min	Lung tissue	PE: ↑ Mn-SOD activity in YRE and ARE in comparison to their control subjects; ↑ Cu,Zn-SOD and CAT activity in YRE and ↓ reactive carbonyls derivative in ARE in comparison to their control subjects
Huang et al. 2008 [110]	Supplementation with L-Arg on pulmonary inflammation and oxidative damage induced by exercise in aged rats	Sprague-Dawley rats exercised (E) or sedentary (S) with L-Arg (+L-Arg) or without control rats L-Arg (C)	Race on treadmill for groups E at ~70% VO_2max until exhaustion (time for E+L-Arg and E(−L-Arg) and ~2 min, resp.)	Lung tissue	PE: ↑ [NO], ↑ [MPO], and ↑ [MDA] in EC in comparison to SC, with no changes between EC and SC for [SOD], ↑ [CAT], [GSH-Px], [GR], and [GSH]
Kirschvink et al. 2002 [13]	Oxidative stress, pulmonary function and airway inflammation in healthy horses and with recalcitrant exercise-induced pulmonary hemorrhage	Trained healthy horses, affected by exercise clinical remission	Race on treadmill with 2 min to 8, 9, and 10 m/s and 4% inclination, stages interrupted by 2 to go of 8 min to 3.5 m/s (30 min of warming up and 10 min of recovery)	BALF	PE: ↑ [UA] in healthy horses

(a) Continued

Author, year	Aim	Sample characteristics	Exercise protocols	Samples obtained	Oxidative or inflammatory main results
Lin et al. 2015 [111]	Oxidative stress and antioxidant defenses in animals supplemented or not with L-Arg*	Sprague-Dawley rats grouped as exercised (E) or sedentary (S) with L-Arg (+L-Arg) or control rats without L-Arg (C)	Race on treadmill for E groups at 20 m/min for 15 min and 25 m/min for 30 min; then they run at 30 m/min and 10% of inclination (70–75% VO_2max) until exhaustion (EC = 81 min and E + L-Arg = 87 min)	Lung tissue	PE: ↑ activity XO and MPO in IC in comparison to SC; ↑ [DA], ↑ [NO_x] and ↑ [MDA] in IC in comparison to SC; ↑ activity SOD and GR in EC in comparison to SC
Milk et al. 1996 [112]	eNO and VNO during acute exercise	Healthy horses	Maximal incremental race until 9 m/s	EB	DE: positive correlation of eNO and VNO with the race intensity
Radák et al. 1998 [115]	Acute anaerobic exercise and oxidative modification of pulmonary proteins	Exercised Wistar rats (E) and sedentary control rats (C)	Two sessions treadmill at 30 m/min for 5 min; after 5 min of recovery a 3rd race to exhaustion was performed	Lung tissue	PE: > pulmonary carbonyls and [glutamine synthetase] in E versus C
Reddy et al. 1998 [114]	Pulmonary oxidative damage by acute strenuous exercise in rats deficient in Se and VitE	Female Wistar albino rats deficient in Se and VitE and control rats	Intense swimming to exhaustion	Lung tissue	PE: > [SOD] and < [GSH-Px] and < [GST] in rats deficient in VitE and in comparison to control rats
Prigol et al. 2009 [115]	Supplementation with $(PhSe)_2$ and pulmonary oxidative damage caused by the exercise	Adult Swiss albino mice supplemented with $(PhSe)_2$ and not supplemented control mice	Swimming exercise (30 min) for both groups after 7 d of supplementation	Lung tissue	PE: ↑ [MDA] and ↑ of CAT activity in mice not supplemented with $(PhSe)_2$
Turlanche 1999 [116]	Exhaustive swimming and CAT activity in the lungs of male and female rats*	Sprague-Dawley rats	1 h swimming	Lung tissue	PE: ↑ CAT activity in males and females

BALF: bronchoalveolar lavage fluid; CAT: catalase; $(PhSe)_2$: diphenyl diselenide; GR: glutathione reductase; GSH: glutathione reduced; GSH-Px: glutathione peroxidase; GST: glutathione S-transferase; L-Arg: L-arginine; MASL: meters above sea level; MDA: malondialdehyde; MPO: myeloperoxidase; NO: nitric oxide; Se: selenium; SOD: superoxide dismutase; Cu,Zn-SOD: copper, zinc-superoxide dismutase; Mn-SOD: manganese superoxide dismutase; TBARS: thiobarbituric acid reactive substances; UA: uric acid; VO_2max: volume of nitric oxide; XO: xanthine oxidase; VitE: vitamin E; VitC: vitamin C. In "Author" or "Aim," "the effect of exercise was not the primary aim of study.

(b)

Author, year	Aim	Sample characteristics	Exercise protocols	Samples obtained	Oxidative or inflammatory main results
Abas et al. 2009 [117]	SOD activity and [TBARs] postadaptation by training in altitude*	Wistar albino rats divided into trained in hypobaria (THb) and normobaria (TNb) and not trained in hypobaria (Hb) and normobaria (Nb)	Comparison of baseline samples between groups trained with swimming (T: 5 at 30 min/day) for 4 days/week for 9 weeks) or not trained and exposed or not to simulated altitude of 3000 MASL (E: 120 min/day for 4 days/week for 9 weeks)	Lung tissue	PE: ↓ SOD activity in TNb in comparison to Nb; no difference in [TBARS] for the same groups

(b) Continued.

Author, year	Aim	Sample characteristics	Exercise protocols	Samples obtained	Oxidative or inflammatory main results
Asami et al. 1998 [118]	DNA oxidative damage by chronic exercise	Sprague-Dawley rats with spontaneous hypertension (SI), forced (T) exercise and sedentary control rats (C)	Comparison of baseline samples among rats with spontaneous exercise (wheel), trained on treadmill (T: 30–90 m/min/day for 25 days), and control rats	Lung tissue	PE: >[8-OH-dG] in F in comparison to SI; the DNA oxidative damage was related to the exercise intensity
Ajdinet al. 2009 [119]	Long period of dietary restriction and stress produced by high intensity swimming	Sprague-Dawley rats with restricted diet (RD) or ad libitum (AL), grouped in trained (+T), exercised (+E), and sedentary control rats (C)	Comparison of baseline samples of RD and AL in +T (E 8 weeks of swimming with 2% BW as extra load during ~50–80 min). PE in +E (E swimming until exhaustion), and baseline C	Lung tissue	PE: >GSH ratio/cysand >GSH/Prot AL+T compared to ALC; <LPO, >GSH, and GSH-Px in AL+E that AL+T PE: ↑ [MDA], ↓ [ESH], ↓ GR activity, and TGSH-Px of AL+E compared to ALC (acute effect)
Chimenti et al. 2007 [120]	Epithelial remodeling, inflammatory cells, and apoptosis in the AWs after chronic exercise	Trained Swiss mice (T) and sedentary control mice (C)	Comparison of baseline samples among trained mice (T: 5 d/week for 6 wk at moderate to high intensity)	Lung tissue	PE: >apoptosis, >proliferation, >loss of hair cells, and infiltration of leukocytes in the AWs in T versus C
da Cunha et al. 2013 [121]	Chronic exercise can oxidative stress and NF-κβ/p65 pulmonary immunocontent of rats with lung injury	Trained Wistar rats (T) and maintained control rats (C)	Comparison of baseline samples among rats trained on treadmill (T: 20 min at 60% VO_2max during 24 days in 3 months)	BALF and lung tissue	PE: >pulmonary cellular activity in T versus C; there are no changes in [TBARs], carbonyls, dichlorofluorescein, $[NO_2^-]$, and NF-κβ/p65 in the lung
Gündüz et al. 2004 [122]	Oxidant and antioxidant systems in rats organs after a year of training"	Wistar albino rats grouped in young control rats (YC), aged control rats (AC), and aged rats training (AT)	Comparison of baseline samples between AT in swimming (T: 1 h/day for 5 days/week for 1 year) with YC and AC	Lung tissue	PE: >SOD activity and >GSH/Prot AT in comparison to AC; no difference of [TBARs] between the same groups
Lee et al. 2013 [123]	Administration of a ginseng intestinal metabolite (IH-901) and exercise-induced oxidative stress in trained rat"	Sprague-Dawley rats divided into resting control (RC), training control (EC), resting with IH901 consumption, or exercise with IH901 consumption groups	Training was carried out during 8 weeks on a treadmill: two weeks with 0% inclination and 25 cm/sec; then 2 weeks with 10% and 30 cm/sec; then 4 weeks with 15% and 35 cm/sec	Lung tissue	PE: ↑ TBARs and ↑ protein carbonyls in EC versus RC
Moraghi et al. 2009 [124]	Therapeutic effects of physical exercise on histological and oxidative stress markers in animals exposed to cigarette smoke"	CBA/CG57BL/6 mice divided into control (C), training (T), cigarette smoke (CS), and cigarette smoke plus training (CS+T) groups	Training: 30 min/day during one habituation week; then they performed a swimming program 5 days/week for 8 weeks	Lung tissue	PE: ↑ SOD and ↑ CAT activity in E versus C
Oliveo et al. 2014 [125]	Moderate aerobic exercise training prior to *Streptococcus pneumoniae* infection influences pulmonary inflammatory response"	BALB/c mice divided into sedentary untreated (SU), sedentary infected (SI), aerobic trained untreated (ATU), and aerobic trained infected groups (ATI)	Comparison between SU and ATU during 4 weeks after an individual maximal exercise capacity test was performed (0.1 km/h every 2.5 min, 25% inclination); training was for 40 min/day, 5 days/wk for 4 wk at 50% of the maximal speed	BALF and lung tissue	PE: ↑ Cu/Zn-SOD and ↓ Mn-SOD expression in lung parenchyma of ATU versus SU after an individual maximal exercise capacity test

(b) Continued.

Author, year	Aim	Sample characteristics	Exercise protocols	Samples obtained	Oxidative or inflammatory main results
Reis Gonçalves et al. 2012 [15]	Chronic aerobic exercise on pulmonary inflammation, cytokine, and antioxidant enzymes in animal model of acute pulmonary damage*	Trained BALB/c mice	Comparison of samples before and after a low intensity training on treadmill (T: 50% of MS for 60 min/d, 3 d/week for 5 weeks)	BALF, EB, and lung tissue	PT: with no changes in leukocytes, [IL-6], [IL-10], nor [TNF-α] in BALF, with no changes in [NO] in EB; ↑ expression of IL-6 and Mn-SOD in the lung, but no changes of activity of GSH-Px and GR in the lung
Toledo et al. 2012 [126]	Regular physical exercise in an experimental mouse model exposed to cigarette smoke*	C57Bl/6 mice divided into control mice (C), trained (T), exposed to cigarette smoke (Sk), and Sk plus T (Sk+T)	Comparison of baseline samples in T at moderate intensity on treadmill (T: 50% MS for 60 min/d, 5 d/week for 24 weeks)	BALF and lung tissue	PT: <[ROS] in BALF of En compared to C; >GSH-Px activity, but not of Mn-SOD nor CuZn-SOD in lungs of T compared to C; with no changes in the expression of IL-1ra, TNF-α, and IL-10 between T and C
Yang 2011 [127]	Chronic exercise and expression of cytokines related to inflammation in the lung tissue	Old male Sprague-Dawley rats, group with trained rats (T) and sedentary control rats (C)	Comparison of baseline samples between rats trained on treadmill (T: 25 m/min for 120 min/day for 1 week) and control rats	Lung tissue	>expression of mRNA for TNF-α and IL-4 and <expression of mRNA for IFN-γ of group T versus C

BALF: bronchoalveolar lavage fluid; BW: body weight; DEP: diesel exhaust particles; DNA: deoxyribonucleic acid; EB: exhaled breath; 8-OH-dG: 8-hydroxydeoxyguanosine; GR: glutathione reductase; GSH: glutathione reduced; GSH-Px: glutathione peroxidase; IFN-γ: interferon gamma; IL-1ra, IL-4, IL-6, or IL-10: interleukin-1ra, interleukin-4, interleukin-6, or interleukin-10; LPO: lipid peroxidation; MDA: malondialdehyde; MS: maximal speed; mRNA: messenger RNA; MS: maximal speed; NF-κB/p65: factor nuclear kappa-β/p65; NO: nitric oxide; NO_2^-: nitrite; ROS: reactive oxygen species; SOD: superoxide dismutase; CuZn-SOD: copper-zinc-superoxide dismutase; Mn-SOD: manganese-superoxide dismutase; TBARs: thiobarbituric acid reactive substances; TNF-α: tumor necrosis factor alpha. In "Oxidative or inflammatory main results," PE: postexercise and PT: posttraining. In "Aim," * the effect of exercise was not the primary object.

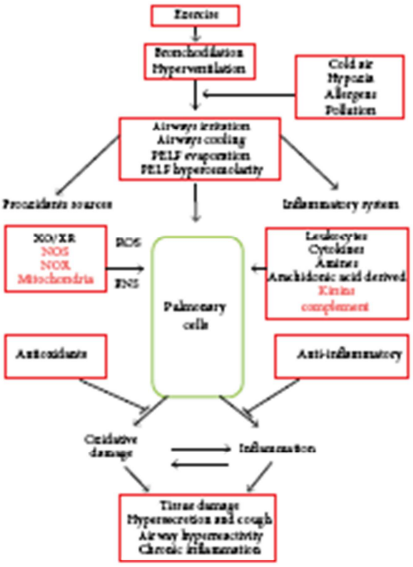

FIGURE 1: Proposed mechanisms related to the process of oxidative damage and pulmonary inflammation induced by exercise. Once the exercise starts the pulmonary ventilation increases and favors bronchodilation. This cools the airways, and also the part of PELF evaporates with subsequent increase of osmolarity and irritation appears. This activates the generating sources of free radicals and the inflammatory system. As a result of the foregoing, oxidative damage and a concomitant inflammatory process are potentially generated at pulmonary cell level; this may involve tissue damage, the increase of cough and the increased secretion of mucus, and the appearance of bronchoreactive phenomena and in the case that this stimulus is repeated (chronic exercise) to establish a process of chronic inflammation and remodeling of pulmonary tissue, particularly in the airways. This process is exacerbated when the exercise is performed in the presence of environmental conditions such as cold and hypoxia, in environments where pollen is abundant or in presence of contaminants (water/air). In red color the prooxidants sources and the parts of the inflammatory system that have not been studied are both appreciated. XO/XR = xanthine-oxidase/dehydrogenase, NOS = nitric oxide synthase; NOX = NADPH oxidase; PELF = pulmonary epithelial lining fluid.

shown controversial results. In swimmers, Bonsignore et al. [57] reported a decreased eNO after 5 km (~179 min) in slightly chlorinated pool; when performing the same test at the sea no changes were observed in this pair but the same distance was maintained at the sea. In other studies, also a decreased eNO after exercise has been observed in healthy subjects [64, 70, 88, 91]. However, in youngsters not trained in swimming, Carbonnelle et al. [58] found increases of eNO after swimming 2 sessions of ~1300 m in 45 min in a pool sanitized with electrical process (nonchlorinated water). Also, De Gouw et al. [61] found an increased eNO in healthy subjects after cycling for 6 min using dry air, while ventilation was kept constant in 40–50% of his or her predicted maximal voluntary ventilation (35 × FEV_1). Other studies showed no changes in the eNO after exercise; Font-Ribera et al. [65] found no differences in eNO concentrations in pool swimmers; the same occurred with eNO in swimmers after an exercise of 45 min [81] and in healthy subjects after either cycloergometer [66, 94] or treadmill incremental exercise test [80].

Through the exhaled breath condensate (EBC) analysis, to observe the oxidative effects of the moderate acute exercise, Nowak et al. [79] subjected a group of healthy subjects to a submaximal exercise on cycloergometer during ~6 min; they found no changes in H_2O_2 and thiobarbituric acid reactive substances (TBARs). Aranéda et al. [46] found no changes of H_2O_2 in EBC after three maximal cycle ergometries of 1 min in elite cyclists carried out at 670 and 2160 masl, but malondialdehyde (MDA) was higher at 2160 meters. Marek et al. [72], in two submaximal cycle ergometries to 60 W (~7 min) and 120 W (~5 min), and later in maximal exercise (~13 min), found no differences in H_2O_2 concentration in EBC [73]; however, in both studies, increases were found in the flow of formed H_2O_2 after exercise. On the same prooxidant, Marcken et al. [76] found an increase after maximal cycle ergometry in healthy subjects, with increments of 10 w/min, but they did not find any differences in subjects with chronic obstructive pulmonary disease after exercise. However, in another study they found no differences in H_2O_2 when healthy subjects performed a cycle ergometry with one leg (40% P_{max}) during 20 min [77]. Marek et al. [74] found that, after 90 min of high intensity running developed at ~18°C and ~−15°C of environmental temperature, the concentration and production rates of H_2O_2 in EBC were higher when the exercise was carried out in a cold environment. Recently an increase in H_2O_2 and nitrite concentrations and correlations between both metabolites in the EBC of 21 and 42.2 km race participants were found. Also in this study, while nitrite increased in EBC, plasmatic nitrite showed no modifications and no correlations between these variables, which suggests a probable localized origin of this process [53].

Until now, only two studies have determined one of the potential sources of prooxidants; thus, it has been described as an increment of xanthine oxidase activity in the pulmonary homogenate of rats that performed strenuous exercise (~15 min) on a treadmill (20 m/min), besides MDA and NO [111]. Likewise, Huang et al. [110] observed an increase of the activity of xanthine oxidase and lung MDA in older rats after running on a treadmill until fatigue, during ~63 min at 70% VO_2 max. Prigol et al. [115] and Akil et al. [105] found increases in TBARs in rats that swam for 20 min and 30 min, respectively, while Reddy et al. [114] found increases in MDA in rats with a vitamin E deficient diet that swam until fatigued. Also in rats, increases of TBARs after swimming during ~2.5 h until fatigue were found [106]. The same result was found in pulmonary homogenates of untrained rats which

swam until exhaustion [119]. A strenuous exercise protocol of ~66 min (80–85% VO_2max) showed no changes in TBARs in rats [107].

In healthy horses, no differences were observed in isoprostane 8-epi-PGF2α of supernatant of bronchoalveolar lavage fluid (BALF) after 50 min of running [13]. An increment of carbonyls in the lungs of rats was observed by Radák et al. [113] after an exercise till exhaustion on the treadmill. However, after an hour of a moderate intensity run in young and old rats, no changes were observed in the lung carbonyls [109].

With regard to the pulmonary antioxidant enzymes, after an hour of acute moderate exercise protocols on treadmills, young rats' lungs showed an increase in the activity of enzymes superoxide dismutase (SOD) of the type CuZn-SOD, Mn-SOD, of the catalase (CAT), without changes in the glutathione peroxidase (GSH-Px). The mRNA expression for these enzymes did not show differences [109]. Lin et al. [111] found an increase in SOD and glutathione reductase (GR) activity with no changes in CAT and GSH-Px activity in rats that ran at 30 m/min and 10% slope until fatigued. Finally, acute and prolonged exercise (more than an hour) at 80–85% VO_2max showed no changes in the activity of GSH-Px and SOD [107]. In acute exercise protocols, using swimming, Reddy et al. [114] found an increase in SOD and glutathione transferase (GST), while mild decreases in GSH-Px activity were observed in rats that swam until fatigued. Frigol et al. [115] found increase in CAT activity in rats that swam for 20 min. In rats that exercise for an hour, Terblanche [116] found increased CAT activity without differences between males and females. In rats 18 months old, Huang et al. [110] described an increase of SOD activity and the maintenance of levels of CAT, GSH-Px, and GR after 51 min on treadmill at 70% of VO_2max. Strenuous exercise increased the activity of GSH-Px, with no changes in GR [119]. In a report of Al-Hashem et al. [106], rats that exercised until fatigue decreased the activity of SOD and CAT.

Acute exercise has also altered the levels of nonenzymatic antioxidants; an increase of uric acid has been described, with no changes in total glutathione, in GSH, and in GSSG in BALF, after 50 min of incremental exercise in healthy horses [13]. In a study of rats that ran during ~81 min at 70–75% VO_2max until fatigue, no variations were found in the homogenized lung GSH [111]. In rats that swam until fatigue (~2.5 h), no differences were found at 600 m of altitude, but there was a decrease of GSH levels at 2270 meters [106]; in this same report, it was found that supplementation with nonenzymatic antioxidants such as VitC (20 mg/kg) and VitE (20 mg/kg), a single dose one hour before starting the exercise, decreases pulmonary lipid peroxidation and SOD and CAT activities increases, in both altitudes. Additionally, supplementation shows higher levels of GSH compared to animals not treated in altitude [106].

Thus, the increase in lung prooxidants and its consequences (lipid peroxidation) due to acute exercise appear to be related to the high intensity and duration of the effort, in terms of either minute ventilation or oxygen consumption, and are enhanced by a hostile environment (hypoxia, pollution, cold, etc.). However, a mainly enzymatic antioxidant adaptive response is still controversial. In contrast, the use of vitamin reducers (C and E) allows the antioxidant capacity to be increased and oxidative damage to be controlled (see Tables 1(a) and 1(b)).

5. Pulmonary Redox Balance and Chronic Exercise

In a first study of pulmonary prooxidants and chronic exercise, Carraro et al. [97] found no differences in eNO of child swimmers (trained 1 h/week during 6 months). Martin et al. [102] observed no differences in eNO of athletes based in pool and not based in pool exposed to pool environment during 5 and 0.5 h/week, respectively. For oxidative damage, Heinicke et al. [47] found a tendency towards increase of 8-isoprostanes in the EBC of biathletes who trained at 2800 meters during 6 weeks (4–6 h/d with 1 d/weeks of rest), which included extensive cross-country skiing, strength training, and shooting technique training.

In a model of physical training of rats, which jogged in 3 months a total of 24 sessions of 20 min/d at 60% VO_2max, no differences were found in pulmonary carbonyls, nitrite, or TBARs [121]. After 24 weeks of training at 50% V_{max} for 60 min/d for 5 d/week, ROS decreased in BALF and no changes of increase were found in pulmonary 8-isoprostanes in trained mice [126]. Using the same load and frequency as before, the levels of eNO and MDA were not altered in lung homogenates of rats trained during 5 weeks [15]. However, during the 8 weeks of training in rats that swam with a 2% of additional body weight during ~50–80 min, an increment of pulmonary carbonyls and MDA was observed [119]. Gündüz et al. [122] found increases of TBARs in older rats (21 months) versus young rats (9 months), without any variations between old rats which were either trained or untrained in swimming during 12 months 1 h/d for 5 d/week. Altan et al. [117] found increases in MDA in rats trained at 3000 meters of altitude (120 min/d for 4 d/week during 9 weeks) compared to sedentary control rats and the ones not trained maintained at sea or height level. In Sprague-Dawley rat that was trained during 8 weeks on a treadmill, an increase in pulmonary TBARs and protein carbonyls was observed [123]. Regarding oxidative stress on nucleic acids, Asami et al. [118] found increases in 8-hydroxydeoxyguanosine in rats after a forced race on treadmill for five weeks in daily sessions with a gradual increase in the time of 30–90 min.

The chronic exercising has also had as a subject of study the potential changes of the expression/activity of the enzymes and nonenzymes pulmonary antioxidant. Likewise, Reis Gonçalves et al. [15] found an increase in the lung Mn-SOD expression of mice subjected to five weeks of training at moderate intensity (60 min/d in 3 d/wk); however, no changes were observed in the GSH-Px, GR, GST, and CAT activities. In another study, Olivo et al. [125] observed an increased expression in pulmonary CuZn-SOD and Mn-SOD postmaximal exercise test of trained mice during 4 weeks at 50% of the maximal speed on treadmill. Altan et al. [117]

found increases of SOD activity after nine weeks of progressive training in a normobaric environment (5 to 30 min/d for 4 d/week), with no differences with a trained group at 3000 meters of altitude. da Cunha et al. [121] observed a higher pulmonary CAT activity in the ones trained on a treadmill during 12 weeks at 60% VO_2max (20 min/d), compared to control rats. In another study, Menegali et al. [124] found an increase of the CAT and SOD activity in lung of trained rat in swimming during 8 weeks. In mice trained on a treadmill for 24 weeks at 50% V_{max} (60 min/d and 5 d/week) increases of GSH-Px were observed without changes of expression of CuZn-SOD, Mn-SOD, and Ec-SOD, studied in sections of pulmonary tissue [126]. In another study, older animals of 21 months that were trained for a year (1 h/d and 5 d/week) had a greater amount of SOD in comparison to control rats of their same age and to young rats. No differences were found in CAT activities, while GSH-Px had a greater activity than a group of their same age [122]. Finally, Aydin et al. [119] observed a decrease in the concentrations of GSH and an increase of GSH-Px activity in pulmonary homogenates of rats, after eight weeks of swimming with overload and progressive weekly time increment (50–80 min).

This reflects the fact that oxidative stress induced by chronic pulmonary exercise in animals is closely associated with high-intensity protocols, but not with those of moderate intensity (see Table 1(b)). However, when moderate chronic exercise was executed while at high altitude, both human and animals presented pulmonary oxidative damage (see Tables 1(b) and 2(b)). In contrast, antioxidant adaptation seems to be more closely related to the animal training time, with an increase in the activity of SOD and CAT in the medium term and the expression of SOD in the short term (see Table 2(b)).

6. Acute Exercise-Induced Lung Inflammation

In horses, Kirchvink et al. [13] found no cellular count variation in BALF after 50 minutes of exercise. In runners' sputum of 10 km (~35.4 min), 12 km (~46.1 min), and 21 km (~89.1 min) a trend of increasing polymorphonuclear neutrophils (PMNs) in samples of induced sputum was found [40]. In the same direction, Bonsignore et al. [56] reported a higher percentage of PMNs in induced sputum, compared to values previous to exercise and an increase in these cells after the marathon (~179 min). Also in induced sputum of runners, Danguezli-Bouzgarrou et al. observed in 2006 [62] and 2007 [63] an increase of PMNs after 60 minutes of moderate racing. In the latter study, higher concentrations of histamine, interleukin-8 (IL-8), LTB_4, and LTE4 were also detected, subsequent to acute exercise during the precompetitive phase versus the competitive phase [63]. Chimenti et al. [5], in a 20-kilometer race (~90 min), reported an increase in IL-8 in the supernatant. Races in smaller time frames (~38 min) showed no changes in the amount of PMNs in induced sputum [93]. In rowers, after a short test of high intensity (1000 m in ~3 min), there was a trend towards an increase of epithelial cells and a positive association between the pulmonary ventilation/body weight (L/kg) and macrophages in induced sputum [78]. In swimmers, increases in lymphocytes and eosinophils and a decrease in macrophages were observed in induced sputum, after a 5 km race in the ocean (hypertonic environment) in relation to the same test performed in an open pool with low concentration of chlorine. However, there is no evidence of the increase in inflammatory cell activation [57]. In a chlorinated pool, in high performance swimmers, no changes were observed in the cellular composition of the induced sputum and the pH in EBC after 45 min at moderate intensity [81]. Larsson et al. [32] found an increase of granulocytes and macrophages in subjects that performed one hour of exercise, on a treadmill, at −23°C, without IL-8 changes in BALF samples. Derivatives of arachidonic acid have been studied in three works; thus, in a maximum acute exercise of approximately 12 min, increases in E_2 prostaglandin and B_2 thromboxane in EBC after exercise were found in men [83]. The leukotrienes in EBC were studied by Bikov et al. [54]; thus, after an eight-minute test on a treadmill no differences in the concentration of cysteinyl leukotrienes were found in normal people. In a test of 4 km of cycling with a 12% hill sloping during ~7 min, an increase of leukotriene B4 in BALF of athletes was found in comparison to the control subjects [67]. Also in EBC, Zietkowski et al. [95] found no changes in high sensitive C-reactive protein after 9 minutes of cycle-ergometry at 85% of HR_{max} in healthy subjects.

The pH in EBC (EBC_{pH}) is a potential marker of pulmonary inflammation that has been used in pathologies that have this condition. In acute exercise, the results have been variable; thus, Marek et al. [73] did not find differences after an exercise until fatigue (~13 min) in EBC_{pH} of amateur athletes. Bikov et al. [55] did not observe changes in the EBC_{pH} of healthy subjects after exercise, while there are other reports that show increases in pH after outdoor exercise [128] and after low-intensity (60% HR_{max}) exercise (~30 min) in nonathlete healthy subjects [84]. In races up to 10 km, no changes have been reported up to 80 min after the race, in both amateur runners [52] and physically active runners [53]. However, there are inverse correlations between changes in prooxidants and changes of EBC_{pH} [53]. In distances that exceed 21 and 42 km, ~101 min and ~246 min, respectively, an acute decreasing trend of EBC_{pH} was observed [52]. However, in an animal study conducted in horses, the group of Cathcart et al. [108] found an increase in EBC_{pH} after running 1.6 km.

In summary, the majority of published papers demonstrate the infiltration of inflammatory cells (macrophages or granulocytes) after acute exercise in humans. A factor that probably influences this is the duration of the exercise, as the increase in PMNs was found only in protocols involving longer periods (see Table 1(a)). Cellular infiltration was found to be due to cold or chlorine. The role of exercise training is difficult to assess, given that the studies were conducted almost exclusively in trained subjects. We must add to this the reported changes in soluble inflammatory mediators. As a whole, these could be an expression of an asymptomatic acute inflammatory process similar to that observed in other tissues (muscle tissue). This would happen in a self-limiting way whenever the necessary conditions of time, environmental factors, and intensity are encountered.

7. Chronic Exercise-Induced Lung Inflammation

Studies in animals have shown that training during 120 min/d for a week on treadmill at 25 m/min increases the expression of mRNA to tumor necrosis factor-alpha (TNF-α) together with promoting a decrease of interferon gamma in pulmonary tissue samples [127]. Chimenti et al. [120] trained mice at moderate intensity for 6 weeks (5 d/week), showing leukocyte infiltration in the airway. At this level of epithelia, an increase of apoptosis and a decrease of the ciliated cells were also observed. In mice that trained 60 min/d to 50% V_{max} for 24 weeks (5 d/week), no variation was observed in the number of macrophages in BALF, but it was possible to see a decrease of the capacity of these cells to form free radicals [126]. However, it is possible that the elaboration of training programs at moderate intensity (66% VO_2max) generates a reduction of the inflammatory response after the completion of ischemia and pulmonary reperfusion, which was evidenced as a decrease of the release of interleukin 1β and tumor necrosis factor-alpha (TNF-α) at plasmatic level in a model performed in rats [129]. An analogous result was described by Toledo et al. [126], who did not find differences in TNF-α, interleukin 10, monocyte chemotactic protein, and interleukin 1 receptor antagonist, quantified in lung sections of mice, after training to 50% V_{max} for 1 h/day, 5 days per week, for 24 weeks.

In studies conducted in humans, it has been reported that the participation in a long distance race training program over the course of a year generates a persistent inflammatory process with no apparent clinical repercussion and an increase in PMNs and in IL-8 concentrations, leukotriene E_4, and histamine in the supernatant of induced sputum samples [130]. Subjects who participated in high performance athletic training in sessions of 1 h/day for 10 days, interspersed with rest 5 days, had lower pH values in EBC compared to healthy control subjects [98]. The same result in this parameter was reported in runners by Grunewald et al. [128]. In the same direction, in amateur runners (~50 km/week) low levels of pH were reported compared to values of healthy control subjects [52]. High performance pool swimmers showed no differences in basal inflammatory parameters when compared with non-pool-based athletes; however, the analysis of the subgroup of athletes that had a positive result in the voluntary hyperventilation test (exercise-induced bronchial hyperreactivity indicator) presented a higher concentration of eNO and a higher count of eosinophils and of epithelial cells when compared to the group that had negative results on this test [102]; among other factors, this could be related to the number of years of practice of pool swimming, since no differences in eNO, in EBC pH, and in cellularity of induced sputum in adolescents were found when compared to normal subjects [131]. Elite swimmers, who trained between 800 and 3380 km/year, had more eosinophils and PMNs in induced sputum compared to nonathlete control subjects [99]. The cessation of the training for 3 months of swimmers decreases eosinophils and lymphocytes in induced sputum compared to active swimmers (~1870 km/year) [100]. The comparison between healthy athletes who are swimmers and others who are engaged in land exercise has shown an increased number of PMNs in induced sputum samples [96]; the same comparison showed no differences in PMNs and eosinophils in induced sputum [102]. Chronic inflammation can be associated with pulmonary epithelial damage; thus, increases of clear cell protein (CC16) in plasma of swimmers who trained during 20 weeks in a chlorinated pool have been reported [132].

In skiers, who trained 435 h/year, increase of lymphocytes and mast cells has been found, with no differences in the concentration of TNF-α and myeloperoxidase in BALF compared to nonathlete control subjects [103]. Karjalainen et al. [101] reported, through the study of bronchial biopsies, an increase in neutrophils, eosinophils, macrophages, and T lymphocytes in elite skiers (435 h/year) compared to healthy control subjects, along with air tract remodeling indicators as an increase in collagen I and collagen III deposits in the submucosa, a hyperplasia of racket cells, and a higher expression of type 5 mucin. The use of anti-inflammatories (800 micrograms/day of budesonide) by cross-country elite skiers (~427 h/year) during 20 weeks did not generate differences regarding the placebo (~468 h/year) in the cellularity (PMNs, macrophages, lymphocytes, eosinophils, and mast cells), studied in BALF and in endobronchial biopsy [104].

In summary, animal models of physical training show increases of soluble inflammatory mediators, which include TNF-α. Human studies have focused on subjects who have greater contact with irritants in the airway due to the specificity of their sport, whether runners (large ventilation volumes), skiers (cold), or swimmers (chlorine gas in the pool room). In these subjects, permanent tissue infiltration of granulocytes, macrophages, and lymphocytes has been observed. Evidence of these changes has been found in both noninvasive samples, such as induced sputum, and in biopsies in the bronchial region. At the same time, an increased presence of soluble proinflammatory substances has been reported. Overall, this suggests that these athletes in particular may suffer from persistent changes in tissue (chronic inflammation and airway remodeling) that have been associated with pulmonary symptoms and functional changes (see the bottom of Figure 1).

8. Oxidative Damage and Inflammation, Relations, and Potential Effects

The generation of prooxidant substances and the establishment of tissue oxidative damage are closely associated with inflammatory processes; thus, inflammatory cells are a known source of prooxidants derived from both oxygen and nitrogen [133]. At the same time, the increase of prooxidants has been involved in the intracellular signaling which leads to inflammatory cell activation, increased secretion of soluble mediators of inflammation [134], endothelial activation, and also increased expression of adhesion molecules and endothelial permeability [135]. This relation implies that, in many situations, the increase of prooxidants participates in the activation of inflammation and vice versa, demonstrating the close relationship between both phenomena [134].

The establishment of both oxidative damage and inflammation in the lungs has been involved in the origin/evolution of various pathological states; for example, both phenomena are a fundamental part of adult respiratory distress [136], asthma [137], chronic obstructive pulmonary disease [138], pulmonary hypertension [139], and viral infectious processes [140]. In the lungs, the relationship between oxidative changes and inflammation has rarely been studied as a main goal, but it is presumed that, in view of the studies conducted in other organs, it must be closely related. This is particularly important in subjects practicing sport, as both inflammation damage and oxidative damage have been implicated in the pathogenesis of phenomena of high prevalence in athletes such as rhinitis, bronchial hyperreactivity, asthma, and airway remodeling [27, 141]; so, most respiratory symptoms (coughing, wheezing, breathlessness, and chest tightness) in endurance athletes such as cross-country skiers are known [142]. In addition, cross-country skiers show a presence of PMNs and lymphocytes infiltration in the airways [101]. This phenomenon can also be extrapolated to other endurance athletes [143] such as marathon runners, cyclists, and swimmers, the latter of which are also exposed to the chlorine in swimming pools, which could be one of the main factors inducing increased eosinophils and leukocytes in the sputum.

9. Methods for the Study of Lung Inflammation/Oxidative Damage by Exercise

The study of the oxidative/inflammatory damage in the lungs is challenging due to both anatomic functional limitations and the limitations of currently applied techniques. Current evidence on this topic focuses primarily on the study of lung diseases, while studies on the effect of exercise as a trigger effect of this phenomenon in healthy people are scarce. Summarizing what is known to date for the species analyzed, the determinations made and the samples obtained are shown in Tables 1 and 2. Lung tissue microenvironment has challenged developers of study methodologies, so, although systemic markers have been proposed (CC16, surfactant proteins A and B, and Krebs von den Lungen-6), they do not yet have sufficient capacity to indicate minor damage, which implies that the processes of the lung itself cannot always be ascertained. For this reason, it is preferable to test samples originated from the lung; those currently under study are exhaled breath (whether direct or condensate), fluids (BALF, induced sputum, and nasal lavage), and cells and portions of whole tissue (biopsies, tissue homogenates, and cut pieces of tissue). Unfortunately, today there is still much controversy regarding the interpretation of the results obtained with these methods. In relation to oxidative/inflammatory exercise phenomenon, in animals, exhaled breath [112], lung tissue homogenates [113, 114, 117, 118, 120, 121, 127], bronchoalveolar lavage [121, 126], and lung tissue sections [126] have been used. In humans, most methods are focused on noninvasive methods and, among these, the induced sputum is the most widely used [40, 56, 57, 62, 63, 78, 81, 93, 96, 99, 100, 102, 144]. Another sample studied corresponds to exhaled breath, which was analyzed whether directly [56, 57, 59, 65, 71, 75, 81, 89, 97, 102] or after being condensed at low temperature [46, 53, 65, 72–74, 77, 79, 81, 83, 84, 128, 130]. Very few studies have used bronchoalveolar lavage [32, 103, 104] and lung tissue obtained by endobronchial biopsy [101, 104].

10. Discussion

In summary, we found that in acute exercise (see Tables 1(a) and 2(a)) there is more evidence of changes in cellularity (predominantly granulocytes) when it (was) is a prolonged high-intensity exercise. This change was not so evident in animals; however, this should be resolved in further studies because it is a parameter measured recently in this population. Long-term of acute moderate exercise (>60 min) in humans stimulated an increase of pulmonary inflammatory mediators (IL-8, LTB_4, and LTE_4). Now, regarding prooxidants, a systematic increase in humans is observed after more than thirty minutes of exercise. It is noteworthy that, in acute exercise in animals, reports of an increase in lung lipid peroxidation are the majority, while it has not been observed in humans, except for intense exercise at high altitudes. This may be partially explained by the techniques used: while tissue samples were analyzed in animals, EBC samples were analyzed in humans; in another aspect, the change with greater support in relation to the enzymatic activity corresponds to the maintenance or decreased levels of GSH-Px and to the increase in SOD.

With regard to chronic exercise (training) and its effects (see Tables 1(b) and 2(b)), the number of studies is still very small, but there is a tendency observed, seen in humans, towards changes in cellularity compatible with chronic inflammation of the airways, particularly in subjects exposed to cold and chlorine. In animals, changes in pulmonary cellularity (leukocyte infiltration) were observed in only one study [120]. For soluble inflammatory mediators, in animals the scientific evidence has shown an increase in the concentration of these substances (IL4, IL6, and mRNA TNF-α) subsequent to chronic exercise. The oxidative damage was observed in animals following moderate chronic exercise (>4 sem), specifically in older rats, and cold or altitude environment. In humans, only one study showed oxidative damage by altitude training [45, 47]. With regard to enzymatic antioxidants, a tendency towards higher levels in SOD and GSH-Px is observed in humans. As for nonenzymatic antioxidants, only one study showed a decrease in the concentration of pulmonary GSH in trained rats [119].

The problem requires further study to clarify numerous questions in order to have a more definitive overview; thus, several challenges for researchers in the field have arisen. Likewise, the activity of the sources of production of free radicals in the lung (mitochondria, xanthine oxidase, NADH oxidase, and NOS) should be studied and the knowledge of the status of antioxidant systems, particularly in humans, where there are no records available, should be improved. Regarding inflammatory parameters, the study of soluble mediators of inflammation should be extended; in addition, the effect of both substances with antioxidant and

anti-inflammatory effect should be explored. Furthermore, it is necessary to generate research projects which explore the parameters of oxidative/inflammatory mechanisms simultaneously in order to establish the interrelation mechanisms between both processes. It is also necessary to characterize the effect of time and intensity of performed exercise, the role of environmental conditions, and the level of training of the subjects on oxidative damage/lung inflammation by exercise. Finally, to advance the resolution of this problem, it is urgent to improve the technical conditions to allow obtaining representative samples of lung environment in its different compartments, and it is also necessary for these methods to be noninvasive and contribute to monitoring the athletes.

Conflict of Interests

The authors have no conflict of interests to declare.

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8.1.2 Artículo científico 2 publicado

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ORIGINAL PAPER

Increase of pro-oxidants with no evidence of lipid peroxidation in exhaled breath condensate after a 10-km race in non-athletes

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Abstract It is a well-established fact that exercise increases pro-oxidants and favors oxidative stress; however, this phenomenon has been poorly studied in human lungs. Pro-oxidative generation (H_2O_2, NO_2^-), lipid peroxidation markers (MDA), and inflammation (pH) in exhaled breath condensate (EBC) have been determined through data from 10 active subjects who ran 10 km; samples were obtained immediately before, at 20, and at 80 min post-exertion. In EBC, the concentration of H_2O_2 at 80 min post-exertion was increased. NO_2^- concentration showed a tendency to increase at 80 min post-exertion, with no variations in MDA and pH. No variations of NO_2^- were found in plasma, while there was an increase of NO_2^- at 80 min post-exertion in the relation between EBC and plasma. NO_2^- in EBC did not correlate to plasmatic NO_2^-, while it did correlate directly with H_2O_2 in EBC, suggesting a localized origin for the exercise-related NO_2^- increase in EBC. MDA in plasma did not increase nor correlate with MDA in EBC. In conclusion, high-intensity exercise increases lung-originated pro-oxidants in non-athlete subjects with no evidence of early lipid peroxidation and changes in the pH value in EBC.

Keywords Exhaled breath condensate · Runners · Lung oxidative stress · Lung inflammation

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Introduction

It is a well-documented fact that exercise favors the increase of pro-oxidants and that in some situations it produces oxidative stress [21, 30]. A reduced group of studies on animals have been focused on the impact of exercise on pulmonary redox equilibrium state, reporting evidence of oxidative stress [4, 36].

Exercise increases lung ventilation and favors higher contact with cold air, air pollutants, and chlorine in swimming pools [22, 43]; at the same time, it favors immune system activation [28]. The aforementioned may be particularly important in subjects who have regimes of long training hours. Consequently, previous studies in humans have demonstrated inflammation and redox state changes in the lungs of athletes such as swimmers [14], skiers [41], and runners [3, 11].

The study of redox state changes in the lungs resulting from exercise is difficult because sampling involves some risks to the participants; for this reason, the use of exhaled breath condensate (EBC), extensively studied in lung diseases and which has been proposed for the evaluation of various tissue processes (oxidative damage, cancer, remodeling, and inflammation) located in this organ [18, 20], can be a useful tool in the characterization of this phenomenon in athletes. Using EBC samples, no changes in the concentration of H_2O_2 ($[H_2O_2]_{EBC}$) were found during exercise [32], but changes in their flow [25] were found. It has also been reported that there was an increase in $[H_2O_2]_{EBC}$ on climbers exposed to altitudes of 6,125 m [1] and in biathletes who trained for 6 weeks at an altitude of 2,800 m [17]. In both protocols, an increase and a trend to the increase of lipid peroxidation measured as malondialdehyde (MDA) and 8-isoprostane, respectively, was shown. Recently, our research group compared amateur long distance runners who trained 50 km a week in 10-, 21.1-, and 42.2-km races, reporting increases in $[H_2O_2]_{EBC}$ and NO_2^- concentration in EBC ($[NO_2^-]_{EBC}$) for the 21.1- and 42.2-km races with no modifications in the participants of the 10-km race. In the three evaluated distances, no increase in lipid peroxidation, measured as the MDA concentration in EBC ($[MDA]_{EBC}$), was shown [3]. In this paper, we extend the description of redox state changes which occur in EBC from participants of long distance races to physically active but non-athlete subjects. We hypothesized that, in this group, a 10-km race could generate an increase in pro-oxidants and favor lung lipid peroxidation since they are not chronically exposed to the pulmonary effects (irritation, dryness, inflammation, cell damage) of the distance runners' training regime. A second objective was to advance in the characterization of EBC markers as originated either locally or from the systemic environment; for this purpose, we compared the concentrations of NO_2^- and MDA in both EBC and plasma.

Materials and methods

Subjects

Ten non-smoking students of Physical Education (see Table 1) with no history of high or low respiratory tract inflammation during the month previous to the study were made subjects of this study. They also had no history of chronic respiratory diseases (asthma or allergic rhinitis) and did not consume nutritional supplements, antioxidants, or anti-inflammatory medicaments. They practiced 9.2±3.3 h/week of moderate to intense exercise. The distribution of total exercise time, expressed in hours per week, is presented as mean±standard deviation, and the percentage of the total sample performed in this activity is shown in parentheses: running 2.5±0.5 (80 %), swimming 1.2±1.4 (40 %), football 1.5±1.7 (30 %), mountain bike 1.5±6.3 (20 %), tennis 0.9±2.1 (20 %), handball 0.6±1.4(20 %), volleyball 0.5±0.7(20 %), and basketball 0.5±0.7 (20 %). Participants were informed orally and in writing, before signing an informed consent. This study was approved by the Ethics Research Committee of the Universidad de los Andes.

Table 1 General description of participants

	Values
Men/Woman	9/1
Age (years)	20.50±1.60
Weight (kg)	62.64±6.8
Height (cm)	172.4±5.3
VO_2 max (ml kg^{-1} min^{-1})	47.37±6.0
Time of race (min)	50.65±4.63

Values are shown as mean ± SD

Protocol

After being evaluated at rest, they went through a 10-min warm up before running 10 km at maximum effort in an open 330-m racetrack. On each complete turn, the cardiac frequency was determined (Polar, model T31) in order to quantify the intensity of the exercise. All subjects performed this test simultaneously. EBC samples were taken using the previously described device [1, 2]. Subjects were at rest, wearing a nasal clip, and having previously washed their mouths with distilled water. Sampling time was between approximately 10 to 15 min or until 1.5 mL of EBC was obtained. Also, venous blood was drawn, heparinized, and then centrifuged at 3,000 rpm to obtain plasma. Once samples were obtained, they were stored in liquid nitrogen and later at −80 °C until they were analyzed. EBC or plasma samples were taken before (pre) exercise, 20 min after exercise completion (20-post), and 80 min after exercise completion (80-post).

Malondialdehyde in EBC and plasma

MDA concentration was measured according to Larstad et al. [23]. EBC at 300 µL or 50 µL of plasma was mixed with 100 µL of 25 mM thiobarbituric acid. The mixture was incubated for 1 h at 95 °C. After cooling, first in ice for 5 min and then for 40 min at room temperature, the mixture was submitted for high-performance liquid chromatography (Shimadzu LC10AD, Corporation), where a C-18 column 150-mm long and 4.6-mm I.D. (Supelcosil LC-18, Supelco) was used. The mobile phase (1 mL/min) was a 20:80 (v/v) mixture of acetonitrile in 20 mM potassium phosphate buffer (pH 6.8). Measurements were performed with a fluorescence detector (RF-551, Shimadzu), excitation and emission wavelengths, being at 532 and 553 mm, respectively. Malondialdehyde bis (diethyl acetal) from Merck was applied as standard.

Hydrogen peroxide in EBC

It was measured using FOX2 [31] reagent. This reagent contains Fe^{+2} (250 µM), which in an acidic medium ($HClO_4$ 110 mM), and is oxidized to Fe^{+3} by the presence of H_2O_2. The amount of H_2O_2 is monitored through the reaction between the ferric ion and the xylenol orange indicator (250 µM). Sorbitol (100 mM) was added to the original reagent according to Gay and Gebicki [15]; this method has been previously used by our research group [1–3]. For measurements, 350 µL of EBC and 150 µL of modified FOX2 were taken, then the sample was incubated for 1 h at room temperature, and absorbance was read at 560 nm (Jenway 6405). Three calibration curves were performed for each group's measurements using H_2O_2 (Merck) as standard.

pH in EBC

It was measured using the protocol of Paget-Brown et al. [33]. EBC at 100 µL was bubbled with argon for 8 min at a flow rate of 350 mL/min, and pH was later measured using a 3×38 mm (Diameter×Length) microelectrode (Cole and Palmer) connected to a pH meter (Oakton® Acorn pH 6).

Nitrites in EBC and plasma

Nitrite concentration was measured using spectrophotometric test based on the Griess reaction [16]. Griess reagent at 300 µL (0.1 % naphthylethylenediamine–dihydrochloride, 1 % sulphanilamide, 3 % H_3PO_4) was added to 300 µL of EBC or plasma deproteinized with $NaOH/ZnSO_4$. The mixture was incubated for 10 min, and absorbance was measured at 550 nm. Three calibration curves were performed for each group's measurements using sodium nitrite (Merck) as standard.

Statistics

Using the Shapiro–Wilk normality test, it was observed that the samples did not come from a Gaussian distribution; therefore, non-parametric tests were applied. The Friedman test was used for repeated samples, and Dunn's test was used as a further test for all the measured parameters. Correlations were determined by the Spearman correlation coefficient. The significance level used was of $p<0.05$. For statistical analysis, GraphPad Prism, USA software was used.

Results

Exercise intensity estimated as the percentage of cardiac reserve was at 91.2±4.7 %. The race time was 50.6±4.6 min. Both variables are expressed as mean and standard deviation. An increase in $[H_2O_2]_{EBC}$ (Fig. 1) as compared with the pre-value at 80-post ($p<0.05$) was seen. $[NO_2^-]_{EBC}$ showed a trend to significance; it had

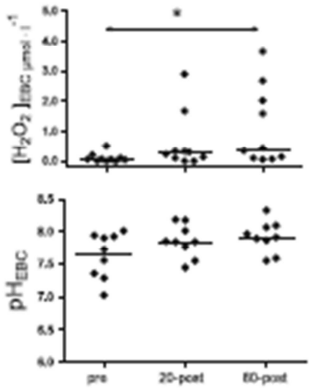

Fig. 1 $[H_2O_2]_{EBC}$ and pH_{EBC} in participants of a 10-km race. The *line* represents the median value. *$p<0.05$ is different from the pre-value

a value of $p=0.045$ in the Friedmann test, with no differences between groups in the posteriori test. No changes in nitrites in EBC and plasma ($[NO_2^-]_P$) after the race ($p=0.97$) were seen. The relation $[NO_2^-]_{EBC}/[NO_2^-]_P$ showed increases (Fig. 2) on the pre-value in the 80-post ($p<0.05$). No differences in $[MDA]_{EBC}$ ($p=0.60$), in the values of malondialdehyde in EBC and plasma ($[MDA]_P$; $p=0.83$), or in the relation between $[MDA]_{EBC}/[MDA]_P$ ($p=0.60$) as shown in Fig. 3 were observed. The pH in EBC (pH_{EBC}; $p=0.39$) showed no post-race differences (Fig. 1).

Correlations were made between absolute values and their absolute changes (deltas). A first group of absolute deltas was obtained from the difference between the absolute values of the 20-post-stages minus pre-stages. The second group of deltas was obtained from the differences between the absolute values of the 80-post-stages minus 20-post-stages; in the performed delta correlations, both sets of data were considered together.

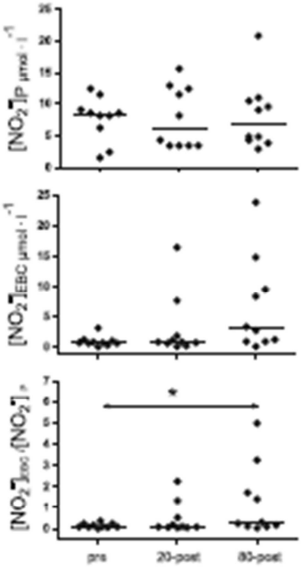

Fig. 2 $[NO_2^-]_P$, $[NO_2^-]_{EBC}$, and $[NO_2^-]_{EBC}/[NO_2^-]_P$ in participants in a 10-km race. The *line* represents the median value. *$p<0.05$ different from pre-value

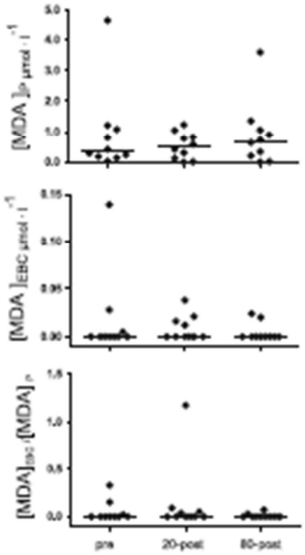

Fig. 3 $[MDA]_P$, $[MDA]_{EBC}$, and $[MDA]_{EBC}/[MDA]_P$ in participants in a 10-km race. The *line* represents the median value

Regarding nitrite, no significant correlations between absolute values of $[NO_2^-]_P$ versus $[NO_2^-]_{EBC}$ ($r=0.21$, $n=30$, $p=0.26$) and for absolute changes between these same variables ($r=0.18$, $n=20$, $p=0.46$) were observed. A similar result was found for $[MDA]_P$ versus $[MDA]_{EBC}$ for absolute values ($r=-0.22$, $n=30$, $p=0.24$) and between absolute changes ($r=-0.16$, $n=20$, $p=0.49$). Both the relation between absolute values of $[H_2O_2]_{EBC}$ versus $[NO_2^-]_{EBC}$ ($r=0.69$, $n=30$, $p<0.0001$) and absolute changes ($r=0.73$, $n=20$, $p<0.0002$) showed a significant association as shown in Fig. 4. No significant correlations between absolute values of $[H_2O_2]_{EBC}$ and $[NO_2^-]_{EBC}$ with pH_{EBC} were found. Correlations between absolute changes of $[H_2O_2]_{EBC}$ and pH_{EBC} showed a trend to significance ($r=-0.45$, $n=18$, $p=0.06$), while absolute changes between $[NO_2^-]_{EBC}$ versus pH_{EBC} were significant ($r=-0.61$, $n=18$, $p=0.007$; see Fig. 5). No significant correlations between the race time and intensity (measured as the percentage of cardiac reserve) with the studied variables in EBC and plasma in 20-post and 80-post were found.

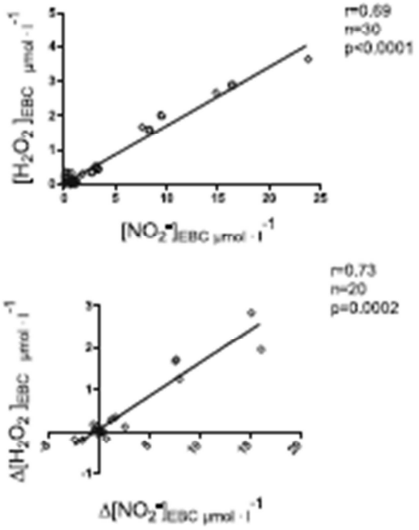

Fig. 4 Relationship between $[NO_2^-]_{EBC}$ versus $[H_2O_2]_{EBC}$ (top) and $\Delta[NO_2^-]_{EBC}$ versus $\Delta[H_2O_2]_{EBC}$ (bottom) in participants of a 10-km race

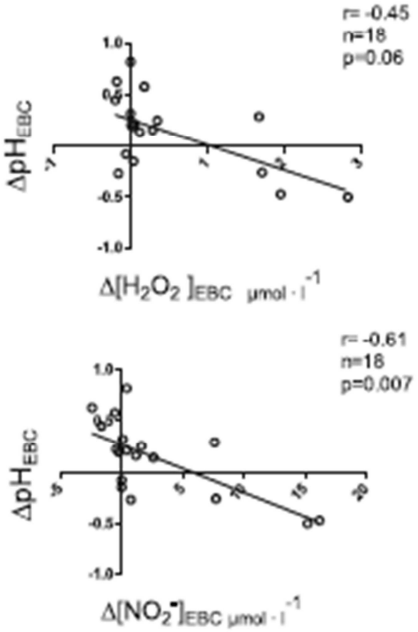

Fig. 5 Relationship between $\Delta[H_2O_2]_{EBC}$ versus $\Delta[pH]_{EBC}$ (top) and $\Delta[NO_2^-]_{EBC}$ versus $\Delta[pH]_{EBC}$ (bottom) in participants of a 10-km race

Discussion

Exercise increases lung ventilation and the speed with which the surrounding air reaches our lungs. High-intensity and prolonged exercising, typical of endurance races, inflame the airways [6, 42] and increase its pro-oxidants [3] as it has been previously described. One of the factors that have an influence on oxidative stress produced by exercise is the fitness degree of the participants; Brooks et al. [7] showed higher NO and superoxide anion formation because of acute exercise in sedentary rats' perfused/infused muscle versus muscles of trained rats. In patients with chronic obstructive pulmonary disease, there was less oxidative stress induced by acute exercise after their participation in a physical training program [34]. In this sense, it is possible that subjects, such as those from the present study, physically active but not subjected to high endurance athletes' regime (long sessions of prolonged aerobic exercise), are more prone to increase in pro-oxidative formation since the intensity of the performed exercise reached 90 % of the cardiac reserve (see the "Results" section). In this study, the main result is the increase in $[H_2O_2]_{EBC}$ and $[NO_2^-]_{EBC}$($[NO_2^-]_P$; both parameters show a tendency at 20 min that becomes significant at 80 min post-race. At the same time, despite the increase of these species, there is no lipid peroxidation increase, as it has been previously described in the lungs of animals [38], nor pH_{EBC} decrease as an indicator of tissue inflammation, as it has been described in pathologies such as asthma, bronchiectasis, and adult respiratory distress [20].

Regarding $[H_2O_2]_{EBC}$ results, there are few similar experiences in the literature to the one presented here, especially because of the increased exercise time (over 30 min) of our protocol, which makes it difficult to compare; Nowak et al. [32], Marek et al. [25], and Mercken et al. [26] conducted submaximal and maximal

exercises finding no differences in $[H_2O_2]_{EBC}$, but they used a protocol with only 6- and 15-min exercising times, respectively. Data presented here in are comparable to our previous report on amateur long distance runners [3]. In that report, no changes in 10-km runners were observed and absolute changes were minor in this distance in comparison to 21.1- and 42.2-km races. In this report, we found that in a group of non-runner subjects, $[H_2O_2]_{EBC}$ does increase after the race, as measured on the same times as of the aforementioned work. The fact that trained subjects do not show any increase in this pro-oxidant in this distance may be partly explained by the induction of anti-oxidant defenses [37] and by a lower inflammatory response as a result of chronic exercise [47]. This finding shall be later reevaluated in subjects with different levels of training directly compared under the same conditions.

During physical exercise, in the lungs, nitric oxide is involved in both dilatation of airways to increase mobilized airflow and vasodilatation in order to avoid excessive increase in pulmonary artery pressure [45]. In the pathological context, nitric oxide participates in lung redox imbalance that occurred in inflammatory processes [44]. Nitric oxide has a short half-life; therefore, in many experimental models, more stable metabolites such as nitrite and nitrate are determined [46]. Nitric oxide and its related compounds have a complex metabolism; thus, it is not yet fully clarified. This happens, among other aspects, because of its multiple origins; it can be formed from typical lung cells (epithelial cells, endothelial cells, smooth muscle cells) as well as in leukocytes and erythrocytes [8].

Regarding the effect of a long distance race on $[NO_2^-]_{EBC}$, to our knowledge, this parameter has been previously reported only by our research group, and no changes in $[NO_2^-]_{EBC}$ after a 10-km race were observed, while changes in 21.1- and 42.2-km races were observed [11]. Unlike this previous work, we currently report a trend to increased $[NO_2^-]_{EBC}$ and an increase in the relation between $[NO_2^-]_{EBC}/[NO_2^-]_P$ which means an increase in this pro-oxidant in the lungs by exercise in subjects who are not accustomed to this physical effort unlike usual runners. In the particular case of the time in which the increase of the relationship, $[NO_2^-]_{EBC}/[NO_2^-]_P$ (80-post-stage) is potentially observed; it can result from nitric oxide increases that occurred during exercising, since NO_2^- may remain without being completely debugged, in the increase of the lung endothelial nitric oxide synthase activity as seen in animal models and/or in the increased activity of this enzyme as described in human leukocytes after exercise [24, 29].

Contrary to the few measurement reports of $[NO_2^-]_{EBC}$, nitrite has been previously measured in plasma during exercise. In this regard, some reports have observed increases in plasma levels of nitrite+nitrate combination after 10 min of exercise at 75 % of maximal oxygen consumption [10]; however, this finding is not systematic for acute exercise. Bloomer et al. [5] found no nitrite increases in plasma after 30 min of treadmill exercise. In both athletes and sedentary people, Poveda et al. [35] found no changes in $[NO_2^-]_P$ after maximum exercise on a treadmill. In our study, $[NO_2^-]_P$ determination was done to evaluate if the eventual $[NO_2^-]_{EBC}$ increase could be explained by simultaneous increases in plasma (something that was not observed). This lack of NO_2^- increase in plasma and the lack of correlation between individual values and $[NO_2^-]_{EBC}$ and $[NO_2^-]_P$ absolute changes indicate that it is likely that the increase of EBC, because of exercise in these species, may be a localized phenomenon. This idea is also supported by the increase in $[NO_2^-]_{EBC}/[NO_2^-]_P$ relation and by the fact that $[NO_2^-]_{EBC}$ correlates with another exhaled air marker such as $[H_2O_2]_{EBC}$ (see Fig. 4). The finding of this statistically significant association is consistent with our previous report on long distance runners [3]. We believe that our collected data, as a whole, strongly supports the idea that intense prolonged exercise in this population—under the described conditions—alters the redox state of the pulmonary microenvironment.

The increase of the described pro-oxidants was not concomitant with $[MDA]_{EBC}$ increases (lipid peroxidation/oxidative damage indicator) and pH_{EBC} decreases (indicator of tissue inflammation) that were expected to occur. Regarding pH, Riediker and Danuser [39] reported a pH_{EBC} increase immediately and until 60 min post-exercise (30 min of fast walking at 60 % of maximal cardiac frequency).

A later report by Marek et al. did not report any changes in pH_{EBC} that was measured immediately after performing a maximal exercise to exhaustion in cycling (time is not reported) [25]. Ferdinands et al. [13] reported the absence of pH_{EBC} changes after acute exercise; however, they found lower pH_{EBC} values in regular runners. In a recent report on racehorses, Cathart et al. found increased pH_{EBC} 20 to 30 min after running 1.6 km at a moderate to high intensity [9]. The tendency to maintain pH_{EBC} values after exercise, herein reported or

alkalinization reported by other groups, has no explanation yet; however, some authors have suggested the hypothesis that this phenomenon is due to the increase in ammonium secretion (buffer) of the airway epithelium in response to exercise [9]. In the pathological context, subjects with chronically inflamed airways, like asthmatic patients, have lower ammonium levels [19]. Similarly, Mickleborough et al. [27] did not find any changes in the pH_{EBC} in asthmatic subjects after hyperventilation at 85 % of maximal voluntary ventilation for 6 min. These patients decreased their levels of airway inflammation after ingesting a preparation rich in omega three fatty acids for 3 weeks. So, a decrease of exhaled nitric oxide and an increase in the basal value of pH_{EBC} was evidenced, and in contrast to that previously observed in the first hyperventilation test, an alkalinization of pH_{EBC} after the said test was evidenced, which can be interpreted as a better response to acidosis of the airway [27].

In another aspect, our previous data obtained on amateur runners showed the same trend to the increase (proved herein) of pH value in the 10-km runners' group, while there is a trend to a decrease in the groups of 21.1- and 42.2-km races [3]. The difference in the pH_{EBC} response between 10-km races and longer distances may be related to the greater intensity of the inflammatory response against the increased stimulus (distance of the race) and the time necessary to establish an inflammatory process in the tissue; thus, the time in a 10-km race is about 1 h, while a marathon of amateurs takes about 4 h. In this regard, it will be a great contribution, in the future, to extend the follow-up time of this parameter after the race and to include specific markers of inflammation such as cytokines. Furthermore, the different acids and bases found in the EBC samples should be more specifically analyzed.

Although there was no decrease in pH_{EBC} or inverse correlations between the absolute values of the pro-oxidants as we found in our report [3], in this work, we found inverse associations between the absolute changes of pro-oxidants (trend to H_2O_2 and significance for NO_2^-) studied in EBC and the absolute changes of pH_{EBC} (see Fig. 5), which supports the hypothesis that pro-oxidant changes are related to inflammation at this level.

Regarding $[MDA]_{EBC}$, previous results showed no differences at low heights (670 m) after high-intensity cycloergometric exercise [1]. Similar results were found in a 120-W cycloergometric protocol [32]; both findings are equivalent to those reported here but with protocols dissimilar to ours. Failure to find an expected relation between the increase in pro-oxidants and the increase in lipid peroxidation may take place because changes in this parameter occur at a later time as compared to our measuring. Sentürk et al. [40] found MDA increases in plasma 12 h after 10 min of extenuating exercise; Fatouros et al. [12] found the highest $[MDA]_P$ at 24 h after a soccer match. Another possibility is that this pro-oxidative increase may be part of a physiological process in the described groups and conditions and is not associated to tissue damage in this organ. In this work, $[MDA]_P$ is also determined (see Fig. 3) in order to advance in the elucidation of the localized or systemic origin of this marker in EBC. In this respect, similar to that observed in $[MDA]_{EBC}$, we did not find any changes in neither $[MDA]_P$ nor changes in the relation between $[MDA]_{EBC}/[MDA]_P$ (see Fig. 3), so the interpretation of our findings becomes difficult. However, the lack of correlation between absolute values as well as between their absolute changes support the hypothesis that $[MDA]_{EBC}$ is not related to $[MDA]_P$; this was also observed in a previous work in which cyclists performed a maximal exercise at 2,160 m of altitude, showing increases in $[MDA]_{EBC}$ with no changes in MDA measured in serum. In the aforementioned work, no significant correlations between the said parameters were found [1].

In conclusion, unlike the previous results obtained in amateur runners, in physically active subjects, 50 min of high-intensity race (10 km) produces an increase in oxygen- and nitrogen-derived pro-oxidative species. Probably, this could be related to a stronger reaction response regarding the formation of pro-oxidant/inflammatory factors which are common in subjects less adapted to high-intensity and prolonged exercise. Despite the increase of pro-oxidants, we did not find any early modifications in lung lipid peroxidation and pH value in EBC. Nitrites in EBC most likely originated from a localized process in lungs.

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8.1.3 Artículo científico 3 publicado

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ORIGINAL PAPER

Effect of exercise duration on pro-oxidants and pH in exhaled breath condensate in humans

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Abstract Exercise promotes pulmonary oxidative imbalance. In this regard, some evidence has been obtained from the study of exhaled breath condensate (EBC) during urban races, in which the factors involved in the occurrence of this process are still not characterized. In this paper, under laboratory conditions, both the role of time of exercise on the generation of pro-oxidants (H_2O_2, NO_2^-) and pH have been assessed in EBC of 16 under-trained subjects who completed three tests of cycloergometric exercise at low intensity (30 % of VO_2 max) with a duration of 10, 30, and 90 min. Samples were obtained as follows: immediately before and at 80 min post exertion in each test. In the 90-min test, an increase in H_2O_2, NO_2^- concentration in EBC at 80 min post exertion with no changes in the pH was observed. Total O_2 consumption and total ventilation weakly correlated with the changes in H_2O_2 and NO_2^-. In conclusion, the concentration of pro-oxidants in the EBC depends on the duration of the exercise when it is performed at low intensity under laboratory conditions.

Keywords Exhaled breath condensate · Pro-oxidants · Lung · Time of exercise

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Introduction

Physical exercise is a recognized trigger of changes in the redox state, particularly when this activity takes place under special environmental conditions (cold, altitude, and pollution) and when the intensity is high or the exercise is performed for a prolonged period of time [2, 23]. So, redox state changes have been previously described, with the muscle tissue being the main focus of study, in view of its major functional changes during exercise. Thus, free radicals have been involved in the contractile activity, cell damage, inflammation, and fatigue [25, 26, 29]. Another organ that undergoes great changes in its activity due to exercise is the lung; hence, the flow of mobilized air increases, the temperature of the airways decreases [19, 31], the contact with environmental pollutants increased [10], and blood flow is also increased [9]. More details of the mechanisms were

recently extensively reviewed by our group [1]. Although some of these changes have been suggested as destabilizers of the redox state, few works have ventured to study this issue, and those are mostly in animal models [27]. In humans, the difficulty in obtaining samples, on the one hand, has limited the study of the lung and fostered the development of non-invasive methods (induced sputum, exhaled air, exhaled breath condensate) to study the lung tissue microenvironment. In exercise, there are previous reports regarding exhaled breath condensate (EBC) to study changes in the redox state of both athletes and physically active subjects [6]. Thus, EBC analysis has yielded an increment of H_2O_2 and malondialdehyde on climbers [2] and subjects training at medium altitude [15], showing that exercise in hypobaric conditions implies oxidative lung damage [5]. In a subsequent study, an increase in the concentration of pro-oxidants and a tendency towards airway acidification (a phenomenon associated with lung inflammation) were found in the EBC of runners of 21.1- and 42.2-km urban races [3]. In the same report, direct correlations between the running time and the absolute changes in the concentration of nitrite and hydrogen peroxide in EBC are found. At the same time, there was an inverse correlation between the race time and the absolute changes of pH in EBC [3]. The relations described were found in a field study, under different environmental conditions, in different subjects, and the exercise was performed with varying intensity, which could have affected the results. Consequently, this study aimed to measure EBC samples, pro-oxidants, and pH of subjects who exercised under controlled laboratory conditions, and the main focus was how exercise duration affected changes in these parameters.

Methods

Subjects

Sixteen male, healthy, active (see Table 1), non-smoker subjects with no history of rhinitis or asthma, without respiratory infection during the last month and who had not participated in any scheduled aerobic physical activity such as urban races, swimming, or cycling. They also did not consume anti-inflammatories, antioxidants, or any other nutritional supplements. Participants were informed orally and in writing, before signing an informed consent. This study was approved by the Ethics Research Committee of the University of Los Andes.

Protocol

Evaluations were performed as described as follows: (1) Survey of habits and anthropometric assessment (Antropometic Gaucho Kit, Rosscraft™, USA); (2) Determination of maximum oxygen uptake (VO_2max) on a cycle ergometer (VIAsprint™ 150/200p, Viasys™, USA) using exhaled gases analysis (Oxycon mobile, Jaeger™, Germany); (3) In the following three visits, exercise was performed on a cycle ergometer at a stable load equal to 30 % of VO_2 max for 10, 30, and 90 min, in which ventilation, VO_2, heart rate, perceived exertion, and pedaling cadence (60 rpm) were controlled. Participants appeared between 8:00 and 12:00 a.m., at least 1 h after a light breakfast and they hydrated only with the same isotonic electrolyte replacement drink, free of stimulating substances, antioxidants and/or anti-inflammatories, after exercise was completed. All the described physical tests were performed at a temperature between 18 and 22 °C and humidity between 60 and 70 %. To obtain EBC samples, exhaled air was cooled and condensed through an instrument designed and previously validated by our group [2, 4]. Subjects were at rest, wearing a nasal clip and having previously washed their mouths with distilled water. Then, they were asked to breathe at tidal volume for approximately 15 min or until 1.5 ml was obtained. The team had a saliva trap to avoid contamination with some mediators that occur in the mouth. Once samples were obtained they were stored in liquid nitrogen and later at −80 °C up until their analysis. In all three protocols, EBC samples were taken before (pre) exercise and 80 min after exercise completion (80-post), given that previous studies

Table 1 General description of participants

	Values
Age (years)	22.3 ± 4.2
Weight (kg)	73.5 ± 8.4
Height (cm)	1.75 ± 0.1
VO_2 max (ml kg^{-1} min^{-1})	46.0 ± 8.2
BMI (kg m^{-2})	24 ± 2.2

Values are shown as mean ± SD

conducted by our group have typically shown changes in this time of sampling [3, 6].

Hydrogen peroxide

The hydrogen peroxide in EBC was measured using a FOX2 reagent [21]. This reagent contains Fe^{+2} (250 μM), which, in an acidic medium ($HClO_4$, 110 mM), is oxidized to Fe^{+3} by the presence of H_2O_2. The amount of H_2O_2 is monitored through the reaction between the ferric ion and the xylenol orange indicator (250 μM). Sorbitol (100 mM) was added to the original reagent according to Gay and Gebicki [12]; this method has been previously used by our research group [3, 6]. For measurements, 350 μL of EBC and 150 μL of modified FOX2 were taken, then the sample was incubated for one hour at room temperature and absorbance was read at 560 nm on a microplate spectrophotometer (EPOCH™, BioTek Instruments, USA). Three calibration curves were performed for each measurements' group by using H_2O_2 (Merck) as standard.

pH

The pH was measured using Paget-Brown et al. protocol [24]. One hundred microliters of EBC were bubbled with Argon for 8 min at a flow rate of 350 mL/min, and pH was later measured using a 3×38 mm (diameter × length) microelectrode (Cole and Palmer) connected to a pH meter (Oakton™ Acorn pH 6).

Nitrites (NO_2^-)

Nitrite concentration was measured using the spectrophotometric test based on the Griess reaction [13]. Three hundred microliters of Griess reagent (0.1 % naphthylethylenediamine-dihydrochloride, 1 % sulphanilamide, 3 % H_3PO_4) were added to 300 μL of EBC. The mixture was incubated for 10 min, and absorbance was measured at 550 nm on a microplate spectrophotometer. Three curves were made for each measurement, with sodium nitrite as standard.

Statistics

Once individual values were tabulated, the Shapiro-Wilk test was applied to evaluate the distribution of the samples. When a normal distribution was obtained, a Student's *t* test for paired samples was applied to the mean values; otherwise, the Wilcoxon test was applied. The absolute changes were compared using ANOVA or the Friedman test. Correlations were determined using the Spearman correlation coefficient or the Pearson test according to the distribution. For the parameters measured in the EBC, the average and range of the intra-day coefficients of variation were obtained from the pre-exercise values of the three assessments for the same subjects. The significance level used was of $p < 0.05$. For statistical analysis, GraphPad Prism 6.0, USA software was used.

Results

With regard to the parameters pertaining to physical exercise (see Table 2), no differences in mean heart rate ($p = 0.24$), external load ($p = 0.69$), or pedaling cadence ($p = 0.47$) were observed. Regarding the minute ventilation and relative oxygen consumption, a higher average value was observed in the 90-min test, when compared to the 10-min test. The perceived effort had a greater value for the 90-min test than in the 10- and 30-min tests. For both total mobilized air and total oxygen consumed, a smaller value was observed for the 10-min test in comparison to the 30- and 90-min tests. Also, the 90-min test showed higher values in both parameters when compared to the 30-min test.

With respect to the markers analyzed in EBC, no differences were observed in the pre-exercise values of the three tests $[H_2O_2]_{EBC}$ ($p = 0.15$), $[NO_2^-]_{EBC}$ ($p = 0.44$), and pH_{EBC} ($p = 0.12$). From these values, the intra-day coefficient of variation was 51 % (0.65–115) for $[H_2O_2]_{EBC}$, 47 % (12–92) for $[NO_2^-]_{EBC}$, and 1.58 % (0.9–2.8) for pH_{EBC}. An increase in $[H_2O_2]_{EBC}$ at 80-post ($p = 0.0007$) was found in the 90-min protocol, with no differences in the 10-min ($p = 0.47$) and 30 min ($p = 0.23$) protocols, respectively (see Fig. 1). A similar result was found in $[NO_2^-]_{EBC}$; thus, no differences in the 10 min ($p = 0.14$) and 30 min ($p = 0.60$) tests were found, showing increases of this species in the 90 min ($p = 0.047$) protocol, as shown in Fig. 1. The pH_{EBC} values showed no differences when comparing pre-values versus 80-post values in the 30-min ($p = 0.35$) and 90-min ($p = 0.34$) tests, while there is a tendency to increase in the 10 min ($p = 0.051$) test as shown in Fig. 2. Absolute changes (Δ), calculated as the difference between 80-post and pre-exercise, showed a higher value for nitrite between the 90-min

Table 2 Workload and the physiological response in three different exercise protocols duration

	10 min	30 min	90 min
Load (W)	59.93 ± 5.1	59.94 ± 5.1	59.80 ± 6.2
Cadence (rpm)	59.69 ± 0.7	59.95 ± 0.2	59.86 ± 0.8
RPE	1.96 ± 1.1	2.25 ± 1.0	5.72 ± 1.6* **
HR (beats min^{-1})	103.2 ± 8.6	105.1 ± 8.1	106.7 ± 11.5
VE ($L\ min^{-1}$)	26.18 ± 2.6	27.21 ± 1.7	28.51 ± 1.8*
Total ventilation (L)	261.6 ± 25.96	816.3 ± 53.26*	2568 ± 168.30* **
VO_2 ($mL\ kg^{-1}\ min^{-1}$)	12.66 ± 2.5	13.79 ± 2.4	14.43 ± 2.2*
Total VO_2 (mL kg)	126.5 ± 25.57	413.80 ± 73.50*	1297.0 ± 200.0* **

Values are expressed as mean ± SD

*$p < 0.05$ difference from the 10-min test; **$p < 0.05$ difference from the 30-min test

versus 10-min protocol. $\Delta[H_2O_2]_{EBC}$ showed differences between the 30-min versus 90-min protocol (see Table 3). Finally, no differences between the $\Delta\ pH_{EBC}$ of the three protocols ($p = 0.21$) were observed (see Table 3). As for correlations, a significant correlation between $\Delta[NO_2^-]_{EBC}$ versus $\Delta[H_2O_2]_{EBC}$ ($r = 0.32$, $n = 16$, $p = 0.023$) was observed; no significant correlations between ΔpH_{EBC} and $\Delta[H_2O_2]_{EBC}$ or $\Delta[NO_2^-]_{EBC}$ were found. Minute ventilation showed no significant correlations with pro-oxidants and pH_{EBC}. Total ventilation correlated with $\Delta[H_2O_2]_{EBC}$ ($r = 0.30$, $n = 48$, $p = 0.041$) and $\Delta[NO_2^-]_{EBC}$ ($r = 0.38$, $n = 48$, $p = 0.007$). No significant correlation between this parameter versus the changes in pH was observed. The total oxygen consumption during the test correlated with $\Delta[NO_2^-]_{EBC}$ ($r = 0.33$, $n = 48$, $p = 0.02$) and showed a tendency to show significance with $\Delta[H_2O_2]_{EBC}$ ($r = 0.26$, $n = 48$, $p = 0.06$) minute-relative oxygen consumption did not correlate with pro-oxidants nor with pH_{EBC}.

Discussion

Exercise requires more body oxygen consumption; therefore, physiological modifications necessary to increase the provision of this element are brought up. Thus, an increase in ventilatory activity is generated by increasing both depth of inspiration/exhalation and respiratory rate. Under exercise conditions, our group has previously reported increases in the pro-oxidants generation measured in EBC and correlations between the time of outdoor exercise and the production of these species in different groups of subjects [3, 6]. In this report, we created a protocol that involved three tests of different duration in which the subjects performed exercise where the temperature, moisture and contaminants from the ambient air were controlled. Moreover, unlike the tests performed in the field in the current experimental set, it was possible to continuously measure relevant physiological parameters and to contrast them against pro-oxidants. Thus, we find that in front of intensity close to 30 % of VO_2 max, a cycloergometric protocol, developed at a fixed external load, produced increases in minute ventilation, minute-relative VO_2, and perceived effort which are probably associated to the fatigue showed in the last part of 90 min test. Although we cannot rule out that these changes are involved in the phenomenon under study, we note that the big difference of stimulus to our subjects was the increase in total ventilation and total relative VO_2 to which they were exposed to; so, in both parameters, nearly 10-fold differences between the 10 and 90 min stages were observed. The large increase in the total described ventilation is of particular interest, since this implies the possibility of lowering the temperature of the airway, favoring mechanical damage and promoting evaporation of fluid from the epithelial surface; it has been suggested that these factors in exercise are involved in the irritation and inflammation of the airway [1, 11, 19]. As for total relative VO_2 during the test, it is relevant since an association between the highest oxygen consumed and the increase of reactive oxygen and nitrogen species has been described [18].

In the lungs, both normal cells and inflammatory-type cells can form pro-oxidants derived from oxygen and nitrogen. H_2O_2, which is one of the reactive oxygen derivatives, has been determined in EBC samples, in

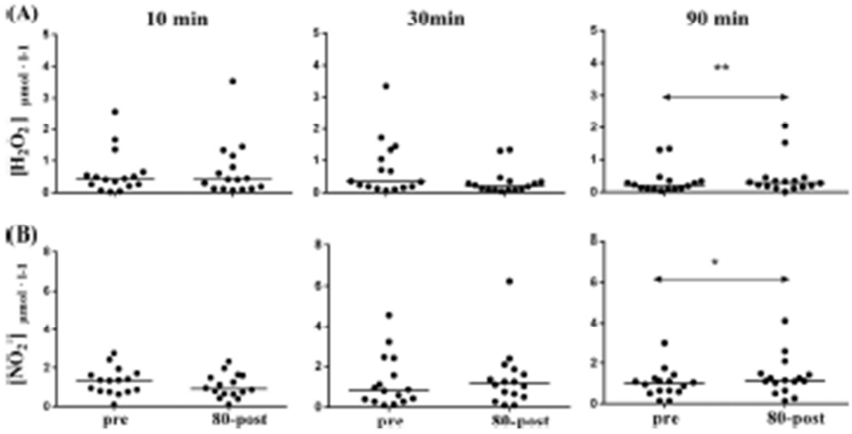

Fig. 1 $[H_2O_2]_{EBC}$ (A) and $[NO_2^-]_{EBC}$ (B) in participants of a cycloergometric exercise of 10, 30, and 90 min. Values are expressed as median, *$p < 0.05$ different from pre-value

both patients with COPD or asthma [20, 35] and in subjects who perform physical exercise [2, 6, 17, 22]. Although its origin is not clear, there is a history that links its concentration in EBC with both blood phagocytes [32] and inflammatory cells in induced sputum samples [16], which are active producers of pro-oxidants and characteristic of defensive processes. In the present study, an increase in $[H_2O_2]_{EBC}$ in the 90-min test was observed. Furthermore, the $\Delta[H_2O_2]_{EBC}$ was higher in the 90-min test, when compared to the 30-min test. Taken together, these data suggest that exercise time determines the $[H_2O_2]_{EBC}$. The influence of time on exercise has not been the focus of a study previously; however, there are reports of brief protocols of exercises; in this sense, Nowak et al. [22] in a 6-min protocol at 120 W no change was found. Similarly, in samples obtained during the performance of a submaximal exercise (60 and 120 W) lower than 10 min, increases in $[H_2O_2]_{EBC}$ [17] were not found, either. In prolonged exercise, our group has previously reported increases in $[H_2O_2]_{EBC}$, 80 min post exercise in runners who exercise between 1 (10 km) and 4 h (42.2 km). Specifically, we can compare the current 90-min protocol with a 21.2-km race, so while in the first mentioned example, increases of +40 % in 21.1 km (about 100 min) are found, runners showed changes from +200 % to the 80-post [3] at similar baseline of H_2O_2. The foregoing suggests the probable influence of exercise intensity on the generation of H_2O_2 at this level. In this direction, Marcken et al. [20] reported increases in H_2O_2 production, measured in the EBC, in healthy people, after about 12 min when the exercise was maximal, while no changes were observed during the same time of exercise performed at 40 % of maximum output (80 W approx.).

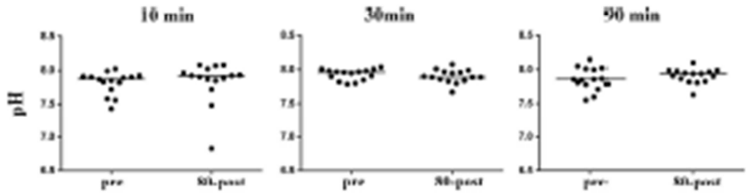

Fig. 2 pH_{EBC} in participants of a cycloergometric exercise of 10, 30, and 90 min. Values are expressed as median

Table 3 Effect of exercise duration on the absolute changes in pro-oxidants and pH in exhaled breath condensate

	10 min	30 min	90 min
$\Delta [H_2O_2]$ (μmol L^{-1})	−0.03 (−0.18, 0.07)	−0.03 (−0.12,0.025)	0.06 * (0.03,0.12)
$\Delta(NO_2^-)$ (μmol L^{-1})	−0.26 (−0.59, 0.0)	0.12 (−0.48, 0.61)	0.21 ** (0.0, 0.85)
Δ pH	0.10 (−0.02, 0.19)	−0.02 (−0.12, 0.04)	0.00 (−0.08, 0.23)

Values are expressed as median with interquartile range in parentheses

*$p < 0.05$ difference from the 30-min test; **$p < 0.05$ difference from the 10-min test

Nitrite is another chemical of great importance in the study of redox state; this substance is part of nitric oxide metabolism, a chemical species with multiple physiological and pathological functions. This substance has also been observed in the EBC from asthmatics [28] and healthy people who exercise [3, 6]. In this report, $[NO_2^-]_{EBC}$ increased in the 90-min protocol. This same exercise time showed higher $\Delta[NO_2^-]_{EBC}$ values than in the 10-min protocol. According to our judgment, these results support the idea that the increase of time doing exercise, at low intensity, increases $[NO_2^-]_{EBC}$. This parameter has also been previously reported by our group and increases in $[NO_2^-]_{EBC}$ in exercise for more than 1 h duration have been described in both poorly trained people who ran 10 km [6] and runners of 21.2- and 42.2-km races [3]. In this last work, correlations between time of exercise and $\Delta[NO_2^-]_{EBC}$ have been found, which somehow led to specifically study the effect of exercise time. As in the case of H_2O_2, it is seen that the magnitude of changes observed for $[NO_2^-]_{EBC}$ for 90 min of exercise, under controlled conditions, is 13 %, while for the 21.2-km race, increases of 90 % are found. This reinforces the idea that exercise intensity (after total ventilation) is involved in generating this difference.

Airway acidity has been studied by determining the pH_{EBC}, which is lower in the case of pulmonary inflammatory processes [7, 28]. It has a high rate of reproducibility [34], which we found in our sample, too. In this report, a tendency to increase after 10 min is observed, with no changes at a longer exercise time; deltas showed no differences, either. The reported tendency to increase, in the current work, is similar to that found in by another authors [8, 14, 30]. For example, Riediker et al. [30] in an exercise on a treadmill at a slight higher intensity to the one presented here (calculated as 60 % of maximum heart rate), where an increase in pH_{EBC} was found, measured 1 h after exercise.

Along the same lines, we have also found a tendency for pH_{EBC} to increase in participants of 10 km race in both untrained [6] and runners [3]; the last two races were performed at maximum effort. Contrary to what we expected, the result of low pH_{EBC} by prolonged exercise was not reproduced, as it was previously found in runners [3]. In part, this can probably be explained due to the low intensity of our current protocol.

In the search for the mechanisms of the observed variations in pro-oxidants and pH in the EBC as described above, we correlate their absolute changes versus some measured relevant physiological parameters. In first place, it was of particular interest to assess the minute-relative oxygen consumption and total consumption in these samples during exercise, as well as the known relationship between oxygen consumption and formation of pro-oxidants [18]. In this regard, we found a significant but weak correlation between $\Delta[NO_2^-]_{EBC}$ and total relative VO_2 in addition to a tendency to significance for $\Delta[H_2O_2]_{EBC}$; according to this, it is likely that, at least in part, the increased consumption of O_2 may explain the increase in the pro-oxidants in this organ. Regarding ventilatory changes, as mentioned in the first paragraph, these would be the primary source of changes that occur in the lung microenvironment measured in the EBC. In this way, while minute ventilation did not correlate with any of the markers measured, we did find significant correlations, albeit weak, between the total ventilation and changes in the studied pro-oxidants. From our point of view, this helps to support the idea that the total amount of mobilized air inflames and generates oxidative changes on airway epithelium, but it probably only constitutes one of the factors involved in the phenomenon described. In another aspect, it is likely that the low

correlations are also influenced by the reproducibility found (similar to other authors [33]) for $[NO_2^-]_{EBC}$ and $[H_2O_2]_{EBC}$. In this regard, an advance in the search of strategies (improvement of sampling devices, protocols, obtention of fractions) should be made, in order to use these samples in the non-invasive monitoring of athletes in the future.

In conclusion, a low-intensity cycloergometric exercise, performed under laboratory conditions, the concentration of pro-oxidants in EBC depends on the exercise time. Moreover, the increase of the observed pro-oxidants, depends, in part, on the total oxygen consumed and the total air mobilized through the airway during exercise.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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8.2 Apéndice 2

Presentación de Póster en el Congreso Chileno de Neurociencia, Ciencias Fisiológicas y Farmacología.

94) **Hydrogen peroxide and nitrite increase in exhaled breath condensate after low-intensity aerobic exercise in non-trained active subjects**

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It is a well-established fact that exercise increases pro-oxidants and promotes oxidative stress; however, this phenomenon has been poorly studied at lung level. In non trained subjects, it has been observed an increase on pulmonary pro-oxidants after high intensity aerobic exercise for nearly an hour, but it has not been studied in low-intensity exercise and less in under-trained subjects. Pro-oxidative generation (H_2O_2, NO_2^-), lipid peroxidation markers (MDA) and inflammation (pH) in exhaled breath condensate (EBC) were obtained from 11 active under-trained subjects. All subjects completed two sessions of cycloergometric exercise at low intensity (30-40% Heart Rate Reserve) and equal lung ventilation during 30 and 90 minutes respectively. Samples from both protocols were obtained immediately before, at 20 and 80 minutes post exertion. There were no differences in lung ventilation between both exercise tests, moreover heart rate remained within the established ranges for both protocols. On $[H_2O_2]_{EBC}$ an increasement was observed at 80 post in the 30 min protocol (Pre: 0.13±0.13 µmol·l^{-1} and $Post_{80}$: 0.24±0.17 µmol·l^{-1};p<0.05). This same finding was observed on the 90 min protocol (Pre: 0.08±0.08 µmol·l^{-1} and $Post_{80}$: 0.15±0.01 µmol·l^{-1}; p<0.05). $[NO_2^-]_{EBC}$ showed a tendency towards an increase at 80 post in the 30 min protocol, while there was an increase in the 80 post on the 90 min protocol (Pre: 0.35±0.49 µmol·l^{-1}and 0.92±1.66µmol·l^{-1};p<0.05). There were no differences in $[MDA]_{EBC}$ on both protocols. pH_{EBC} values showed no variations in the 30 min protocol (p=0.35), while there was a tendency towards increase in the 90 minute protocol (p=0.086). In conclusion, low intensity exercise increases lung originated pro-oxidatives in under-trained subjects. There was no evidence of changes on lipid peroxidation or early inflammation.

8.3 Apéndice 3

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Differences in energy expenditure, amount of physical activity and physical exertion level during a Zumba fitness class among adult women who are normal weight, overweight and obese

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