Remodelación cardiovascular inducida por hipoxia intermitente. Reversibilidad y efecto de la edad

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REMODELACIÓN CARDIOVASCULAR INDUCIDA POR HIPOXIA INTERMITENTE
Reversibilidad y efecto de la edad

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“La fuerza sin amor
es energía gastada
en vano”

Albert Einstein

A mi abuela nona
A toda mi familia del corazón

En el jardín de mi vida
nació mi primera flor
una nieta muy bella que
cantándole la Santa Ana
acunaba con amor.

Han pasado ya los años y
esa flor creció, has forjado
tu propia vida siempre con
mucha pasión

Ani..ni la distancia ni
el tiempo impedirán que yo te
brinde todo mi amor.

Es por eso que cuando tenga
que partir a tu lado "Señor"…
solo te pido que siempre
protejas a esta mi primera flor

(Abuela Yoli. 2018)

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..Hoy soy quien soy gracias a ustedes..

RESUMENES

RESUMEN

El Síndrome de apnea obstructiva del sueño (SAOS) es una enfermedad crónica prevalente en la población adulta y es considerado un problema de salud pública de primera magnitud debido principalmente a su morbimortalidad cardiovasculares. La hipoxia intermitente (HI) crónica es considerada el principal factor deletéreo involucrado en las consecuencias cardiovasculares asociadas al SAOS.

El primer estudio estuvo dirigido a evaluar la reversibilidad de la remodelación cardiovascular inducida por la HI en un modelo murino de SAOS. Las alteraciones observadas a nivel aórtico y cardíaco tras la exposición a seis semanas de HI fueron normalizadas tras un período en condiciones de normoxia, como modelo traslacional del tratamiento efectivo de la enfermedad.

Continuando en la misma línea de investigación, se estudió el efecto de la edad en la remodelación cardiovascular inducida por HI. Los resultados mostraron una protección cardiovascular en los ratones con edad avanzada, y en ratones jóvenes se observó una remodelación cardiovascular similar a la que ocurre con el declive natural asociado al envejecimiento. En vista de lo observado, el tercer estudio estuvo dirigido a definir los mecanismos moleculares que explican la remodelación cardiovascular inducida por hipoxia intermitente crónica observada en el segundo estudio y como son modulados por la edad. En este estudio se encontró que la regulación de la expresión génica ante la exposición a HI crónica es órgano-dependiente y es modulada por la edad.

El cuarto y último estudio se realizó en pacientes con SAOS tratados con el tratamiento de elección, presión positiva continua en la vía aérea (CPAP). En primer lugar se evaluó el efecto de la CPAP en variables clínicas y biológicas; en segundo lugar se consiguió definir un modelo de predicción de respuesta al tratamiento en términos de cambios de la presión arterial nocturna. Los resultados sugirieron el uso de la monitorización de la presión arterial ambulatoria como herramienta clave en la identificación de pacientes que se beneficiarán del tratamiento con CPAP para el control de la presión arterial.

RESUM

La Síndrome d'apnea obstructiva de la son (SAOS) és una malaltia crònica prevalent en la població adulta i és considerat un problema de salut pública de primera magnitud a causa principalment a la seva morbimortalitat cardiovasculars. La hipòxia intermitent (HI) crònica és considerada el principal factor deleteri involucrat en les conseqüències cardiovasculars associades a la SAOS.

El primer estudi va estar dirigit a avaluar la reversibilitat de la remodelació cardiovascular induïda per la HI en un model murí de SAOS. Les alteracions observades a nivell aòrtic i cardíac després de l'exposició a sis setmanes de HI van ser normalitzades després d'un període en condicions de normòxia, com a model translacional de l'tractament efectiu de la malaltia.

Continuant en la mateixa línia d'investigació, es va estudiar l'efecte de l'edat en la remodelació cardiovascular induïda per HI. Els resultats van mostrar una protecció cardiovascular en els ratolins amb edat avançada, i en ratolins joves es va observar una remodelació cardiovascular similar a la que ocorre amb el declivi natural associat a l'envelliment. En vista del que observat, el tercer estudi va estar dirigit a definir els mecanismes moleculars que expliquen la remodelació cardiovascular induïda per hipòxia intermitent crònica observada en el segon estudi i com són modulats per l'edat. En aquest estudi es va trobar que la regulació de l'expressió gènica davant l'exposició a HI crònica és òrgan-dependent i és modulada per l'edat.

El quart i últim estudi es va realitzar en pacients amb SAOS tractats amb el tractament d'elecció, pressió positiva contínua en la via aèria (CPAP). En primer lloc es va avaluar l'efecte de la CPAP en variables clíniques i biològiques; en segon lloc es va aconseguir definir un model de predicció de resposta a el tractament en termes de canvis de la pressió arterial nocturna. Els resultats van suggerir l'ús del monitoratge de la pressió arterial ambulatòria com a eina clau en la discriminació de pacients que a l'rebre tractament amb CPAP reduiran el risc cardiovascular, d'aquells pacients que tindran un efecte perjudicial a l'ésser tractats.

ABSTRACT

Obstructive sleep apnea syndrome (OSAS) is a chronic disease prevalent in the adult population and is considered a major public health problem due mainly to its cardiovascular morbidity and mortality. Chronic intermittent hypoxia (CIH) is considered the main deleterious factor involved in the cardiovascular consequences associated with OSAS.

The first study was directed to assess the reversibility of CIH-induced cardiovascular remodeling in a murine model of OSAS. The alterations observed at the aortic and cardiac level after exposure to six weeks of CIH were normalized after a period under normoxia conditions, as a translational model of the effective treatment of the disease.

Continuing in the same line of research, the effect of age on cardiovascular remodeling induced by IH was studied. The results showed cardiovascular protection in the mice with advanced age, and in young mice a cardiovascular remodeling was observed similar to the natural decline associated with aging. In view of what was observed, the third study was aimed at defining the molecular mechanisms that explain the cardiovascular remodeling induced by CIH observed in the second study and how they are modulated by age. In this study it was found that the regulation of gene expression under CIH exposure is organ-dependent and is modulated by age.

The fourth and final study was conducted in patients with OSAS treated with the treatment of choice, continuous positive airway pressure (CPAP). First, the effect of CPAP on clinical and biological variables was evaluated; secondly, it was possible to define a prediction model of treatment response in terms of changes in nocturnal blood pressure. The results suggested the use of ambulatory blood pressure monitoring before CPAP treatment as a key tool in the discrimination of patients who will reduce cardiovascular risk from those patients who will have a detrimental effect when are treated.

ABREVIACIONES

LISTA DE ABREVIACIONES

CPAP	Presión Positiva en la vía aérea
COX-2	Ciclooxigenada 2
eNOS	Oxido nítrico sintasa endotelial
FiO_2	fracción inspirada de oxígeno
FoxO3	Forkhead Box O3
HIF-1	Factor 1 hipoxia inductivo
HI	Hipoxia intermitente
ICAM-1	Molécula de adhesión intercelular 1
IGF1R/mTORC	Insulin-like growth factor receptor **1** and mammalian target of rapamycin
IAH	Indice de apneas-hipopneas
IL	Interleuquina
IMC	Índice de masa corporal
MAPA-24h	Monitoreo ambulatorio de presión arterial de **24h**
NADPH	Nicotinamida adenina dinicleótido fosfato oxidasa
NF- κ B	Factor nuclear - κ B
Nrf2	Factor de transcripción
PA1	Proteína activadora 1
P53	Tumor protein p53
MCP-1	Monocyte chemoattractant protein-1
ROS	Sustancias reactivas de oxígeno
SAOS	Síndrome de Apnea Obstructiva del Sueño
SHHS	Sleep Heart Health Study
SOD2	Superóxido dismutasa
TNAF-a	Tumor necrosis factor-α
VCAM-1	Molécula de adhesión vascular 1
VEGF	Factor de crecimiento endotelial vascular

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INTRODUCCIÓN

1. SÍNDROME DE APNEA OBSTRUCTIVA DEL SUEÑO

1.1. Definición y concepto

El síndrome de apnea obstructiva del sueño (SAOS) se incluye dentro de los desórdenes de la respiración durante el sueño. Es una enfermedad crónica que se caracteriza por la aparición de episodios recurrentes de limitación del paso del aire durante el sueño, como consecuencia de una alteración anatómico-funcional de la vía aérea superior que conduce a su colapso[1,2]. El Documento Nacional de Consenso sobre el SAOS[3] lo definió como un cuadro de somnolencia excesiva, trastornos cognitivo-conductuales, respiratorios, cardiacos, metabólicos o inflamatorios secundarios a episodios repetidos de obstrucción de la vía aérea superior durante el sueño.

Los episodios de obstrucción pueden ser totales o parciales. El colapso total de la vía aérea se denomina apnea y se define como la ausencia o reducción de más del 90% de la señal respiratoria durante más de 10 segundos de duración. Un colapso parcial de la vía aérea hace referencia a una hipopnea y se define como una reducción de la señal respiratoria de más del 30% y menor al 90% que ocurre con una disminución de la saturación de oxígeno mayor al 3% y/o un microdespertar en el electroencefalograma.

Tanto las apneas como hipopneas pueden ser obstructivas cuando se acompañan de un aumento del esfuerzo toraco-abdominal, centrales si este esfuerzo está ausente, o mixtas como combinación de ambas, siendo frecuente que comiencen por un componente central y terminar con un componente obstructivo.

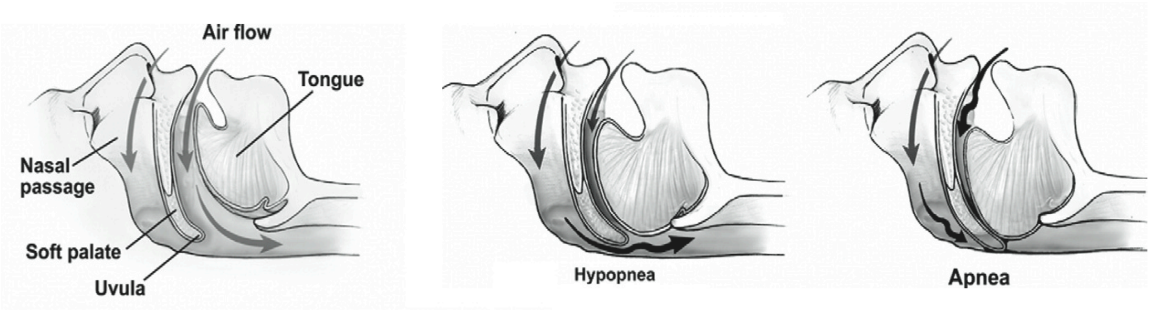


Figura 1. Mantenimiento de la permeabilidad de la vía aérea superior durante la respiración normal (izquierda). Colapso parcial (hipoapnea, centro) o completa (apnea, derecha) de la vía aérea durante eventos respiratorios (figura adaptada de Somer V 2008).[4]

1.2. Epidemiología

El SAOS ocurre a lo largo de toda nuestra vida, desde los recién nacidos hasta los ancianos, aunque la edad media de diagnóstico es a los 40 y 50 años de edad[5–7]. Es considerado una enfermedad muy prevalente en la población general adulta[8]. A finales de 1980s y principios de 1990s, se llevaron a cabo tres grandes estudios de cohorte en USA: Winconsin Slep Cohort Stury, Sleep Heart Health Study, y Penn State Cohort. Dichos estudios evidenciaron una prevalencia de trastornos de respiración durante el sueño (IAH>5 eventos/hora) de entre un 6.5% y un 9% en mujeresy entre un 17% y un 31% en hombres. Un estudio reciente sugiere un creciente aumento de la prevalenciadel SAOS, lo cual puede reflejar la creciente prevalencia de sobrepeso y obesidad[6]. Dicho estudio, HypnoLaus Sleep Cohort estimó una prevalencia de trastornos de respiración durante el sueño (IAH>15 eventos/hora) 23.4% en mujeres y 43.7% en hombres (refHypnoLaus). Así, estudios llevados a cabo en España evidencian que entre un 4,7 y un 7,8% de la población general mayor de 40 años presenta SAOS grave. En población mayor de 65 años, la prevalencia de SAOS grave asciende al 26% en hombres y 21% en mujeres, por lo que una vez alcanzada la menopausia, las mujeres tienen riesgo similar[8–10].

Tabla 1. Prevalencias específicas según edad y sexo según el índice de apnea-hipopneas (IAH) a partir de resultados de polisomnografías (tabla adaptada de Lévy P 2014).[11]

Edad (años)	Prevalencia (%)				
	IAH ≥5	IAH ≥10	IAH ≥15	IAH ≥20	IAH ≥30
Hombre					
30-39	9-17	8-12	3-6	2	2
40-49	25-26	18	11-16	10	7
50-59	28-31	14-24	9-19	15	11
60-70	52	32	24	15	9
Mujer					
30-39	3-7	3-7	1-4	NA	NA
40-49	9-15	5-10	4	NA	NA
50-59	16-35	6-16	4-9	8	4
60-70	47	26	16	13	6

Son factores de riesgo la obesidad, edad y sexo masculino. Diferentes estudios epidemiológicos han evidenciado una prevalencia del 3 al 7% para la población adulta masculina y del 2 al 5% para las mujeres (Tabla 1), por lo que es 2-3 veces más prevalente en hombres que en mujeres[12–14]. Esta prevalencia aumenta claramente con la edad[9].

La obesidad tiene una clara asociación con el SAOS, independientemente del sexo. En estudios de población, un aumento en el 10% del peso corporal aumenta el riesgo de SAOS seis veces. Otros factores de riesgo son el tabaquismo, el consumo de alcohol, predisposición genética, factores anatómicos, la deprivación de sueño o el sueño en decúbito supino, entre otros.

Factores genéticos tienen un rol en el desarrollo del SAOS[15]. La prevalencia en los familiares de primer grado de pacientes con SAOS es dos veces mayor en comparación con los familiares de primer grado de los controles sanos[16]. La susceptibilidad al SAOS aumenta con el número de familiares afectados[17]. El análisis de segregación en el estudio familiar de Cleveland mostró que, independientemente del índice de masa corporal (IMC), hasta el 35% de la varianza en el IAH depende de factores genéticos[17]. Los factores hereditarios incluyen la morfología craneofacial y de las vías respiratorias superiores, además de las diferencias en la distribución de la grasa corporal y el control de la respiración[16].

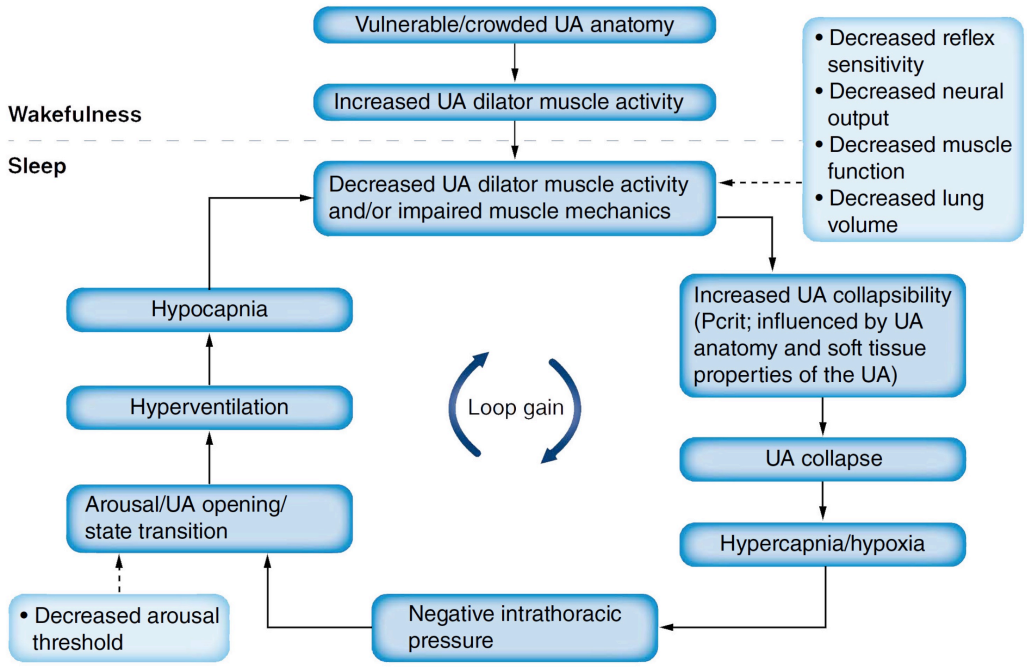
El SAOS se ha visto asociado a un deterioro de la calidad de vida y distintas comorbilidades. Enfermedades cardiovasculares y desórdenes metabólicos se han observado hasta en un 50% de los pacientes SAOS[18]. De hecho, el SAOS es la causa más prevalente en pacientes con hipertensión resistente[19] y perfiles de presión arterial nocturna non-dipping[20]. Además el SAOS se asocia con deterioro cognitivo, accidentes de tráfico y un exceso de mortalidad[21]. Se ha evidenciado que los pacientes SAOS no diagnosticados duplican el consumo de recursos sanitarios con respecto a los diagnosticados y tratados. Por todo ello, se considera el SAOS como un problema de salud pública de primera magnitud.

1.3. Fisiopatología

Los mecanismos fisiopatológicos del SAOS son diversos y complejos, por lo que se sugiere un origen multifactorial donde interaccionan factores anatómicos y funcionales,

esquematizado en figura 2[1,21,22]. El colapso de la vía aérea superior es producido debido a un desequilibrio entre las fuerzas que tienden a cerrarla y aquellas fuerzas que la mantienen abierta. Existen factores que tienden a cerrar la vía aérea superior secundarios a una deficiencia en sus reflejos, en los centros respiratorios o en la propia musculatura, causando los diferentes eventos respiratorios y los trastornos fisiopatológicos y biológicos secundarios[11,23]. No obstante, se puede decir que este trastorno es el resultado de la interacción de los distintos factores anatómicos, funcionales, neuronales y genéticos que pueden influir de distinta manera según cada individuo[24,25].

Figura 2. Mecanismos fisiopatológicos al ciclo de colapso de la vía aérea superior en la apnea obstructiva de sueño.



Las líneas sólidas indican los principales mecanismos implicados en el colapso de la vía aérea, mientras que las líneas discontinuas hacen referencia a los cambios fisiológicos que contribuyen al colapso. Pcrit, presión crítica; VA, vía aérea (modificada de Lévy 2014)[11].

1.3.1. Factores anatómicos

La vía aérea superior humana es una estructura compleja que permite funciones de fonación, deglución y respiración. Se pueden diferenciar cuatro segmentos anatómicos:

nasofaringe (fosas nasales a paladar duro), velofaringe (paladar duro a paladar blando), orofaringe (paladar blando a epiglotis) e hipofaringe (epiglotis a laringe). Anomalías anatómicas en uno o más segmentos pueden comprometer la estabilidad de la vía aérea superior y contribuir a un estrechamiento o cierre durante el sueño. El colapso también puede ser secundario a un aumento del volumen del tejido blando local (como las amígdalas y las adenoides), un incremento del depósito de grasa parafaríngea y alteraciones en la estructura craneofacial (como el paladar duro estrecho y arqueado, la acroglosia y el aumento de los puntajes de mallampati, entre otras)[26].

Otro factor importante a nivel anatómico es la longitud de la vía aérea superior, en donde una mayor longitud genera un incremento en la predisposición a la obstrucción en hombres. Otro aspecto a tener en cuenta es la posición supina, que promueve la redistribución de fluidos y tejidos blandos en dirección anterosuperior originando mayor tendencia al colapso[27]. Por último una disminución del volumen pulmonar, también se asocia a una predisposición al colapso de la vía aérea superior[28].

1.3.1. Factores funcionales

El dinamismo de la vía aérea superior se encuentra explicado por el modelo Starling. [29] En la parte superior de la faringe hay presión atmosférica y en la parte inferior presión traqueal. Al mismo tiempo se genera la presión intraluminal que promueve la apertura de la vía aérea superior, y la presión extraluminal que la cierra. La diferencia entre ambas presiones resulta en la presión transmural, la cual define el diámetro de la vía aérea superior. La presión a la cual se genera una obstrucción o colapso se llama presión crítica, siendo más positiva en paciente SAOS que en individuos sanos[28].

Durante la inspiración, se genera presión subatmosférica en la vía aérea al contraerse el diafragma, promoviendo el flujo de aire a su interior. Las vías respiratorias inferiores permanecen permeables debido al soporte intramural de los anillos cartilaginosos en el árbol traqueobronquial. En cambio, la hipofaringe carece de dicho soporte por lo que su permeabilidad es vulnerable a diversos factores como el tono muscular, la masa tisular y la cantidad de tejido adiposo. Durante la vigilia, la hipofaringe se mantiene permeable debido a la actividad de numerosos músculos dilatadores. Sin embargo, durante el período de sueño, se produce una reducción de la actividad muscular y como

consecuencia el diámetro de la vía aérea se reduce y se produce un colapso en los casos de SAOS[30].

Otro factor causal de SAOS corresponde a la inestabilidad del control respiratorio, conocido como *high loop gain*. La disminución del estímulo neurológico respiratorio central provoca una reducción de la actividad dilatadora de los músculos de la vía aérea superior, aumentando su resistencia y promoviendo una predisposición al colapso[31].

Otro factor importante es la propensión para despertarse, conocido como umbral de despertar. Tras los episodios de obstrucción y una disminución del flujo aéreo, la presión arterial de dióxido de carbono aumenta (hipercapnia) y la presión arterial de oxígeno disminuye (hipoxemia). Como consecuencia, los quimioreceptores periféricos sensibles a la hipoxemia y los quimioreceptores centrales sensibles a la hipercapnia perciben dichas fluctuaciones gaseosas y envían una señal de alarma al sistema nervioso central, el cual aumenta el impulso respiratorio central, lo que se traduce en un aumento del tono muscular de la vía aérea para conseguir su dilatación. Si no es suficiente, se activa la corteza cerebral promoviendo un despertar o microdespertar con el fin de elevar el tono de los músculos estriados con dilatación de la vía aérea y finalización del evento obstructivo. La repetición frecuente de este fenómeno explica el fraccionamiento del sueño, el sueño poco reparador y la somnolencia diurna que presentan los pacientes con SAOS. Una vez finalizada la apnea obstructiva, la presión negativa es muy elevada, lo cual permite el ingreso de una gran cantidad de aire (fase hiperpneica), y permite el intercambio gaseoso de una manera muy rápida. Esto hace que la concentración de CO_2 en la sangre pueda caer por debajo del umbral de apnea, lo que el sistema nervioso central interpreta como una hiperventilación y responde generando una apnea central[30].

1.4. Manifestaciones clínicas

La clínica relacionada con el SAOS aparece como consecuencia de dos procesos fisiopatológicos fundamentales: por una parte, se generan ciclos de hipoxia-reoxigenación (hipoxia intermitente, HI) que puede ocasionar la aparición de problemas cardiovasculares, desregulación metabólica, cáncer, entre otras comorbilidades; y por otra parte, la disrupción de la arquitectura del sueño que conduce a hipersomnia diurna, alteraciones cognitivas y psiquiátricas.[13,21,32–34]

Ningún parámetro clínico aislado o en combinación con otros ha demostrado suficiente valor en el diagnóstico del SAOS, aunque la valoración clínica y exploración física exhaustivas permiten clasificar a los pacientes en alta, media o baja probabilidad clínica pretest, lo que ayuda a valorar posteriormente el método diagnóstico a utilizar.

La triada clínica principal del SAOS la componen tres síntomas:

1. Roncopatía crónica: es el síntoma con mayor sensibilidad (su ausencia hace poco probable el diagnóstico de SAOS). Sin embargo, la mayoría de roncadores no tienen SAOS (roncan el 40% de los varones y el 20% de las mujeres de la población general). Por ello, la presencia de roncopatía crónica como síntoma único no es suficiente para la realización de una prueba de sueño con intención diagnóstica de SAOS.

2. Apneas presenciadas*:* es el síntoma con mayor especificidad, la cual aumenta si son observadas de forma repetida durante la misma noche y si son prolongadas.

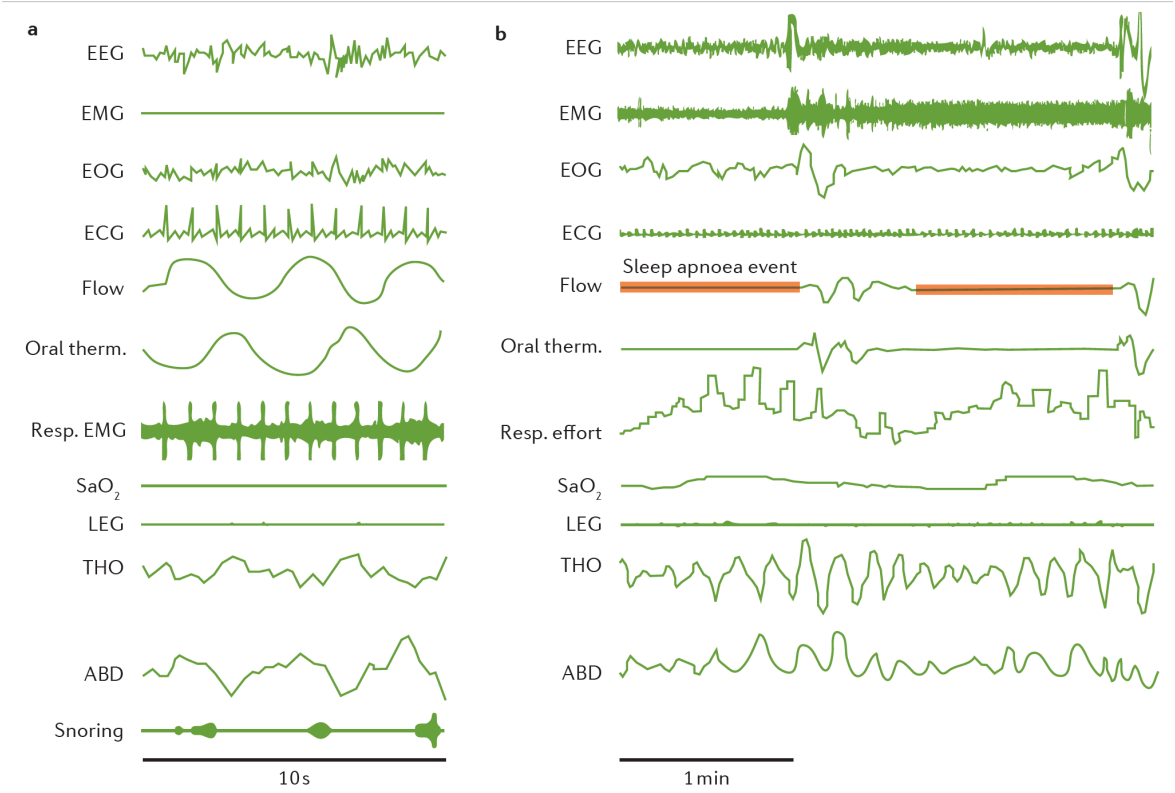
3. Somnolencia diurna: es un síntoma poco específico y sensible pero el de mayor importancia ya que marca la intensidad clínica del SAOS. Su presencia no explicada por circunstancias evidentes es suficiente aun en ausencia de otros síntomas o signos para la realización de un estudio de sueño con carácter diagnóstico.

Otros síntomas y signos frecuentes son: sueño no reparador, cuello ancho y corto, obesidad, despertares frecuentes, nicturia, cefalea matutina, hipertensión arterial, entre otros.

1.5. Diagnóstico

El método diagnóstico de referencia es la polisomnografía que consiste en un registro continuo del electroencefalograma, electrooculograma y electromiograma mentoniano (para cuantificar las fases del sueño y microdespertares), así como de otras variables para cuantificar los trastornos respiratorios y sus repercusiones, como se ve en la figura 3 (pulsioximetría, flujo aéreo nasobucal mediante cánula nasal y termistor, ronquidos, movimientos toracoabdominales y electrocardiograma)[3,35].

Figura 3. Metodo diagnotico



A) Polisomnografía correspondiente a 10 segundos de un individuo normal (a), ausencia de apneas o hipoapneas, solo presencia de ronquidos. B) Polisomnografía procedente de un paciente con SAOS. Se visualizan dos eventos hipóxicos, marcados en color naranja. Cada evento se asocia con una reducción de fujo de aire, aumento del esfuerzo respiratorio y saturación de oxígeno. Además los eventos respiratorios se asocian con arousal visible en electroencealograma (EEG), electromiograma (EMG) y electro-oculograma (EOG). ABD, movimientos abdominales; ECG, electrocardiograma; Flow, flujo nasal; Leg, detección movimiento piernas; SaO2, saturación de oxígeno transcutanea; THO, movimientos torácicos. Figura procedente de la revisión Lévy P et al 2015).[21]

Si bien es la prueba de referencia, es una técnica relativamente cara, laboriosa y técnicamente compleja, que dificulta la disponibilidad en todos los centros. Dada la alta prevalencia del SAOS, se usan métodos diagnósticos simplificados como es la poligrafía respiratoria. Este equipo portátil registra variables respiratorias y cardíacas sin registrar variables neurofisiológicas y es utilizada en pacientes con una baja probabilidad clínica de SAOS para descartar la enfermedad, y en los enfermos con una alta probabilidad diagnóstica. Tanto la polisomnografía como la poligrafía respiratoria cuantifican el índice de apneas-hipopneas (IAH), el cual define la gravedad de la enfermedad. El Documento de

Consenso Nacional sobre el SAOS[3] sugiere como clasificación: SAOS leve (IAH entre 5 y 14,9), SAOS moderado (IAH entre 15 y 29,9) y SAOS grave (IAH mayor de 30).

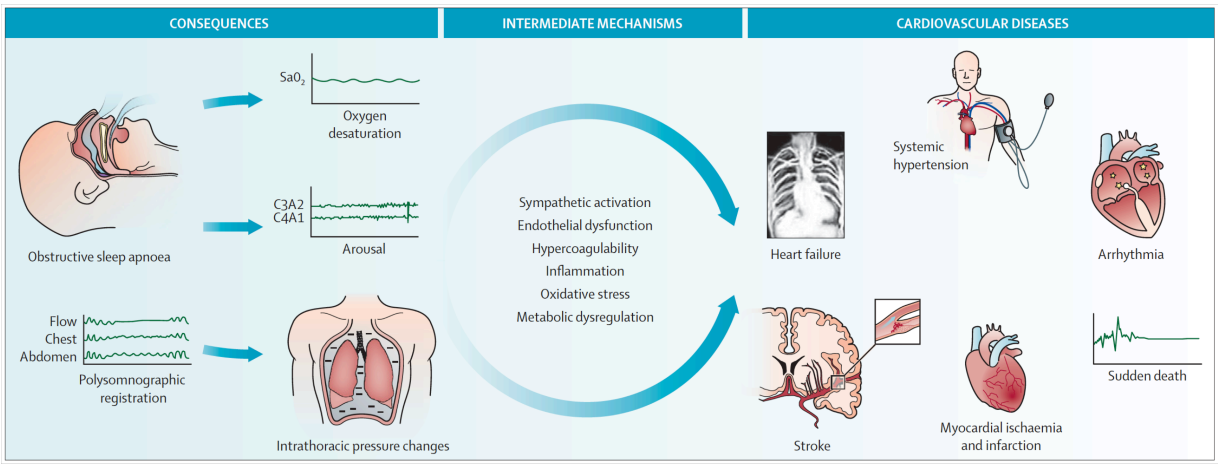
1.6. Tratamiento

El tratamiento del SAOS tiene como objetivo resolver los signos y síntomas de la enfermedad, normalizar la calidad de sueño, IAH y desaturación de oxígeno. Reducir el riesgo de complicaciones sistémicas y, en el área de la salud pública, disminuir los costes directos e indirectos generados[3]. Como medidas terapéuticas generales encontramos higiene de sueño, reducción de peso en pacientes obesos o con sobrepeso, evitar la posición de decúbito supino, abandonar el consumo de alcohol y de tabaco, así como evitar fármacos como las benzodiacepinas, ya que disminuyen la respuesta ventilatoria a la hipoxia y la hipercapnia durante el sueño y vigilia[3].

Cuando el paciente presenta un SAOS grave o un SAOS moderado asociado a riesgo cardiovascular, la medida terapéutica es tratamiento con presión positiva continua en la vía aérea (CPAP). La CPAP fue desarrollada por Colin Sullivan en 1981 y consiste en una turbina que trasmite una presión positiva continua a través de una mascarilla nasal o nasobucal[27]. El sistema genera un flujo de aire constante que por acción mecánica evita el colapso de la vía aérea superior. Es un tratamiento efectivo para remisión de las manifestaciones clínicas del SAOS, recuperación de la capacidad de atención entre otras variables cognitivas y mejoría de la calidad de vida[36,37]. La mejoría depende de la adherencia al tratamiento[38]. Como tratamientos alternativos encontramos oxígeno, dipositivo mandibular, cirugía o somnoplastia.

2. SÍNDROME DE APNEA OBSTRUCTIVA DEL SUEÑO Y ENFERMEDAD CARDIOVASCULAR

Los eventos de apnea obstructiva incorporan una variedad de factores estresantes que



activan mecanismos que contribuyen al inicio y la progresión de enfermedades cardíacas, vasculares y metabólicas[4,21,39]. La obstrucción de la vía aérea induce una presión intratorácica marcadamente negativa que estira las estructuras intratorácicas, en particular las aurículas del corazón y los grandes vasos sanguíneos. Las obstrucciones de la respiración también inducen hipoxemia e hipercapnia[40]. El estrés hipoxémico se amplifica aún más por la reoxigenación posterior (hipoxia intermitente, HI), lo que resulta en la generación de especies reactivas de oxígeno (ROS) e inflamación[41]. Los eventos obstructivos también están acompañados por despertares del sueño, con la consiguiente fragmentación del sueño y posiblemente privación, que puede activar una amplia gama de mecanismos de enfermedad cardiovascular. A continuación se resumen (figura 4) algunos de los mecanismos claves involucrados en la fisiopatología del SAOS (apartado 2.1) y que podrían potenciar el desarrollo de enfermedades cardiovasculares en pacientes con SAOS (apartado 2.2).

Figura 4. Síndrome de Apnea Obstructiva del sueño, consecuencias y mecanismos intermedios que contribuyen potencialmente al riesgo de enfermedad cardiovascular

Figura extraída de la revisión de Sánchez-de-la-Torre M et al 2012[39].

2.1. Mecanismos fisiopatológicos

2.1.1. Estrés oxidativo

El estrés oxidativo es el resultado del incremento del la ratio pro-oxidante/antioxidante, es decir, un desequilibrio en el estado redox entre la producción y eliminación de especies reactivas de oxígeno (ROS). Los radicales libres juegan un importante rol en la regulación de la función celular y traducción de la señal. Sin embargo, un aumento de la producción de ROS representa una amenaza celular, ya que pueden reaccionar con estructuras intracelulares y dañar directamente biomoléculas vitales como proteínas, lípidos y ácidos nucleicos, siendo la base patofisiológica de las enfermedades crónicas y relacionadas con la edad, como son el cáncer, las enfermedades cardiovasculares, la diabetes, la inflamación crónica y los trastornos neurodegenerativos.

En el SAOS el aumento de estrés oxidativo se atribuye principalmente a la reducción de la concentración de oxígeno disponible durante los eventos apneicos y la formación de ROS durante la reoxigenación cuando se restablece la respiración[42,43]. El estrés oxidativo inicia un ciclo vicioso en el cual se promueve una sobreactivación simpática e inflamación, que a su vez potencia el estrés oxidativo. La combinación de estrés oxidativo, activación simpática e inflamación promueven la aparición de disfunción endotelial, hipertensión y aterosclerosis[44,45]. Además, el estrés oxidativo contribuye al desarrollo de otras comorbilidades asociadas al SAOS, como son la hiperlipidemia, la resistencia a la insulina, diabetes y tiene un rol importante en la obesidad. Sin embargo, las interacciones dependientes de ROS son complejas[46].

El aumento de la producción de ROS asociado con la HI se atribuye a una disfunción mitocondrial, la activación de la enzima NADP oxidasa (nicotinamida adenina dinicleótido fosfato oxidasa, NOX) y xantina oxidasa y el desacoplamiento de la enzima oxido nítrico sintasa (NOS), la cual comienza a generar una mayor cantidad de ROS y menor cantidad de óxido nítrico[42,46]. Ensayos controlados no aleatorizados en pacientes con SAOS han mostrado aumentos en la producción de ROS en monocitos y subpoblaciones de granulocitos, por un aumento de la actividad NOX[47,48]. Además de un aumento en la producción de ROS, algunos investigadores han sugerido que la apnea del sueño podría aumentar el estrés oxidativo al reducir la capacidad antioxidante[49,50].

Estudios recientes también indican la importancia de ciertos polimorfismos genéticos de NOX, los cuales afectan a los niveles de estrés oxidativo y déficits cognitivos en pacientes SAOS[51]. Otros estudios han evidenciado un aumento de marcadores de peroxidación lipídica[52–55], oxidación del DNA y proteínas[46,54] en pacientes SAOS, cuyos valores correlacionan con la severidad del IAH[56,57], y son parcialmente normalizados con el tratamiento con CPAP.

2.1.2. Activación Simpática

Durante las apneas en pacientes con SAOS, la hipoxemia y la hipercapnia actúan a través de los quimiorreceptores periféricos induciendo una activación del sistema nervioso simpático[58]. Los pacientes con SAOS, incluso en ausencia de comorbilidad, tienen un aumento de la actividad nerviosa simpática, que persiste en la vigilia diurna normóxica[58,59]. Como consecuencia, los pacientes SAOS presentan una presión arterial y frecuencia cardíaca elevadas durante el período de sueño y durante la vigilia en reposo[60]. Varios estudios randomizados han demostrado que el tratamiento con CPAP reduce la actividad del sistema nervioso simpático y atenúa el aumento del tono simpático en pacientes con SAOS[61–63]. Además se ha evidenciado que la administración de oxígeno al 100% (para eliminar el impulso quimiorreflejo tónico) reduce significativamente la actividad simpática, la frecuencia cardíaca y la presión arterial en pacientes con SAOS durante la vigilia diurna[64].

2.1.3. Inflamación

El SAOS muestra una asociación con inflamación local y sistémica. Células sanguíneas de pacientes con SAOS presentan un fenotipo proinflamatorio y protrombótico, el cual facilita el daño y disfunción endotelial, aterosclerosis y trombosis. Una mayor activación de leucocitos y plaquetas se ha encontrado en pacientes SAOS en comparación a controles sanos[47,65]. Los monocitos circulantes también muestran un fenotipo proinflamatorio, caracterizado principalmente por una mayor producción de ROS y moléculas de adhesión. Este aumento de las moléculas de adhesión a nivel de la membrana celular de los monocitos circulantes, contribuyen a aumentar la afinidad de unión con células endoteliales, y sus niveles dependen de la severidad del SAOS (IAH), y son reducidos con tratamiento con CPAP[47,65]. Asimismo, linfocitos T citotóxicos de pacientes SAOS muestran

un fenotipo activado y proinflamatorio dependiendo de la severidad (IAH), con una mayor expresión de citoquinas proinflamatorias como tumor necrosis factor-α (TNF-α), y una menor expresión de citoquinas anti-inflamatorias como interleuquina-10 (IL-10)[66–68]. También se ha observado que las plaquetas derivadas de pacientes SAOS se encuentran activas y con un fenotipo pro-fibrótico, el cual es atenuado con el tratamiento con CPAP.

Estudios observacionales muestran un incremento de marcadores inflamatorios circulantes que confirman una activación de la inflamación vascular en SAOS. Marcadores como proteína C reactiva, moléculas de adhesión, citoquinas proinflamatorias (TNF-α, IL-6, IL1-b, proteína C reactiva), marcadores de coagulabilidad[43,69,70]. El incremento de los niveles de marcadores inflamatorios implica la participación de factores de transcripción sensibles a redox, como el factor 1 hipoxia inductivo (HIF1), la proteína activadora 1(PA1) y el factor nuclear κB (NF-κB). NF-κB es uno de los reguladores clave de la inflamación, la respuesta inmune y la supervivencia celular. Un estudio no aleatorizado sugirió que NF-κB está altamente activado en pacientes con SAOS en comparación con los controles sanos, y que el tratamiento con CPAP reduce la activación de NF-κB[71].

Aunque es clara la asociación entre SAOS e inflamación, el impacto de la CPAP en marcadores inflamatorios es compleja. Algunos estudios han evidenciado una reducción de las citoquians con CPAP[72–75], pero no se confirma en otros[76–78].

Dentro de la fisiopatología de la asociación SAOS e inflamación, juegan un papel importante las adipocinas, involucradas en procesos de inmunidad e inflamación[79]. Estas adipocinas proinflamatorias contribuyen al estado inflamatorio de los pacientes obesos. La obesidad es la comorbilidad más común y se considera una situación inflamatoria crónica en sí misma, por lo que podría ser el factor de confusión más importante en la asociación entre la apnea del sueño y la inflamación. De hecho, Guilleminault y colaboradores[80] mostraron que la obesidad tenía una fuerte asociación con la proteína C reactiva pero no con el SAOS. Un estudio clínico aleatorizado realizado por Kohler y colegas[75] no mostró una mejoría en los marcadores inflamatorios después del tratamiento con CPAP. Los resultados sugirieron que la asociación entre SAOS e inflamación está estrechamente relacionada con la obesidad, siendo el tejido adiposo la mayor fuente de intermediarios inflamatorios.

2.1.4. Disfunción endotelial

El endotelio vascular es una monocapa celular confluente que recubre todo el compartimento vascular en la interfaz entre la sangre y la pared del vaso. El endotelio vascular está íntimamente involucrado en el control del tono vasomotor y es el principal regulador de la hemostasia vascular. El endotelio ajusta continuamente el equilibrio entre la vasoconstricción y la vasodilatación. Si este equilibrio se inclina hacia la vasoconstricción, se produce una disfunción endotelial que causa daño a la pared arterial. La disfunción endotelial ocurre en respuesta a factores de riesgo cardiovascular y puede preceder o acelerar el desarrollo de la aterosclerosis[39,81].

Existe un deterioro en la función endotelial en pacientes con SAOS[82–84]. Varios estudios muestran evidencia indirecta de una disponibilidad reducida de óxido nítrico y altas concentraciones plasmáticas de moléculas de adhesión, lo que sugiere que la inflamación y la disfunción endotelial vascular contribuyen al desarrollo de enfermedades vasculares en pacientes con SAOS[47]. Además, el aumento de la activación simpática y el estrés oxidativo, podrían contribuir al desarrollo de disfunción endotelial. El aumento del estrés oxidativo reduce la disponibilidad de óxido nítrico y aumenta la expresión de ROS, que activa a su vez vías inflamatorias que facilitan el reclutamiento y la acumulación de células sanguíneas en la vasculatura del endotelio[42,47]. Diversos estudios observacionales no randomizados sugieren una mejora en la función endotelial tras el tratamiento con CPAP[85,86].

Además de la disfunción endotelial, la vasculatura puede sufrir un procesos de remodelación estructural, pérdida de elasticidad y aumento de la rigidez arterial. Asociaciones entre SAOS y aumento de la rigidez vascular también han sido reportadas[83,87,88]. Algunos estudios sugieren una mejora en la rigidez arterial con CPAP[89,90]. Sin embargo, un estudio prospectivo de la cohorte Wisconsin Sleep Cohort falló en demostrar una asociación independiente entre SAOS y el desarrollo de rigidez arterial [91].

2.1.5. Desregulación metabólica

Tanto un aumento de la actividad simpática, como la fragmentación del sueño y la hipoxia intermitente son los principales factores relacionados con el SAOS que contribuyen al desarrollo de la desregulación metabólica. Síndrome metabólico hace referencia a un

conjunto de factores, incluido resistencia a la insulina, dislipemia, hipertensión, y obesidad abdominal, que en conjunto resultan en un aumento del riesgo cardiovascular. El Síndrome metabólico es común en pacientes SAOS, así como el SAOS es frecuente en condicione asociadas con anormalidades metabólicas[92].

Estudios muestran que los pacientes SAOS presentan un aumento en la concentración de los niveles de ácidos grasos, e independientemente del IMC, un aumento de la resistencia a la insulina, intolerancia a la glucosa, disfunción de las células beta del páncreas, y dislipemia[93–95].

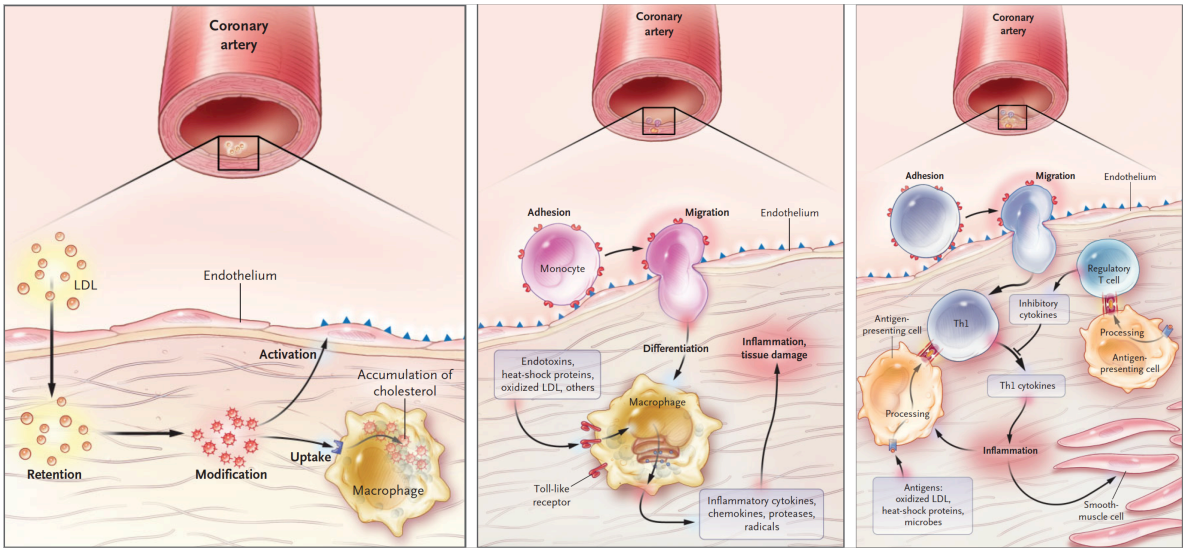
En cuanto a la asociación entre SAOS y riesgo a desarrollar diabetes tipo 2 y si el tratamiento con CPAP podría revertir la resistencia a la insulina, existen resultados variables. Algunos autores muestran una reducción posprandial de los triglicéridos y colesterol tras el tratamiento con CPAP[63]. Sin embargo, otros ensayos clínicos han mostrado un beneficio parcial del tratamiento con CPAP para componentes metabólicos[96–98]. Dado que la obesidad coexiste con el SAOS, no es del todo claro si la presencia de desorden metabólico es consecuencia del SAOS o simplemente refleja los efectos de la obesidad grave coexistente[99].

2.1.6. Aterosclerosis

Las lesiones ateroscleróticas (ateromas) son engrosamientos focales asimétricos de la capa más interna de la arteria, la íntima o endotelio, desarrolladas como resultado de un proceso de remodelación de la pared vascular y orquestado por un proceso de inflamación. Los ateromas consisten en células, elementos de tejido conectivo, lípidos y residuos. Las células inflamatorias e inmunes transmitidas por la sangre constituyen una parte importante de un ateroma, el resto son células endoteliales vasculares y de músculo liso vascular. El ateroma está precedido por una estría grasa, una acumulación de macrófagos cargados de lípidos debajo de la pared endotelial. Las estrías grasas son prevalentes en personas jóvenes, nunca causan síntomas y pueden progresar a ateromas o eventualmente desaparecer.

En el centro de un ateroma, las células de espuma y lípidos extracelulares forman una región central, que está rodeada por una capa de células de músculo liso vascular y una matriz rica en colágeno. Las células T, los macrófagos y los mastocitos se infiltran en la lesión, se activan y producen citoquinas inflamatorias. El infarto de miocardio ocurre cuando el proceso ateromatoso impide el flujo de sangre a través de la arteria coronaria, ya sea por estrechamiento luminal progresivo (estenosis) o la formación de un trombo oclusivo por ruptura de placa.

Figura 5. Principales características del proceso aterosclerótico.



Efecto activador de la infiltración LDL de la inflamación arterial (izquierda). Transposición de monocitos desde el torrente sanguíneo a la pared vascular, diferenciación de monocitos a macrófagos activos, liberación de citoquiinas inflamatorias, producción de ROS que promueven inflamación y daño tisular (centro). Activación de linfocitos T, liberación de citoquinas Th1, activación de células de musculo liso vascular, magnificación de la inflamación local (derecha).

El SAOS se asocia con múltiples procesos que conforman la base patogénica de la aterosclerosis, como son el estrés oxidativo, la inflamación, la sobreactivación del sistema simpático, la disfunción endotelial y rigidez arterial[21]. Además se asocia con un incremento en el grosor intima-media y aparición de lesiones ateroscleróticas tempranas, independiente de factores de riesgo cardiovascular o comorbilidades metabólicas y cardiovascular asociadas[100].

2.2. Consecuencias cardiovasculares del síndrome de apnea obstructiva del sueño

2.2.1. Hipertensión

Tanto el SAOS como la hipertensión son condiciones comunes, y diversos individuos presentan ambas enfermedades. Existe una prevalencia del 50% de hipertensión en pacientes SAOS, y un 30% de pacientes hipertensos presentan SAOS no diagnosticado[20,101]. En la cohorte de Wisconsin, distintos autores encontraron una relación lineal entre la presión arterial 24 horas y el IAH, independientemente de factores de confusión[102,103]. Un estudio de 2677 adultos que referían a la unidad de sueño, la odds de hipertensión aumentaba 1% por aumento de cada unidad de IAH en los niveles de prevalencia de hipertensión, siendo 22.8% en controles, 36,5% en SAOS leve, 46% en SAOS moderados, y 53,6% en SAOS grave (después de ajustar por edad, IMC y género)[104]. En un estudio reciente, el SAOS, definido como IAH>15 eventos por hora, fue la condición más común asociada a pacientes con hipertensión resistente, siendo la prevalencia del 64%[19].

Como consecuencia del aumento de la presión arterial nocturna y diurna en pacientes con SAOS, muestran una alteración en el patrón circadiano de presión arterial predominantemente non-dipping y una mayor incidencia de hipertensión nocturna[105,106], que está asociada con daño en el órgano diana y empeoramiento de los consecuencias cardiovasculares[106–108].

Según diferentes metanálisis, el tratamiento con CPAP reduce la presión arterial en pacientes normotensos e hipertensos con SAOS[109–112]. Sin embargo, el impacto del tratamiento con CPAP en la presión arterial no es regular. En pacientes mínimamente sintomáticos, la CPAP tiene un efecto neutral sobre la presión arterial[112], mientras que en sujetos con hipertensión resistente, la CPAP puede disminuir la presión arterial sistólica en 5–7 mmHg[113]. Además, aunque el efecto del tratamiento con CPAP sobre la presión arterial está relacionado con el cumplimiento del tratamiento, existe una variabilidad individual que podría estar relacionada con los factores epigenéticos, al menos en parte[114]. Por lo tanto, un perfil de pacientes con SAOS grave, dificultad para controlar la hipertensión, y una adherencia al tratamiento son los que presentan una mayor reducción de los niveles de presión arterial al recibir tratamiento con CPAP.

2.2.2. Enfermedad coronaria e infarto de miocardio

La prevalencia del OSA en individuos con enfermedad coronaria es de aproximadamente del 30 al 60%[115], considerablemente más alta que la prevalencia en la población general. Entre los hombres hospitalizados por infarto agudo de miocardio , se ha informado que la prevalencia del SAOS de casi el 70%, destacando aún más la asociación de estos trastornos.

Los principales hallazgos prospectivos del Sleep Heart Health Study (SHHS) basado en la comunidad proporcionan evidencia epidemiológica del papel causal del SAOS en la incidencia de enfermedades cardiovasculares y la mortalidad relacionada con la enfermedad cardiovascular. Durante un seguimiento de 8 años de más de 6.000 individuos, el riesgo de muerte relacionada con la enfermedad coronaria en el subconjunto masculino fue 70% mayor en aquellos individuos con un AHI ≥15 en comparación con individuos no afectados[116]. Se observó una relación similar entre el IAH y la enfermedad coronaria incidente (infarto de miocardio, revascularización o mortalidad cardíaca) en varones de 40 a 70 años en la cohorte de SHHS. En comparación con los que no tenían SAOS, aquellos con SAOS grave (AHI ≥30) tenían casi un 70% más de probabilidades de desarrollar enfermedad coronaria[117]. Un estudio con menor tamaño muestral (n ~ 1500) y un seguimiento más corto (~ 3 años) también mostró un mayor riesgo de cardiopatía coronaria con un aumento de IAH, incluso cuando se ajustó para la hipertensión y el IMC[118]. A diferencia de los hallazgos de SHHS, este estudio mostró un mayor riesgo incluso en SAOS leve (IAH=5-15). Tomados en conjunto, estos estudios proporcionan evidencia convincente de que el SAOS es un factor de riesgo para la enfermedad coronaria incidente y para la mortalidad relacionada con la enfermedad coronaria. Los datos observacionales sugieren que el tratamiento con CPAP en aquellos pacientes con SAOS grave reduce el riesgo de eventos de enfermedad coronaria[109].

Un estudio intrigante de los investigadores de SHHS sugiere que la asociación entre SAOS y enfermedad coronaria puede ser bidireccional[119]. Este análisis encontró que en la fracción muy pequeña de participantes que desarrollaron enfermedad coronaria incidente (~ 3%), el IAH empeoró modestamente (>3 eventos / hora) en la evaluación de seguimiento en relación con la línea de base. El efecto fue más pronunciado en aquellos

pacientes con SAOS al inicio del estudio y en aquellos que no eran obesos ni tenían sobrepeso.

2.2.3. Accidente cerebrovascular

En un estudio transversal de 6000 sujetos, la prevalencia de accidente cerebrovascular fue mayor en pacientes con SAOS moderado (IAH>11). Otro estudio evidenció que el SAOS se asocia con accidente cerebrovascular o muerte con un cociente de riego de 2.24, incluso después de ajustar por edad, sexo, IMC, diabetes, hipertensión y dislipemia. Susan Readline y colaboradores siguieron durante 8.7 años a un total de 5.422 sujetos sin historia de accidente cardiovascular a nivel basal y sin tratamiento para el SAOS. Encontraron una asociación positiva significativa entre accidente cerebrovascular isquémico e IAH en hombres (p=0.016), siendo los pacientes con un IAH>19 los que presentaban un riesgo casi 3 veces mayor de tener un accidente cerebrovascular isquémico. La fuerza de esta asociación apoya la necesidad de ensayos prospectivos que evalúen el tratamiento con CPAP para la prevención accidente cerebrovascular isquémico en hombres con SAOS.

2.2.4. Fallo cardíaco

En dos grandes series de casos, el SAOS fue detectado en un 37% de 450[120] y 11% de 81[121] pacientes con fallo cardíaco resultante de disfunción sistólica. La prevalencia de SAOS fue mayor en pacientes hombres (38%), con la obesidad como principal factor de riesgo, mientras la prevalencia en mujeres fue de 31% y siendo la edad avanzada el principal factor de riesgo[120]. Se ha reportado que el SAOS predice el fallo cardíaco incidente en pacientes hombres, presentando un riego del 58% de desarrollar fallo cardíaco aquellos pacientes SAOS con un IAH>30[122]. Aunque la mayoría de estudios documentan una prevalencia del 50% de SAOS en pacientes con fallo cardíaco con función sistólica preservada[123], la mayoría de los estudios recientes muestran una prevalencia aumentada (30-62%) en pacientes SAOS con una fracción de eyección ≤45[116,124]. De hecho, el tratamiento con CPAP atenúa alteraciones en la función diastólica[125], sugiriendo un rol etiológico potencial del SAOS en fallo cardíaco diastólico. Entre los mecanismos involucrados en la asociación entre SAOS y desarrollo de fallo cardíaco se encuentran aumentos en la presión arterial, sobrecarga ventricular izquierda e

hipertrofia, así como aumento del riesgo de infarto de miocardio[109]. Dado el número de evidencia disponible, es evidente que el SAOS se relaciona con diversas enfermedades cardiovasculares, por lo que su tratamiento es una necesidad.

2.3. Síndrome de apnea obstructiva del sueño y consecuencias cardiovasculares no fatales

En un análisis transversal del Sleep Heart Health Study (SHHS), más de 6.000 pacientes de la población general, sujetos con SAOS mostraron un 42% de tener al menos un evento cardiovascular[116]. En un estudio longitudinal de más de 4.000 participantes, que a nivel basal no tenían fallo cardiaco y/o enfermedad coronaria, seguidos durante 8,7 años, la incidencia de ambas condiciones aumentaron con el aumento de la severidad del SAOS en hombres[117].

En un estudio observacional, Marin et al[109] realizaron el seguimiento de 1.651 hombres durante 10 años, y mostraron que tener SAOS grave no tratado aumentaba el riesgo de tener un evento cardiovascular no fatal , en comparación con los sujetos sanos. Aquellos pacientes con un cumplimiento alto del tratamiento con CPAP (al menos 4 horas de uso) reducían el riego al nivel de sujetos sin SAOS. El estudio aleatorizado realizado por Barbé y colaboradores[50], no mostró una mejoría en cuanto a nuevos casos de hipertensión o evento cardiovasculares de pacientes SAOS tratados con CPAP. Sin embargo, cuando se analizaba según la adherencia al tratamiento, aquellos pacientes que usaban la CPAP al menos 4 horas por noche mostraron una disminución significativa de la incidencia de nuevos casos.

2.4. Síndrome de apnea obstructiva del sueño y mortalidad cardiovascular

Un reciente trabajo de Lavie et al, sugiere una reducción del riesgo de muerte en SAOS con la edad. Estos autores evaluaron 372 pacientes fallecidos que habían sido seguidos previamente una media de 4,6 años. Encontraron que los hombres menores de 50 años con un IAH > 30 tenían un significativo aumento del riesgo de muerte comparado con los hombres con IAH < 10. Este riesgo no se vio en los pacientes mayores de 50 años, lo que apoya las teorías previas de que es posible que en esta población el riesgo cardiovascular sea menor que en las edades medias.

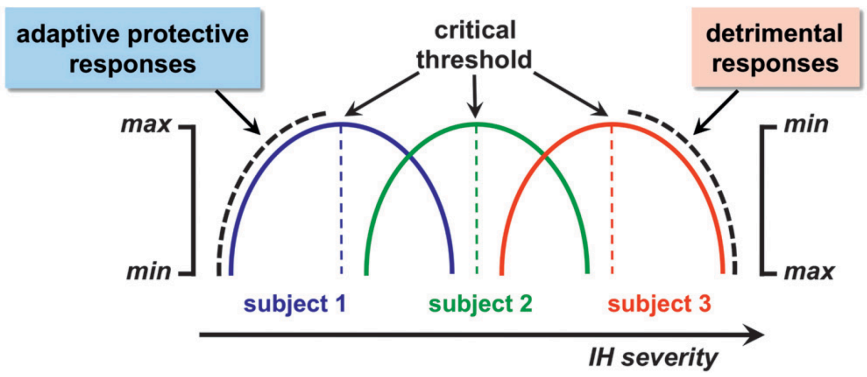
A pesar de lo atractivo de estas hipótesis, algunos estudios apuntan que los sujetos de edad avanzada también están sujetos a riesgo cardiovascular. Por todo ello, a pesar de que las evidencias sugieren que es posible que los pacientes de edad avanzada con apneas-hipopneas durante el sueño puedan diferir, en algunos aspectos, de los observados en sujetos más jóvenes, aún no se dispone de datos concluyentes.

3. HIPOXIA INTERMITENTE

La HI se define como episodios repetidos de hipoxia intercalados con episodios de normoxia y es considerada el principal componente implicado en las complicaciones cardiovasculares asociadas al SAOS. En los último 20 años, se han desarrollado diferentes protocolos de HI, tanto en modelos animales como en humanos, con el fin de estudiar las consecuencias de la HI y los mecanismos celulares y moleculares implicados en estas alteraciones. De esta manera se logra evaluar el efecto de la hipoxia de manera aislada y controlada, sin la presencia de factores de confusión como ocurre en la investigación clínica realizada en pacientes.

En general, las respuestas biológicas a la HI pueden ser adaptativas o maladaptativas, dependiendo de la gravedad, frecuencia y duración de la hipoxemia.

Figura 6. Efecto dual de la hipoxia intermitente.



La severidad del patrón de HI determina su efecto, siendo protector en condiciones de baja severidad, correspondiente a SAOS leve-moderado, o efecto patológico en condiciones de severidad grave.

3.1. Modelo animal de hipoxia intermitente

Los modelos de HI se inducen mediante el control del contenido de oxígeno y nitrógeno del aire que se respira. De esta forma los animales de experimentación respiran intermitentemente un aire enriquecido con nitrógeno para crear hipoxia, alternando con oxígeno o aire para la fase de reoxigenación. La duración de las fases de hipoxia y normoxia, así como las pendientes de la disminución y el aumento de la saturación de oxígeno, están condicionadas por el tamaño de la jaula / cámara y los flujos de gas y mezclas que dan como resultado diferentes paradigmas de HI. Los animales de control generalmente se colocan en el mismo tipo de jaula o cámara y se exponen a estímulos de flujos de aire similares para asegurar niveles similares de ruido y turbulencia relacionados con la circulación de gas.

Los modelos animales más utilizados son los roedores. El estímulo de HI se aplica durante el período de sueño, en el caso de los roedores durante el día. La duración del estímulo depende del protocolo experimental de cada estudio, variando desde 4horas al día[126] a 12 o 24 horas/día [127,128], así como de la frecuencia de los eventos hipóxicos durante ese período de tiempo. La elección del número de eventos va desde 120 ciclos/hora (ciclos de 30 segundos) a 60 ciclos/hora[129–131]. La elección de la frecuencia y patrón del estímulo resulta en diferentes saturaciones de oxihemoglobina, que va desde 60% al 80% en ratones y ratas expuestos a ciclos con fracción inspirada de oxígeno (FiO_2) del 5% cada 30 segundos y desde 83% a 86% en ratones expuestos a ciclos de HI más largos y progresivos con FiO_2 del 6-10%. De esta manera, se pueden realizar protocolos experimentales diferentes que simulan distintos grados de severidad de la enfermedad, correspondiendo SAOS leve, moderado o grave. El modelo murino de HI más utilizado es el modelo que simula SAOS grave con 60 eventos hipóxicos por hora durante el período de sueño. Además de la severidad de los estímulos hipóxicos, es muy importante el tiempo de exposición, variando desde días (efecto agudo) a semanas o meses (efecto crónico). Las diferencias en el protocolo experimental de HI elegido, puede explicar en parte las discrepancias observadas en la literatura donde el efecto de la HI va desde beneficioso a deletéreo.

3.2. Maladaptación a hipoxia intermitente: efecto patológico

Existe una creciente evidencia que define a la HI como el principal mecanismo en el desarrollo de las consecuencias cardiovasculares, metabólicas, y cognitivas en el contexto del SAOS, mediante la activación de vías de estrés oxidativo e inflamación. El modelo animal de HI más utilizado tiene como objetivo principal imitar los eventos hipóxicos que sufren los pacientes con SAOS grave, definido por el IAH y el índice de desaturación de oxígeno. Por lo tanto el patrón elegido suele

tener ciclos hipóxicos con alta frecuencia, baja saturación de oxígeno y restringido durante el período de sueño[132]. Dentro de los efectos patológicos a nivel cardiovascular encontramos:

Aumento de la presión arterial y actividad simpática

La homeostasis cardiovascular depende la información periférica recibida la cual inicia ajustes reflejos en respuesta a cambios ambientales. Los cuerpos carotídeos localizados en la bifurcación de la arteria carótida común, actúan como órganos sensores detectando cambios en los niveles de oxígenos en sangre arterial provocando cambios cardiovasculares para asegurar un suministro adecuado de oxígeno en condiciones de hipoxemia. Estas células oxígeno-sensibles regulan la función cardiorespiratoria durante condiciones de hipoxia[133]. Estos sensores periféricos liberan transmisores en respuesta a hipoxia, activando la estimulación de neuronas del núcleo del tracto solitario del tronco cerebral, el cual activa regiones del cerebro encargadas de controlar el sistema autónomo, específicamente la rama simpática[134]. Cuando estos quimiorreceptores y quimioreflejos periféricos son activados por la HI, se produce un aumento de la actividad del sistema nervioso simpático[135], contribuyendo con el aumento de la presión arterial e hipertensión.

Lo modelos de HI han confirmado la relación causal entre el componente hipóxico de la apnea del sueño y la elevación de la presión arterial. En roedores, animales no propensos a desarrollar hipertensión, la HI induce una elevación moderada de la presión arterial, incluso después de varias semanas de exposición[131,136–139]. De hecho, dos estudios diferentes encontraron que ratones C57BL6 mostraron un aumento similar después de 14 días (21,4 mmmHg)[129] y 90 días (19.8mmHg)[128]. Resultados similares se han encontrado en sujetos sanos expuestos a HI durante 14 días, en donde desarrollaron una elevación de la presión arterial sostenida durante el día[135]. El modelo animal de HI predijo por tanto los resultados en humanos, confirmando la relevancia clínica de este modelo experimental.

Inflamación y estrés oxidativo

Los cambios en saturación de oxígeno durante los ciclos de HI pueden provocar oscilaciones en la presión parcial de oxígeno en los tejidos, siendo más susceptibles tejidos con mayor tasa metabólica y perfusión, como son el cerebro, hígado y riñón[140]. Aunque algunas respuestas homeostáticas a la HI pueden desarrollarse con el tiempo, los cambios oscilantes en la disponibilidad de oxígeno en los tejidos son considerados como una fuente importante de ROS. Estas moléculas pueden generarse a partir de diferentes compartimentos subcelulares y orgánulos, como mitocondrias, membrana celular, lisosomas, peroxisomas y el retículo endoplásmico[141–143].

La familia HIF puede explicar, al menos en parte, algunos de los procesos que vinculan la HI con la producción de ROS, la activación de la inflamación y la regulación ascendente de otras moléculas involucradas en la angiogénesis y las respuestas homeostáticas. HIF-1 es un activador transcripcional comúnmente estudiado, que comprende una subunidad α regulada por O2 y una subunidad β constitutiva[144]. Por lo tanto, el HIF-1α puede ser inducido por la hipoxia como consecuencia de la disminución de la degradación dependiente de O2 de HIF-1α[145]. HIF-2α es otro miembro de la familia HIF inducido por la hipoxia continua, que también puede interactuar con HIF-1α[146]. Aunque la hipoxia continua promueve el aumento de la expresión y la actividad de ambas moléculas[146], la HI aguda up-regula HIF-1α y down-regula HIF-2α[147]. Además, si consideramos que HIF-2α regula la transcripción de varias enzimas antioxidantes, incluida la SOD-2 (superóxido dismutasa 2), y el factor de transcripción NRF2 (factor nuclear derivado de eritroide 2)[148], la down-regulación de HIF-2α en el contexto de la HI aguda también puede contribuir al aumento de las ROS mediante la transcripción insuficiente de enzimas antioxidantes.

Ante este incremento del estrés oxidativo, la mayoría de células responderán aumentando la expresión del factor transcripcional sensible a redox NF-κB, una molécula fundamental en la respuesta proinflamatoria. En el contexto de la hipoxia, el NF-κB también puede ser modulado por HIF-1α[149]. En el núcleo, NF-κB regula la transcripción de varios genes proinflamatorios responsables de codificar las citoquinas inflamatorias (TNF-α, IL-6 e IL-8), quimiocinas (MCP-1), moléculas de adhesión (VCAM-1, ICAM-1) y otras enzimas como la ciclooxigenasa-2 (COX-2). Curiosamente, el NF-κBredox sensible puede activar células endoteliales, leucocitos y plaquetas que expresan moléculas de adhesión y citoquinas proinflamatorias y contribuyen a la disfunción endotelial, promoviendo la infiltración de células inmunes a la pared vascular y desarrollo de aterosclerosis, así como otras morbilidades cardiovasculares que se han atribuido a la HI[150–152].

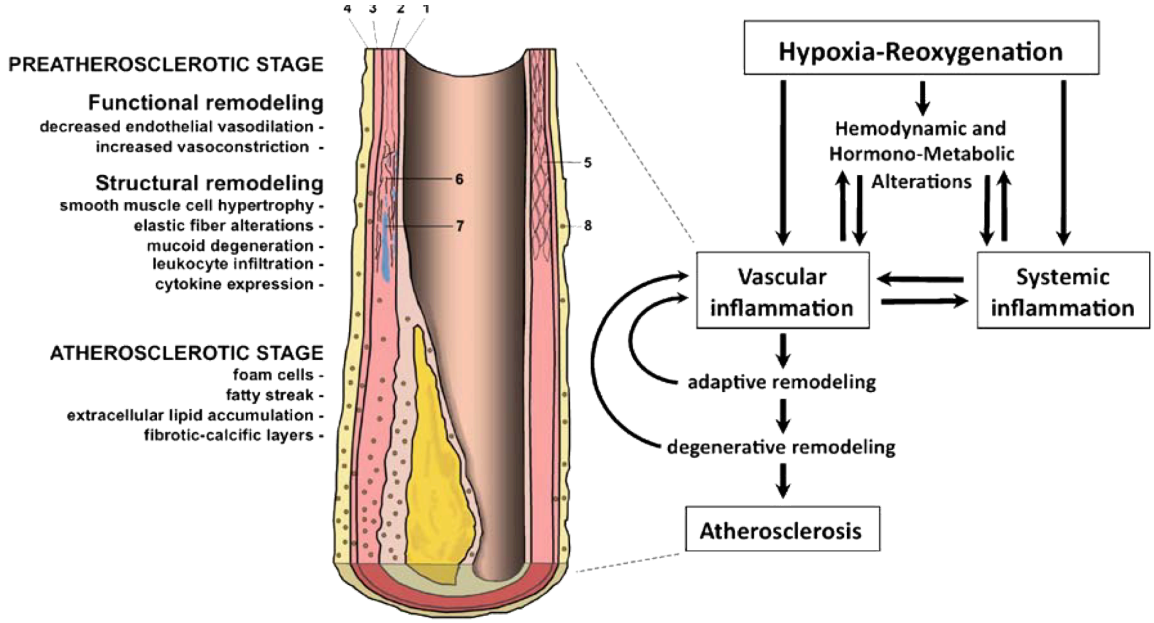
3.2.1. Consecuencias vasculares de la exposición a hipoxia intermitente

Los modelos animales de HI han permitido evaluar el componente hipóxico de la apnea del sueño por separado, estudiando las alteraciones tanto sistémicas como a nivel vascular. La placa aterosclerótica es el resultado final de un acumulo de eventos biológicos sistémicos y localizados de remodelación arterial que puede estar iniciado o agravado por la apnea del sueño.

Diversos estudios han reportado una remodelación preaterosclerótica funcional y estructural inducida por HI. A nivel funcional, ratones expuestos a HI muestran una disminución de la vasodilatación, debido a una reducción de la producción de óxido nítrico [139]. También presentan un aumento en la vasoconstricción inducida por la sobractivación del sistema simpático[129,130]. Este

desequilibrio hemodinámico resulta en un aumento de la rigidez arterial y reducción de la distendibilidad arterial. A nivel estructural, ratones expuestos a HI muestran una remodelación preaterosclerótica que afecta a distintos componentes de la pared vascular. En primer lugar se ha evidenciado un aumento del grosor intima-media, signo temprano de aterosclerosis en apnea del sueño[153]. Además, existe una remodelación de los componentes de la matriz extracelular inducido por la HI. Específicamente ratones expuestos a HI muestran un aumento de la desorganización y fragmentación de las láminas de elastina, aumento de depósitos de mucopolisacáridos y colágeno en la pared vascular[40,129,154]. Todos estos cambios mecánicos adaptativos de la pared vascular están involucrados en una disminución progresiva de su elasticidad, contribuyendo a un aumento de la rigidez de la pared vascular.

Figura 7. Remodelación ateroscletótica inducida por hipoxia intermitente.



La hipoxia intermitente provoca alteraciones hemodinámicas, metabólicas e inflamatorias, induciendo remodelación vascular que resulta en aterosclerosis. Las alteraciones preateroscleróticas incluyen: aumento del grosor intima (1)-media (2) con hipertrofia de las células de músculo liso vascular (5), alteración de las fibras de elastina (6), degeneración mucoide (7), infiltración de linfocitos (8) en la adventicia (3) y peri-adventicia (4). Figura extraída de Arnaud C et al 2009[155]

3.2.2. Consecuencias cardíacas de la exposión a hipoxia intermitente

La exposición a HI induce hipertensión pulmonar resultando en hipertrofia cardíaca del ventrículo derecho[131,156,157], ventrículo izquierda[158], o ambos[136]. La hipertrofia cardíaca inducida por la HI ocurre incluso cuando se induce denervación simpática y se previene el aumento de la presión arterial[156], sugiriendo un efecto directo de la HI en el miocardio. De hecho, la HI afecta tanto cardiomiocitos como la vascularización del tejido cardíaco. Ratones expuestos a HI muestran disfunción endotelial de los vasos cardíacos, y un aumento de la densidad capilar y expresión de VEGF[129].

Ratas expuestas a 5 semanas de HI, presentan hipertrofia del ventrículo izquierdo, dilatación y reducción de la función cardiaca, con un aumento de estrés oxidativo evidente por peroxidación lipídica y disminución de los niveles de superóxido dismutasa[159]. En ratones, 10 días de HI indujo hipertrofia de cardiomiocitos, fibrosis, y un aumento del estrés oxidativo, inflamación y apoptosis[160,161].

3.3. Adaptación a hipoxia intermitente: efectos beneficiosos

Dependiendo de la duración, severidad, y patrón del estímulo hipóxico, la HI puede inducir una respuesta adaptativa que otorga efectos beneficiosos o una respuesta maladaptativa con efectos patológicos. El efecto protector de la HI se induce por la activación y propagación de respuestas adaptativas u homeostáticas promovidas durante la exposición a HI, es un proceso conocido como precondicinamiento. Por lo tanto, exposiciones cortas a una HI moderada (>12% 0_2) puede otorgar protección de células, tejidos y órganos específicos contra eventos graves de hipoxia o isquemia.

Animales que han sido expuestos a HI durante periodos cortos han mostrado mayor resistencia a daño inducido por eventos isquémicos graves producidos posteriormente. En comparación con los controles, ratones expuestos a HI (8% 0_2x10 min / 21% 0_2x10min, 6 ciclos) sobreviven más tiempo cundo son expuestos a hipoxia letal, con daño tisular atenuado. Ratones (6% 0_2x6 min / 21% 0_2x6min, 5 ciclos) o ratas (10% 0_2 x 40seg / 21% 0_2 x 40seg, 4 horas) expuestos a HI mostraron protección frente isquemia inducida por infarto[137,162,163]. Otros investigadores han reportado efecto antihipertensivo en ratas jóvenes, asociado a un aumento de la producción de óxido nítrico a nivel vascular[151]. Además, se ha observado un efecto terapéutico en infarto de miocardio, reduciendo el tamaño del infarto, la fibrosis y apoptosis de cardiomiocitos[164].

La respuesta cardioprotetora otorgada por la HI en contra del daño por isquemia-reperfusión viene dada por una mayor vascularización, aumento de la vasodilatación y flujo coronario, así como una mayor expresión de proteínas antioxidantes que neutralizan el exceso de producción de ROS[165]. Tras la reperfusión después de un período de isquemia en el tejido miocárdico, se genera gran cantidad de ROS, el cual contribuye a aumentar el daño miocárdico[166,167]. Sin embargo, al mismo tiempo, los ROS pueden promover cardioprotección activando diferentes vías de respuesta a estrés oxidativo. Es por ello por lo que la producción de ROS en condiciones de HI podría estar involucrada parcialmente en el fenómeno de pre-condicionamiento isquémico asociado a la HI. Por lo tanto, dependiendo de la severidad de la HI , existe un umbral de concentración de ROS que genera una respuesta adaptativa y promueve protección, que si el aumento del estrés oxidativo persiste, pasa a ser una respuesta maladaptativa con efectos deletéreos[168].

HIPÓTESIS Y OBJETIVOS

ESTUDIO 1

La remodelación cardiovascular inducida por la hipoxia intermitente es revertida por normoxia en un modelo murino de apnea del sueño

Este estudio corresponde al siguiente artículo:

Anabel L. Castro-Grattoni, Roger Alvarez-Buvé, Marta Torres, Ramon Farré, Josep M. Montserrat, Mireia Dalmases, Isaac Almendros, Ferran Barbé, and Manuel Sánchez-de-la-Torre

"Intermittent Hypoxia-Induced Cardiovascular Remodeling Is Reversed by Normoxia in a Mouse Model of Sleep Apnea" CHEST 2016; 149(6):1400-1408

Factor de impacto: 7.652

Hipótesis

La exposición a hipoxia intermitente crónica induce remodelación cardiovascular en un modelo murino de apnea del sueño. Dicha remodelación cardiovascular es normalizada tras un periodo de normoxia, simulando el tratamiento efectivo del SAOS.

Objetivo

Evaluar la remodelación cardiovascular a nivel morfológico inducida por hipoxia intermitente crónica en un modelo murino de apnea del sueño, así como su normalización tras un período en condiciones de normoxia.

ESTUDIO 2

Efecto de la edad en la remodelación cardiovascular inducida por la hipoxia intermitente crónica en un modelo murino de apnea del sueño

Este estudio corresponde al siguiente artículo:

Anabel L. Castro-Grattoni, Monique Suarez-Giron, Ivan Benítez, Marta Torres, Isaac Almendros, Ramon Farré, Josep M. Montserrat, Mireia Dalmases, David Gozal, Manuel Sánchez-de-la-Torre, ; on behalf of the Spanish Sleep Network.

"Effect of age on the cardiovascular remodeling induced by chronic intermittent hypoxia as a murine model of sleep apnea"

En proceso de revisión en Respirology

Factor de impacto: 4.407

Hipótesis

La edad juega un papel fundamental en la remodelación cardiovascular inducida por la hipoxia intermitente en un modelo murino de apnea del sueño. Así, el efecto de la hipoxia intermitente es independiente de la edad del sujeto que se expone a dicho estímulo.

Objetivo

Evaluar el efecto de la edad en la remodelación cardiovascular inducida por la hipoxia intermitente crónica en un modelo murino de apnea del sueño.

ESTUDIO 3

El efecto de la hipoxia intermitente crónica en la expresión génica cardiovascular es modulado por la edad en un modelo de apnea del sueño

Este estudio corresponde al siguiente artículo:

Anabel L. Castro-Grattoni, Monique Suarez-Giron, Ivan Benítez, Lourdes Tecchia, Marta Torres, Isaac Almendros, Ramon Farré, Adriano Targa, Josep M. Montserrat, Mireia Dalmases, Ferran Barbe, David Gozal, Manuel Sánchez-de-la-Torre; on behalf of the Spanish Sleep Network.

"The effect of chronic intermittent hypoxia in cardiovascular gene expression is modulated by age in a mice model of sleep apnea" (Submitted)

Hipótesis

El efecto de la hipoxia intermitente crónica en la expresión génica de distintos marcadores a nivel cardiovascular se encuentra modulada por la edad.

Objetivo

Evaluar el efecto de la edad en la expresión diferencial de mecanismos moleculares que delinean la remodelación cardiovascular inducida por hipoxia intermitente crónica en un modelo murino de apnea del sueño.

ESTUDIO 4

Respuesta de la presión arterial al tratamiento con CPAP en sujetos con apnea obstructiva del sueño: valor predictivo de la monitorización ambulatoria de la presión arterial 24 horas

Este estudio corresponde al siguiente artículo:

Anabel L. Castro-Grattoni, Gerard Torres, Montserrat Martínez-Alonso, Marta Torres, Ferran Barbé, Cecilia Turino, Alicia Sánchez-de-la-Torre, Anunciación Cortijo, Joaquin Duran-Cantolla, Carlos Egea, Manuel Sánchez-de-la-Torre

"Blood pressure response to CPAP treatment in subjects with obstructive sleep apnoea: the predictive value of 24-h ambulatory blood pressure monitoring"
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Hipótesis

Características clínicas a nivel basal permiten identificar aquellos pacientes con SAOS que se beneficiarán del tratamiento con CPAP para el control de la presión arterial.

Objetivo

Identificar características clínicas a nivel basal, incluido un MAPA-24horas, un estudio de sueño y biomarcadores cardiovasculares, con valor predictivo de respuesta al tratamiento con CPAP en términos de presión arterial nocturna.

ARTÍCULOS

Estudio 1

"Intermittent Hypoxia-Induced Cardiovascular Remodeling Is Reversed by Normoxia in a Mouse Model of Sleep Apnea"

Anabel L. Castro-Grattoni, Roger Alvarez-Buvé, Marta Torres, Ramon Farré, Josep M. Montserrat, Mireia Dalmases, Isaac Almendros, Ferran Barbé, and Manuel Sánchez-de-la-Torre. CHEST 2016; 149(6):1400-1408

Intermittent Hypoxia-Induced Cardiovascular Remodeling Is Reversed by Normoxia in a Mouse Model of Sleep Apnea

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ABSTRACT

BACKGROUND: Intermittent hypoxia (IH) is the principal injurious factor involved in the cardiovascular morbidity and mortality associated with OSA. The gold standard for treatment is CPAP, which eliminates IH and appears to reduce cardiovascular risk. There is no experimental evidence on the reversibility of cardiovascular remodeling after IH withdrawal. The objective of the present study is to assess the reversibility of early cardiovascular structural remodeling induced by IH after resumption of normoxic breathing in a novel recovery animal model mimicking OSA treatment.

METHODS: Weinvestigated cardiovascular remodeling inC57BL/6mice exposed to IHfor 6 weeks vs the normoxia group and its spontaneous recovery after 6 subsequent weeks under normoxia.

RESULTS: Aortic expansive remodeling was induced by IH, with intima-media thickening and without lumen perimeter changes. Elastic fi ber network disorganization, fragmentation, and estrangement between the end points of disrupted fi bers were increased by IH. Extracellular matrix turnover was altered, as visualized by collagen and mucoid interlaminar accumulation. Furthermore, left ventricular perivascular fi brosis was increased by IH, whereas cardiomyocytes size was unaffected. These cardiovascular remodeling events induced by IH were normalized after recovery in normoxia, mimicking CPAP treatment.

CONCLUSIONS: The early structural cardiovascular remodeling induced by IH was normalized after IH removal, revealing a novel recovery model for studying the effects of OSA treatment. Our fi ndings suggest the clinical relevance of early detection and effective treatment of OSA in patients to prevent the natural course of cardiovascular diseases.

INTRODUCTION

OSA is a highly prevalent disorder that affects 6% to 15% of the general population and is caused by repetitive upper airway occlusion during sleep.[1,2] OSA is an important public health problem because of its association with increased cardiovascular morbidity and mortality, including hypertension, coronary artery disease, congestive heart failure, heart attack, and stroke.[3,4] The major OSA components associated with cardiovascular consequences are large swings in intrathoracic pressure, post apneic arousals, and intermittent hypoxia (IH). IH is the main detrimental event leading to cardiovascular morbidity and mortality.[5,6]

Sympathetic overactivation, oxidative stress, and systemic inflammation are the main intermediary mechanisms associated with IH.[4,7] These abnormalities all contribute to the development of early and late cardiovascular remodeling, including increased blood pressure, endothelial dysfunction, carotid intima-media thickness (IMT), arterial stiffness, and accelerated progression of atherosclerosis, and induce cardiac rhythm and structural disturbances.[4,8]

Murine models have been used to study the adaptive and degenerative hemodynamic and structural alterations of

the cardiovascular system induced by IH.[9] IH induces blood pressure elevation, endothelial dysfunction, enlargement of aortic IMT, cardiac hypertrophy, and extracellular matrix (ECM) alterations; increased systemic inflammation and activation of proinflammatory pathways in cardiovascular tissue; and increased risk of developing atherosclerotic plaques.[10]

CPAP, the gold standard therapy for patients with OSA, effectively improves daytime symptoms and quality of life, and might be an effective treatment for cardiovascular risk reduction.[11,12] Randomized controlled trials have demonstrated that CPAP therapy reduces blood pressure, sympathetic overactivity, and coagulation abnormalities and improves left ventricular ejection fraction.[13-16] CPAP has also been shown to improve endothelial function, IMT, and arterial stiffness in small studies.[5,17] However, there is no experimental evidence that elimination of IH reverses the cardiovascular remodeling induced by injurious hypoxic challenge.

To address this important issue, we established a murine model of recovery in which normal room air breathing is resumed after chronic IH challenge. We hypothesized that the resumption of normoxic conditions, which mimics CPAP treatment, could reverse the early cardiovascular morphological remodeling induced by IH. This recovery model will enable the study of the mechanisms involved in the therapeutic effects of OSA treatments in reversing injuries induced by IH in different organs.

MATERIALS AND METHODS

Study design

The study was approved by the Ethical Committee for Animal Research of the University of Barcelona and was performed on 6-week-old pathogen-free C57BL/6 male mice (Charles River Laboratories). The animals were housed in standard cages in a temperature- and light-controlled room (22C 24C; 14 hours of light, 10 hours of dark). A total of 40 mice were randomly assigned to IH exposure (n=20 mice) or normoxia (n=20 mice) for 6 weeks. After this IH phase, 10 mice from each group were anesthetized (urethane 20%, 1 g/kg) and euthanized by exsanguination, and aortas and hearts were excised. The remaining IH mice were subsequently subjected to a 6-week normoxic Recovery phase to mimic CPAP treatment of patients with OSA and sacrificed, and tissue samples were excised as described below. The experimental design of the protocol is shown in Figure 1A. The groups were labeled N, normoxia; IH, intermittent hypoxia; N.R, normoxia with recovery phase; and IH.R, intermittent hypoxia with recovery phase.

Intermittent Hypoxia

Chronic IH was applied as previously described.[18] For 6 weeks, mice in the IH group received 60 hypoxic events/h (20 s at 5% O2 per min), during 6 h/d, corresponding to severe OSA. Control mice with normoxic breathing were placed in an identical system, but the hypoxic gas from the reservoir was replaced by room air. In the normoxic recovery phase, all mice were subjected to identical normoxic conditions.

Histomorphological Analyses

The mid thoracic aorta and left ventricle of the heart samples were perfused with phosphate-buffered saline, fixed with 4% paraformaldehyde, and embedded in paraffin for further histological analysis by an investigator blinded to the experimental group. The

samples were stained with hematoxylin and eosin (H&E, Master Diagnostica), Gomori trichrome stain (Artisan Link Special Staining System; DAKO), or Alcian blue (Alcian blue 2.5; Bio- Optica). For measurements, images from four consecutive sections were processed using Image J (National Institutes of Health) and Adobe Photoshop CS6 (Adobe Systems Inc) software. All stained sections were captured with a digital microimaging network instrument (Leica-DMD-108; Leica Microsystems), and aortic autofluorescence was visualized using a fluorescence microscope (Olympus-BX51; Olympus).

Intima-media thickness: The cross-sectional IMT was quantified by morphometric analysis of the H&E stained sections (300 measurements for each animal).

Alcian blue staining: The integrated density of the blue staining was quantified and adjusted to the corresponding aortic wall area to detect mucoid deposition.

Cardiac hypertrophy: The cross-sectional area of the cardiac myofibers with a circular running pattern was analyzed quantitatively using H&E stained sections (300 cardiomyocytes for each animal).

Cardiovascular fibrosis: Gomori trichrome stain was used to detect fibrosis in aortic and cardiac tissue. The fibrotic tissue was determined by measuring the positive collagen area adjusted to the total tissue area.

Elastic-network analysis: The aortic autofluorescence was used to perform elastic fiber analysis. The elastin disruption (ie, the complete fragmentation of one elastic fiber) and the distance between both ends of a fragmented fiber were quantified (adjusted by total aortic area and shown as percent space without fiber). In addition, we quantified the area with elastic fiber disorganization based on the inability to count the amount of organized elastic fiber.[19]

Data Analysis

Results were expressed as the mean ± SEM. Depending on normality and variance homogeneity, analysis of variance and Student t test or Mann-Whitney U test were performed. Statistical significance was set at a probability value of less than .05. Structural parameters were adjusted for body weight using a linear regression model.

RESULTS

Body Weight

The body weight at baseline was similar in both groups. However, 6 weeks of IH decreased animal body weight (P = 0.005). After the normoxic recovery phase, the body weights of mice in the IH+R group were similar to those in the N+R group, suggesting a normalization of body weight after IH withdrawal (Fig 1 B).

Morphological Vascular Remodeling

Intima-media thickness: The aortic IMT was increased by IH exposure vs that of the N group (P=0.03). After normoxia, the IMT of mice in the IH+R group was normalized comparedwith its control, suggesting a Recovery of aortic remodeling (Figs 2A, 2B). The aortic lumen perimeter did not exhibit significant changes, indicating expansive remodeling of the aortic wall induced by IH. Moreover, mice in the N+R and IH+R groups did not show statistically significant differences in lumen perimeter.

Elastin fiber disorganization and disruption: Six weeks of IH exposure induced elastin fiber disruption and increased the distance between both ends of the fragmented fibers (Figs 2C-E). These alterations were reduced compared with those of the N+R group, suggesting that the aortas of the IH+R group were subjected to a recovery remodeling process (Figs 2D, 2E). Furthermore, mice exposed to IH displayed an increase in zones of elastin fiber disorganization in the aortic wall, which was not observed in mice in the IH+R group compared with those in the N+R group (Fig 2F).

Aortic Mucoid deposition: Alcian blue staining revealed greater mucoid deposition in the vascular wall of the IH group between subintimal elastic fibers, specifically in regions neighboring the aortic lumen. Mucoid deposition in the aortic wall in the N+R and IH+R groups was similar to that in the normoxia group, suggesting normalization after normoxic Recovery (Figs 3A, 3B).

Aortic fi brosis: The collagen fiber content in the aortic wall was higher in mice exposed to IH for 6 weeks, suggesting the induction of collagen synthesis during IH exposure. Recovery under normoxic conditions of the IH+R group resulted in a decrease in aortic fibrosis, similar to the N+R group (Figs 3A, 3C).

Morphological Cardiac Remodeling

Mice exposed to IH for 6 weeks exhibited increased cardiac perivascular fibrosis compared with the normoxia group (Figs 4A, 4B). After the normoxic recovery phase, the extracellular collagen content of the IH+R group was no different from that of the N+R group (Figs 4 A, 4 B). The cross-sectional area of the left ventricular cardiomyocytes did not differ significantly between groups (Fig 4 C).

DISCUSSION

This study demonstrates that normoxic breathing after a period of chronic IH spontaneously reverts the early structural cardiovascular remodeling induced by this injurious challenge that characterizes sleep apnea. In the field of experimental sleep apnea, considerable research has focused on analyzing the effects of IH, but few studies have analyzed the extent to which the deleterious effects of IH can be reversed by a period of post-IH normoxia, which mimics the clinical situation in which OSA therapy is applied to restore normal breathing. Our novel experimental setting strongly suggests that effective treatment could normalize early cardiovascular lesions induced by hypoxemia associated with OSA syndrome. OSA integrates various pathophysiological triggers, but IH is the principal injurious factor that plays a pivotal role in the progression of cardiovascular diseases.[20] In addition to the evidence of adverse events caused by IH,[9] other studies have demonstrated benefi cial effects of IH in both animal models and patients with OSA.[21] The opposing effects induced by IH depend mainly on the experimental time; long-term exposure (4-8 weeks) is required to cause detrimental effects.[22] In the present study, we assessed several morphological cardiovascular changes resulting from the direct effect of IH for 6 weeks, a common experimental paradigm to mimic severe OSA in patients. Our results confi rmed the hypothesis that restoring normoxia by removing IH stress facilitates homeostatic cardiovascular restoration.

Vascular remodeling is dependent on dynamic interactions between local growth factors, vasoactive substances, and hemodynamic stimuli and is a response to long-standing changes in hemodynamic conditions.[23] IH[24] and sleep fragmentation[19] are independent factors that promote vascular remodeling in the aorta. IMT remodeling is an early predisposing event in atherosclerosis and plaque formation and is associated with increased cardiovascular risk.[25] Patients with OSA exhibit increased IMT in association with infl ammatory markers and nocturnal oxygen desaturation.[26] Our fi ndings confi rm

previous observations of expansive aortic remodeling with increased IMT without vascular dilatation as a result of IH exposure in mice.[27] Importantly, our novel experimental data on IMT normalization after normoxic recovery are in agreement with clinical data on patients with OSA who were treated with CPAP.[17]

We also observed that IH increased elastic fi ver disorganization and disruption. The increase in the estrangement of the two end points of the disrupted lamina, reported in the present study, suggests a higher tensile stress in the aortic wall exposed to IH, leading to a stronger fi ber break. Perturbations in the continuity of the elastic lamina have been implicated in early phases of atherosclerosis[28] and in vascular remodeling induced by sleep fragmentation.[19] Changes in elastin structure and distribution have been reported in a rat model of IH, but quantitative morphometric analysis was not performed.[29] However, we have quantitatively assessed elastic fiber organization and fragmentation of the aortic wall. Strikingly, our results demonstrate that the normoxic recovery in mice that had been previously exposed to IH enabled a normalization of the vascular elastic fiber network alterations.

Changes in the ECM have been implicated in the pathogenesis of atherosclerosis and play an important role in intercellular networking. These changes can lead to a fibroproliferative response, promoting lipid binding to the vascular wall and inducing foam cell formation.[30] We observed abnormal ECM turnover in the aortic wall in mice exposed to IH, which suggests that IH promotes collagen and mucopolysaccharide (proteoglycans and glycosaminoglycans) synthesis and deposition in interlaminar spaces. Importantly, we observed that this ECM remodeling could be normalized after a Recovery period in normoxic conditions, which indicates the possible activation of inhibitory and degradation pathways of collagen and mucopolysaccharide synthesis.

The ECM response to IH stress also includes morphological myocardial remodeling. We observed that IH induced perivascular fi brosis in the left ventricle, whereas interstitial fi brosis was not increased, in agreement with previous studies.[31] Perivascular fi brosis is substantially associated with the impairment of coronary blood fl ow and is involved in the progression of heart failure.[32] Because of signifi cant independent associations between OSA and heart failure, many studies have evaluated CPAP as a treatment for patients with OSA who have heart failure.[33,34] In the present study, we observed a normalization of

coronary perivascular fi brosis after recovery under normoxic conditions. Normoxia restoration was suffi cient to reduce perivascular fi brosis, most likely because of the reduction of the fi broinfl ammatory response and oxidative stress production in myocardial tissue. This fi nding has clinical relevance and suggests that patients with OSA who have heart disease would benefi t from effective breathing normalization, most likely because of the resulting improved coronary blood fl ow.

Cardiac remodeling includes hypertrophy that can exist in a state of compensation or progress to a decompensated state with time. We did not observe left ventricular hypertrophy, consistent with previous studies.[35] However, other studies have observed cardiac hypertrophy induced by IH.[31,36] The large disparity in results for left ventricular hypertrophy may refl ect differences in species or strain or even the side of the heart,22 which could explain our negative result for left ventricular hypertrophy.

Aortic wall and left ventricular remodeling induced by IH is the result of multiple interactions between intermediary mechanisms, including oxidative stress, systemic and tissue infl ammation, metabolic deregulation, endothelial dysfunction, sympathetic overactivation, and blood pressure overload.[24,37] Our study did not focus on assessing changes in blood pressure; however, two similar studies found that C57BL/6 mice exhibit increments in blood pressure after 14 and 90 days of IH exposure.[22,38] Arterial blood pressure increases (10 to 20 mm Hg) in rodent models of IH are comparable with those of other experimental animal models of hypertension.[39] Thus, in mice that are exposed to IH, increases in blood pressure may induce functional, mechanical, and structural changes in the aortic wall in response to hemodynamic and biomechanical stress. Moreover, IMT, elastin fi ber disruption, and interlaminar collagen accumulation induce arterial stiffness,[40] thereby contributing to systemic vascular resistance and arterial
blood pressure elevation.

Reversibility of structural cardiovascular damage has been demonstrated in several animal models of hypertension through spontaneous reversion or through the use of several forms of antihypertensive treatment.4[1-47] Celiprolol reduced cardiovascular alterations induced by hypoxic stress in mice exposed to IH.[48] The reversal of structural changes induced by elevated blood pressure suggests that several of our results could be

explained by a reduction in blood pressure after the recovery phase in normoxic conditions.

The current study has several limitations. Recurrent apnea in patients results in IH, hypercapnia, sleep arousal, sleep fragmentation, and changes in intrathoracic pressure that may contribute to cardiovascular remodeling. However, our study focused exclusively on IH stress, which is a limitation because the mice model of hypoxemia associated with sleep apnea does not represent the totality of the complex disorder. However, IH is the most important pathophysiological component of sleep apnea that underlies cardiovascular complications, which was the principal outcome of our study. The most common index of cardiac hypertrophy is the measure of heart or ventricular weights related to body weight. We did not assess this parameter, but relating heart to body weight is not valid when the investigated groups do not exhibit similar body growth patterns, as we observed in this study.49 The main strength of this work is that the use of a conventional mouse strain allowed us to assess the cardiovascular impact induced by IH per se and the subsequent recovery process under normoxic conditions, avoiding other confounding factors.

CONCLUSIONS

The current study demonstrates that IH induces preatherosclerotic remodeling characterized by IMT, elastin disruption and disorganization, accumulation of collagen fi bers, and mucoid elements on the aortic wall. We also observed initial myocardial remodeling induced by IH exposure, specifi cally perivascular fi brosis. These cardiovascular remodeling events are virtually reversed when the IH stress was removed and mice were returned to normoxic conditions, mimicking the effective treatment of the hypoxic component of OSA. The clinical relevance of our findings suggests that early detection of patients with OSA and the subsequent therapeutic intervention to normalize breathing may alter the natural course of cardiovascular diseases that are promoted by cyclic hypoxia and reoxygenation. Furthermore, we propose for the first time a murine model of IH followed by normoxia to study the potential benefits of IH resolution with CPAP treatment in patients with OSA, including restoring normal structure and function of the different organs challenged by this sleep breathing disorder. This recovery model may be a useful tool for future studies aimed at identifying possible cellular and molecular

mechanisms and signaling pathways involved in the homeostatic and adaptive response to IH. Additionally, this model may be used in future studies to assess OSA treatments.

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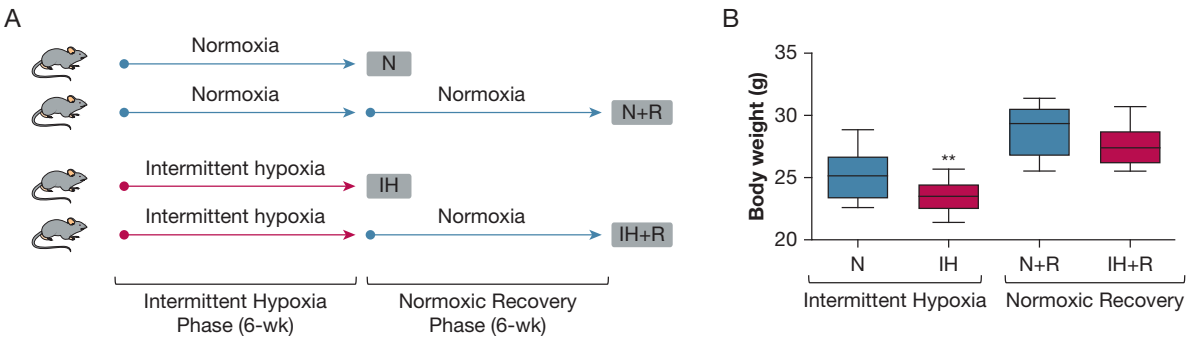
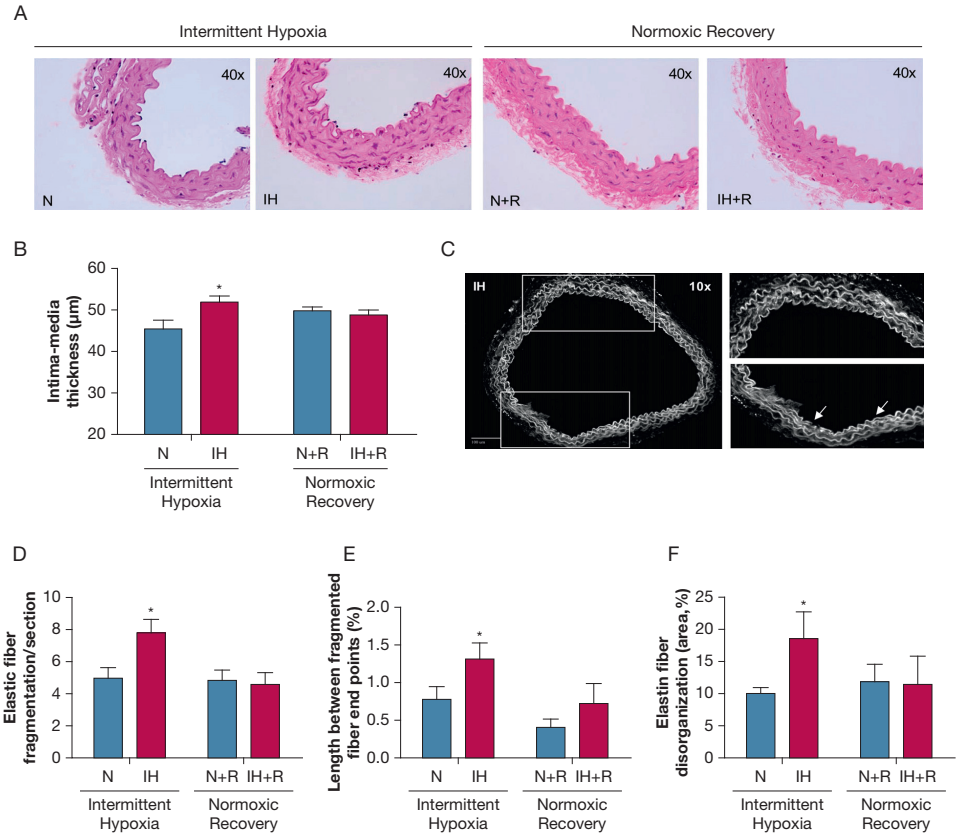
FIGURES

Figure 1- Mouse growth is altered by intermittent hypoxia and normalized after normoxic recovery.



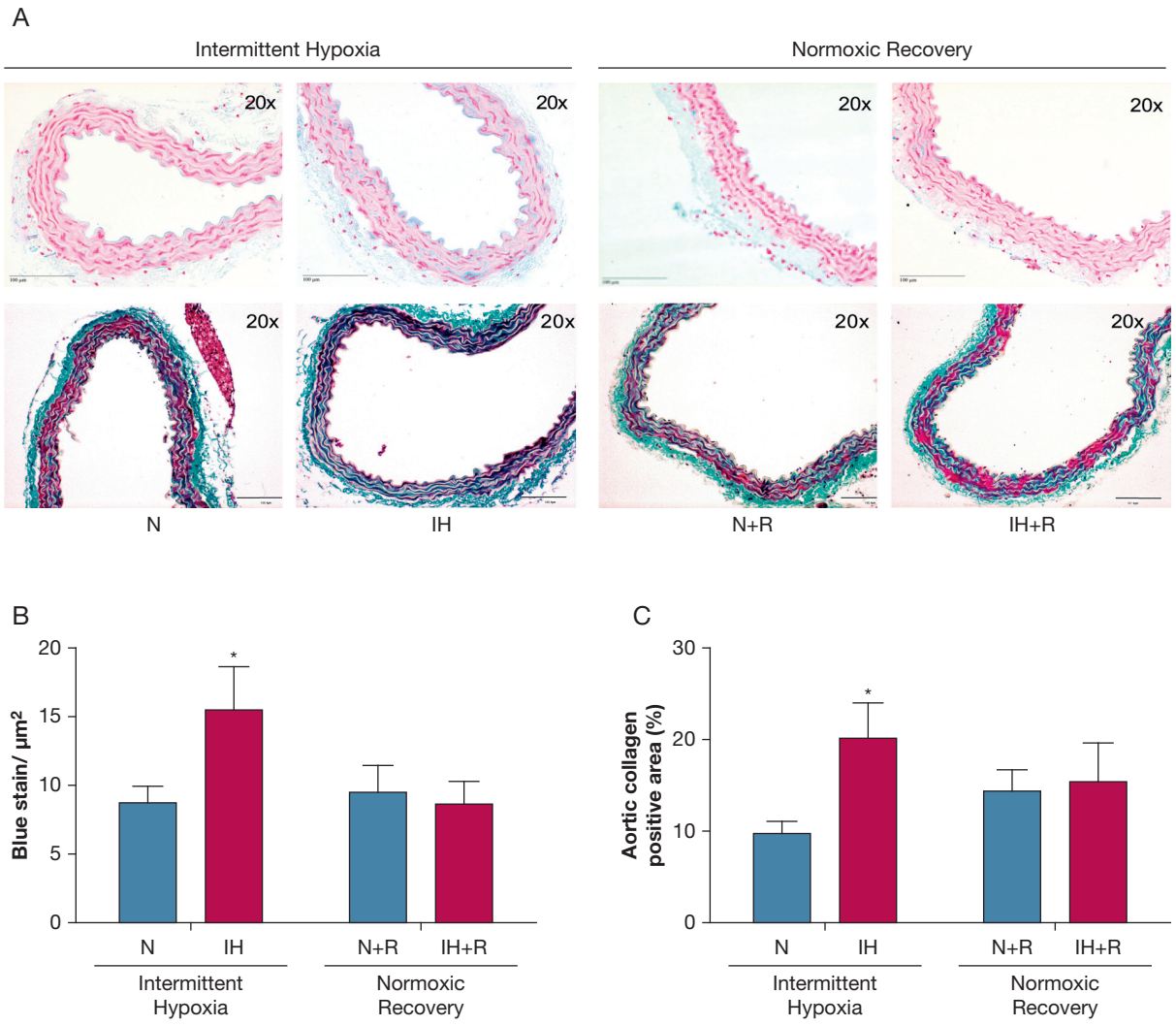
A, Experimental design of the study (n=10, per group): male C57BL/6 mice exposed to room air (N) or to IH for 6 weeks, and mice exposed to N or IH and subsequently subjected to a period of normoxia (6 more weeks; N+R and IH+R). B, Box-plot representation of body weight in N and IH groups at 6 weeks (P=0.005) and in N+R and IH+R groups at 12 weeks (P=0.0136). **P < .01 for intergroup comparisons. IH . intermittent hypoxia; IH+R . intermittent hypoxia plus normoxic recovery; N . normoxia; N+R . normoxia plus normoxic recovery.

Figure 2 - Aortic morphological remodeling associated with intermittent hypoxia and recovery after normoxic conditions.



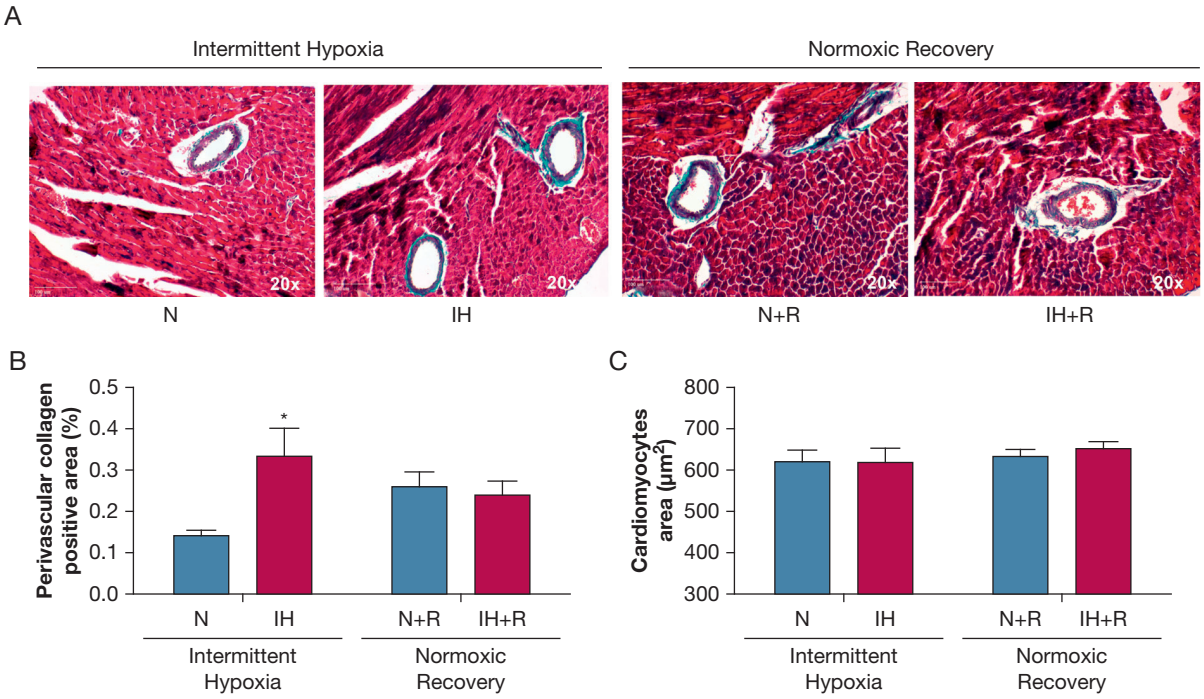
Morphological remodeling of mid thoracic aorta cross sections was assessed in C57BL/6 mice exposed to IH or room air (N) for 6 weeks and the recovery from exposure to IH or N after a period of normoxia (N+R, IH+R). A, Representative images of the aortic wall with H&E staining for each group (original magnificationx400). B, Histomorphometric analysis of intima-media thickness, IH vs N (P =0.03) and IH+R vs N+R (P0.92). C, Representative pictures of the elastic network (original magnificationx100), revealed by autofluorescence, with magnification of zoom elastic fiber disorganization (top inset) and fragmentation (bottom inset, with arrows showing fragmented elastic fiber end points). D, E, Quantification of intima-media elastic fiber breaks (D), and the length between the ends of fragmented fibers adjusted by total aortic wall area (E) (shown as %). F, Elastin fiber disorganization area in the aortic wall adjusted by total area (shown as %). *P < .05; values are mean ± SEM. H&E = hematoxylin and eosin. See Figure 1 legend for expansion of other abbreviations.

Figure 3 – Aorta extracellular matrix remodeling induced by intermittent hypoxia and progression after recovery in normoxic conditions.



Remodeling of extracellular components of the aortic wall was assessed in C57BL/6 mice exposed to IH or room air (N) at 6 weeks and in mice exposed to N or IH and subsequently subjected to normoxia (N+R, IH+R). A, top, Representative images of Alcian blue staining from the mid thoracic aorta to detect aortic wall mucoid deposition (original magnificationx200; mucins in blue); A, bottom, collagen-positive area of the intima-media (%). B, Representative images of Gomori trichrome stain to measure aortic wall fibrosis (original magnificationx200; collagen in green). C, Intima-media Mucoid deposition shown as the ratio of the total blue density to the total aortic wall area. (%).*P < .05; values are mean ± SEM. See Figure 1 legend for expansion of abbreviations.

Figure 4 – Cardiac morphological remodeling associated with intermittent hypoxia and the effect of recovery in normoxic conditions.



Morphological remodeling of cardiac tissue was assessed in mice exposed to IH or room air (N) at 6 weeks and in mice exposed to IH or N that were subsequently subjected to normoxia (N+R, IH+R). A, Representative images of the left ventricle with Gomori trichrome stain to detect perivascular fibrosis (original magnificationx200; collagen in green). B, Analysis of perivascular fibrosis measured as collagen-positive area (%). C, Histomorphometric analysis of the left ventricular cardiomyocyte area of fibers with a circular pattern did not reveal statistically significant differences. *P < 0.05; values are mean ± SEM. See Figure 1 legend for expansion of abbreviations.

Estudio 2

"Effect of age on the cardiovascular remodeling induced by chronic intermittent hypoxia as a murine model of sleep apnea"

Anabel L. Castro-Grattoni, Monique Suarez-Giron, Ivan Benítez, Marta Torres, Isaac Almendros, Ramon Farré, Josep M. Montserrat, Mireia Dalmases, David Gozal, Manuel Sánchez-de-la-Torre. Under review in Respirology

Effect of age on the cardiovascular remodelling induced by chronic intermittent hypoxia as a murine model of sleep apnoea

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Key words: age, ageing, cardiovascular, intermittent hypoxia, obstructive sleep apnoea.

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ABSTRACT

BACKGROUND AND OBJETIVE: Chronic intermittent hipoxia (CIH) is a major determinant of the cardiovascular morbidity associated with obstructive sleep apnoea (OSA), and the magnitude of CIH impact may be influenced by ageing. Here, we assessed the role of ageing in the early cardiovascular structural remodelling induced by severe CIH in a murine model of OSA.

METHODS: Cardiovascular remodelling was assessed in young (2 months old, n = 20) and aged (18 months old, n = 20) C57BL/6 female mice exposed to CIH (20% O2 for 40 s, 5% O2 for 20 s) or normoxia (room air) for 8 weeks (6 h/day).

RESULTS: Early vascular remodelling was observed in young mice exposed to CIH as illustrated by intimamedia thickening (mean change: 4.6 _ 2.6 μm; P = 0.02), elastin fibre disorganization (mean change: 9.2 _ 4.5%; P = 0.02) and fragmentation (mean change: 2.5 _ 0.8%; P = 0.03), and collagen (mean change: 3.2 _ 0.6%; P = 0.001) and mucopolysaccharide accumulation (mean change: 2.4 _ 0.8%; P = 0.01). In contrast, vascular remodelling was not apparent in aged mice exposed to CIH. Furthermore, left ventricular perivascular fibrosis (mean change: 0.71 _ 0.1; P < 0.001) and hypertrophy (mean change: 0.17 _ 0.1; P = 0.038) were increased by CIH exposure in young mice, but not in aged mice. Principal component analysis identified similar cardiovascular alterations among the young mice exposed to CIH and both older mouse groups, suggesting that CIH induces premature cardiovascular senescence.

CONCLUSION: Cardiovascular remodelling induced by severe CIH is affected by the age at which CIH onset occurs, suggesting that the deleterious cardiovascular effects associated with CIH may be more pronounced in younger populations, and such changes resemble chronological age-related declines in cardiovascular structural integrity.

INTRODUCTION

Obstructive sleep apnoea (OSA) is a prominent public health problem that adversely impacts health, quality of life and socioeconomics. OSA is a highly prevalent disease that affects at least 10% of the adult population, and the prevalence increases linearly with age.[1,2] OSA is characterized by repetitive upper airway collapse during sleep, leading to multiple physiological perturbations, including intermittent hypoxia (IH), sleep fragmentation, episodic hypercapnia and increased negative intrathoracic pressure swings.[3] Based on cross-sectional, observational and longitudinal studies, OSA has been defined as an independent risk factor for cardiovascular and cerebrovascular diseases. [4] Clinical and experimental studies indicate that among the cardiometabolic complications of OSA, IH is the most detrimental perturbation.[5,6] Chronic IH (CIH) during sleep induces sympathetic overactivation, oxidative stress, systemic inflammation, insulin resistance and disruption of lipid metabolism, all of which contribute to the development of early and late cardiovascular remodelling, including increased endotelial dysfunction, elevated blood pressure, increased carotid intima-media thickness (IMT), arterial stiffness, accelerated progression of atherosclerosis and cardiac alterations.[4,5,7–11]

A higher prevalence of OSA has been reported in patients aged >65 years than that in subjects aged 30–65 years.12,13 Nonetheless, the aforementioned animal and human experimental IH studies have been exclusively performed in young, healthy adult rodents and humans, and the effect of CIH on aged experimental subjects has not yet been fully explored. The present study aimed to assess the role of ageing in the early cardiovascular structural remodelling induced by CIH in a murine model of OSA. The findings reported here suggest that the detrimental cardiovascular effects induced by CIH are modulated by age, with older mice exhibiting lower susceptibility. In addition, accelerated cardiovascular ageing may be enhanced by CIH in young mice, thus inducing a decline in structural integrity that normally occurs during the natural chronological ageing process.

MATERIALS AND METHODS

Study design

Process The study was approved by the Ethical Committee for Animal Research of the University of Barcelona and performed on pathogen-free C57BL/6 female mice (Charles River Laboratories, Saint Germain sur L'arbresle, France). The animals were housed in standard cages in a temperature- and light-controlled room (25ºC, 12-h light/12-h dark cycles) with food and wáter freely available. Twenty young (2 months old) and 20 old (18 months old) animals were randomly assigned to IH or normoxia (N) conditions.

IH was achieved by varying the nitrogen and oxygen concentrations in the mouse cages (26-cm long, 18-cm wide and 6-cm high) via automated, computercontrolled gas exchange systems as previously described.14 For 8 weeks, 60 hypoxic events/h (40 s of room air at 20% O2 and 20 s of hypoxic air at 5% O2) corresponding to criteria attributable to severe OSA were applied to the mice for 6 h/day during the light period (10:00–16:00 h, corresponding to the usual sleep/rest period of mice). The mice experienced cyclic changes in oxygen saturation levels (SaO2) ranging from a maximum of 95.4 0.1% to a minimum of 62.3 3.5%, as previously described.15 Control mice breathing normoxic gas were placed in an identical system, but the hypoxic gas from the reservoir was replaced by room air.

Histomorphological analysis

The midthoracic aorta and left ventricle of the heart were perfused with PBS, fixed with 4% paraformaldehyde and embedded in paraffin for further histological analyses by an investigator who was blinded to the experimental groups. The samples were stained with haematoxylin–eosin (HE; Master Diagnostica, Granada, Spain) for IMT measurement, Gomori's Trichrome (Artisan Link Special Staining System; DAKO, Glostrup, Denmark) to visualize fibrosis and Alcian blue (Alcian blue 2.5; Bio-Optica, Milan, Italy) for mucoid deposition detection. For each measurement, images from four consecutive sections were processed using ImageJ (NIH, Bethesda, MD, USA) and Adobe Photoshop CS6 (Adobe Systems Incorporated, San José, CA, USA) software. Images of all stained sections were captured with a digital microimaging network instrument (Leica-DMD-108, Leica Microsystems, Wetzlar, Germany), and aortic autofluorescence (Figure S1, Supplementary Information) was visualized using a fluorescence microscope (Olympus-BX51, Olympus, Tokyo, Japan). The histomorphometric analyses were performed following previously published methods,11 which are explained Appendix S1 (Supplementary Information).

Statistical analysis

Continuous variables are summarized as the mean±SE. Depending on the normality and variance homogeneity, analysis of variance and t-tests or Mann– Whitney U-tests were performed. The effects of IH and age on cardiovascular remodelling were assessed using linear regression models. In addition, the structural parameters were adjusted for animal body weight. Principal component (PC) analysis (PCA) was performed using all the variables analysed in the study, and the experimental conditions and age were included as supplementary variables. Furthermore, an agglomerative hierarchical clustering procedure was performed on the results from the PCA to identify different clusters of the animals. The data analysis was performed using R software (R Core Team 2017, Version 3.4.2, Vienna, Austria).

RESULTS

Effects of age and IH on animal body weight

morphological remodelling

At baseline, no significant differences in body weights emerged across all studied groups matched by age. After 8 weeks, the young-IH mice weighed significantly less than the age-matched controls exposed to N (mean: 21.3±0.3 g young-N vs 19.8±0.3 g young-IH; P = 0.005). In contrast, the body weights of the old mice were not affected by CIH exposure (mean: 24.8±0.6 g old-N vs 23.9±0.5 g old-IH; P = 0.4) (data not shown).

Effects of age and IH on cardiac morphological remodeling

Cardiac hypertrophy

The heart weight-to-body weight ratio was significantly higher in the young-IH mice than that in their agematched controls (mean: 4.2±0.04 young-N vs 4.4±0.05 young-IH; P = 0.038). The heart weight-to-body weight ratios in the old mice were similar after IH or N exposure (mean: 4.36± 0.1 old-N vs 4.45±0.1 old-IH; P = 0.33). The differential effect of IH on cardiac hypertrophy between young and old mice did not reach statistical significance (Table S1, Supplementary Information).

Cardiac perivascular fibrosis

Figure 1A shows representative images of the cardiac tissues stained with Gomori's trichrome staining. Young- IH mice exhibited an increased positive area of fibrotic tissue in the adventitia of the coronary arteries and arterioles (perivascular fibrosis) compared to

young-N mice (mean: 0.5±0.1% young-N vs 1.2±0.1% young-IH; P < 0.001). Perivascular fibrosis in the old-IH mice was similar to that in the control old mice (1.07±0.1% old- N vs 1.2±0.1% old-IH; P = 0.27; Fig. 1B). The differential effect of IH on cardiac perivascular fibrosis between young and old mice was significant, suggesting that the effect of CIH on cardiac perivascular fibrosis is agedependent (Table S1, Supplementary Information).

Effects of age and IH on vascular

Intima-media thickness

Morphometric analysis of the IMT showed aortic wall thickening in the young-IH mice (mean: 49.2±1 μ myoung-N vs 52.8 _ 0.6 µm young-IH; P = 0.02; Fig. 2A,B). However, no significant differences in the IMT were found between the old-IH mice and corresponding age-matched controls (64.9±2.7 µm old-N vs 62.3±2.4 µm old-IH; P = 0.35). The linear regression model showed that ageing led to intima-media thickening (P < 0.001) (Table S1, Supplementary Information).

Elastin network alterations

Young-IH mice exhibited elastin fibre disorganization (mean: 2.1±1.2% young-N vs 11.3±3.5% young-IH; P = 0.02) and fragmentation (mean: 4.5±0.8 breaks young-N vs 7±0.3 breaks young-IH; P = 0.03), and a greater area without fibres (mean: 0.6 _ 0.16% young-N vs 1.2 _ 0.1% young-IH; P = 0.045). In contrast, CIH did not induce any significant alterations in elastin in aged aortas (Fig. 2C,D). The differential effect of IH on elastin fibre alterations between young and old mice did not reach statistical significance (Table S1, Supplementary Information). Therefore, the loss of elasticity of the aortic wall observed after 8 weeks of IH was partially attenuated by ageing.

Mucoid deposition

Alcian blue staining revealed greater mucoid deposition in the interlaminar space of the aortic wall in the young-IH mice (mean: 8.4±0.5% young-N vs 10.8±0.4% young-IH; P = 0.045), especially in áreas surrounding the aortic lumen (representative images
are shown in Fig. 3A; positive blue quantification in Fig. 3B). Mucoid deposition in the aortic wall in the old-IH mice was similar to that in the old mice exposed to N (mean: 9.04 _ 0.8% old-N vs 9.18 _ 0.5% old-IH; P = 0.53). The differential effect of IH on mucoid depositio between young and old mice assessed by the linear regression model showed a P-value of 0.08 (Table S1, Supplementary Information).

Vascular fibrosis

The percentage of fibrotic aortic tissue was higher in the young-IH mice (mean: 1.4±0.3% young-N vs 4.5±0.4% young-IH; P = 0.001), suggesting induction of collagen synthesis during IH exposure. In contrast, the percentage of collagen was similar between the old-IH mice and their age-matched controls (mean: 4.53±0.4% old-N vs 4.33±0.6% old-IH; P = 0.53) (Fig. 3A,C). A significant differential effect of IH on aortic fibrosis between young and old mice emerged, suggesting that the effect of IH on aortic fibrosis is agedependent (Table S1, Supplementary Information).

IH as an early promoter of cardiovascular ageing

After unsupervised PCA, different groups were defined according to the characteristics of cardiovascular remodelling. As shown in Figure 4A, all samples from young-N mice were completely separated from those of young-IH mice in the combination of coordinates as demonstrated by the two PC scores that accounted for 27.7% (PC1) and 22.8% (PC2) of the total variance. In fact, the young mice exposed to IH were localized in the same plane of dimension 1 as the old mice (displaced to the right in PC1) that were exposed to either N or IH, both of which were located in the same dimension of the plane. The PC1 was explained mainly by cardiac and aortic fibrosis, which is known as the principal biological process associated with natural chronological cardiovascular ageing.16 These findings suggest possible premature cardiovascular ageing in the young mice exposed to CIH. In addition, ascending hierarchical classification of the animals was performed to visualize different clusters (Fig. 4(B)). Consistent with the unsupervised PCA, old-N and old-IH mice co-localized in the same cluster (cluster 3), suggesting a similar level of cardiovascular remodelling and a neutral effect of IH on aged tissues. In contrast, young-IH mice localized in a different cluster (cluster 2) than their age-matched normoxic controls (cluster 1). On the basis of these findings, the effect of CIH on cardiovascular remodelling is clearly age-dependent (Fig. 4).

DISCUSSION

In the present study, we show that age modulates the deleterious cardiovascular effects associated with CIH mimicking very severe OSA. In young mice (2 months old), 8 weeks of CIH exposure induced early structural remodelling in cardiovascular elements. In contrast, older mice (18 months old) exposed to the same IH protocol showed no evidence of

increased cardiovascular remodelling compared to their respective age-matched controls. Notably, the young and late middle-aged mice are equivalent to ~20-year-old and 60–65-year-old humans, respectively.[17] PCA and ascending hierarchical classification of the animals produced differential clusters of the experimental groups according to morphological cardiovascular characteristics. Following IH, the young mice showed cardiovascular alterations similar to those in the older mice, suggesting that early cardiovascular ageing was induced by CIH.

Prior studies have shown that isolated CIH promotes autonomic nervous system deregulation characterized by both sympathetic hyperactivity and vagal withdrawal, promoting the occurrence of augmented systemic blood pressure values, oxidative stress, systemic and tissue inflammation and metabolic dysregulation, all of which can increase susceptibility to end-organ injury.[18–22] Sustained CIH can therefore induce pathological changes in the structural integrity of cardiovascular tissues, leading to declines in function and reparative capacity and thus resulting in increased cardiovascular-related morbidity and mortality. [23] Our current findings show progressive proatherogenic vascular remodelling and early cardiac remodelling induced by CIH exposure in the absence of additional risk factors such as high-fat, high-sugar diet consumption or genetically induced cardiovascular disease propensity. Following CIH, increased IMT, elastic fibre fragmentation and disorganization, and collagen and mucoid accumulation in the aortic wall were observed in the young mice, similar to our previous study findings, although these features normalized after a period of normal breathing under normoxic conditions. [11] These structural changes are accompanied by loss of the elasticity equilibrium in the aortic wall and therefore promote arterial stiffness, increased blood pressure and atherogenic remodelling.[24,25] In heart tissues, we observed cardiac hypertrophy and increased perivascular fibrosis after CIH, both of which can reduce cardiac output. However, all of these cardiovascular outcomes following CIH occurred in young mice but were conspicuously absent in old mice. Recently, however, macro- and micromechanical properties of the left ventricular myocardium extracellular matrix have been shown to be affected by severe OSA similarly among young and aged mice.[26] Therefore, further indepth investigation of the role of age in the impact of IH at the cardiac level is necessary.

These findings concur with those reported by Quintero et al.[27] who showed that ageing played a protective role against the deleterious effects of CIH.[27] Indeed, these investigators reported that older age wasassociated with improved redox status and redoxresponses to CIH in older rats compared to younger animals, which may account for the relative protection against vascular deficits observed here. In the context of extrapolating the current findings to human disease, we postulate that the absence of cardiovascular remodelling events caused by CIH in old mice may at least partly correspond to the lower mortality rates observed in OSA patients >50 years of age[28–33] and the higher mortality rates reported in younger subjects with severe OSA compared to those in the general population.34

The cardiovascular response to CIH depends on the severity, frequency and duration of IH exposure and generally progresses from adaptation to maladaptation.[5,35–37] Moderate IH has been associated with a beneficial response at the cardiovascular level,[38] including a protective or ischaemic preconditioning effect, while severe CIH exacerbates injury.[35,39] Here, we studied a long-term severe pattern of IH simulating severe OSA. However, lower hypoxia severity due to a higher oxygen fraction during hypoxic events or a shorter duration of such events may have different impacts on young mice, which may show an adaptive response to hypoxic stress with attenuated cardiovascular remodelling. Cardiovascular remodelling induced by moderate IH should be explored at different ages since clinical data suggest that older patients with moderate OSA may have reduced mortality compared to the general population,[33] attesting to a potential preconditioning effect of mild CIH.[40]

Ascending hierarchical classification of mice based on PCA of cardiovascular morphometric characteristics revealed three distinct clusters. On one hand, old mice in the normoxia and CIH conditions showed similar distributions in the analysis dimensions and were defined within the same cluster. On the other hand, young-IH mice were defined as a different cluster separate from their respective age-matched controls and were positioned in the same dimension as the old mice, suggesting similar cardiovascular remodelling to that observed during normal chronological ageing.

This finding led us to postulate that IH may induce age-dependent premature cardiovascular ageing. Indeed, Douglas and Haddad[23] reviewed data from clinical and

experimental studies on the molecular and cellular mechanisms involved in responses to IH and the potential interplay among various pathways that may accelerate the ageing process. More specifically, factors that accelerate ageing may include altered glucose homeostasis, dyslipidaemia and induction of proatherogenic inflammatory mediators and adhesión molecules that are mediated by increased insulin resistance. More recently, Gaspar et al. proposed that OSA may precipitate/aggravate ageing by inducing celular and molecular impairments that characterize senescence, such as stem cell exhaustion, telomere attrition, genomic instability, nutrient sensing dysregulation, loss of proteostasis, mitochondrial dysfunction, selective cellular senescence and epigenetic changes.[41] The assumptions and our current findings support the concept that CIH can influence the biological cardiovascular clock to promote premature vascular and cardiac ageing that may predispose OSA patients to cardiovascular disease.

Our study has several limitations. First, althoughrecurrent apnoea in OSA patients results in IH, hypercapnia, sleep fragmentation and changes in intrathoracic pressures, all of which may contribute to cardiovascular remodelling, our study mainly focused on the CIH effects, although concurrence of sympathetic activation and sleep fragmentation should not be ruled out.[42] However, IH has clearly emerged as the predominant pathophysiological component of OSA underlying cardiovascular alterations, which constituted the main outcome of the present study. Second, we focused on the cumulative organ damage end point at the cardiovascular level and did not evaluate the blood pressure trajectories or any of the putative molecular intermediaries. However, such work has already been conducted in a previous study,[27] which showed a lack of both augmented sympathetic tone and oxidative status in aged animals as an intermediary mechanism accounting for protection against the deleterious effects produced by CIH in young animals. Based on the potential adverse effects of the interferences required for such assessments on the outcomes of interest in the present study, we omitted intermediate end points from our protocol. Finally, we implemented a very severe hypoxia/reoxygenation challenge mimicking severe OSA (a subpopulation of 2–5% of all OSA patients), but this study did not address situations where the level of IH is either mild or moderate. However, it is of note that as a result of the inter-species differences in O2 dissociation curves, lower oxygen saturation levels are required in mice to elicit an

equivalent reduction in oxygen partial pressure among humans.43 Given the dichotomous effect of CIH,[39] future studies investigating the impact of IH at different ages according to the severity of hypoxia and the experimental protocol are needed. The main strengths of our work include the use of older mice that were 18 months of age and the use of a conventional mouse strain, which allowed us to assess the cardiovascular consequences associated with IH exposure without other confounding factors or co-morbidities. In addition, we used female mice rather than male mice due to the absence of relevant studies in female mice and the late emergence of sexually dimorphic cardiovascular outcomes with increased susceptibility in women.[44–46]

In conclusion, the detrimental cardiovascular effects induced by long-term severe IH are modulated by age, with older mice exhibiting lower susceptibility. An accelerated decline in cardiovascular structural integrity with characteristics strikingly similar to those observed during the natural ageing process was observed in young mice exposed to CIH, suggesting premature cardiovascular ageing. Regarding the clinical and translational relevance of our current findings, we propose that young adult subjects with OSA will be predisposed to premature ageing, reinforcing the importance of early diagnosis and treatment, especially considering that the reversibility of vascular disease following CIH may be suboptimal at best.[7,47] Future studies are needed to elucidate the cellular and molecular changes associated with CIH-induced cardiovascular ageing to specifically identify putative therapeutic targets.

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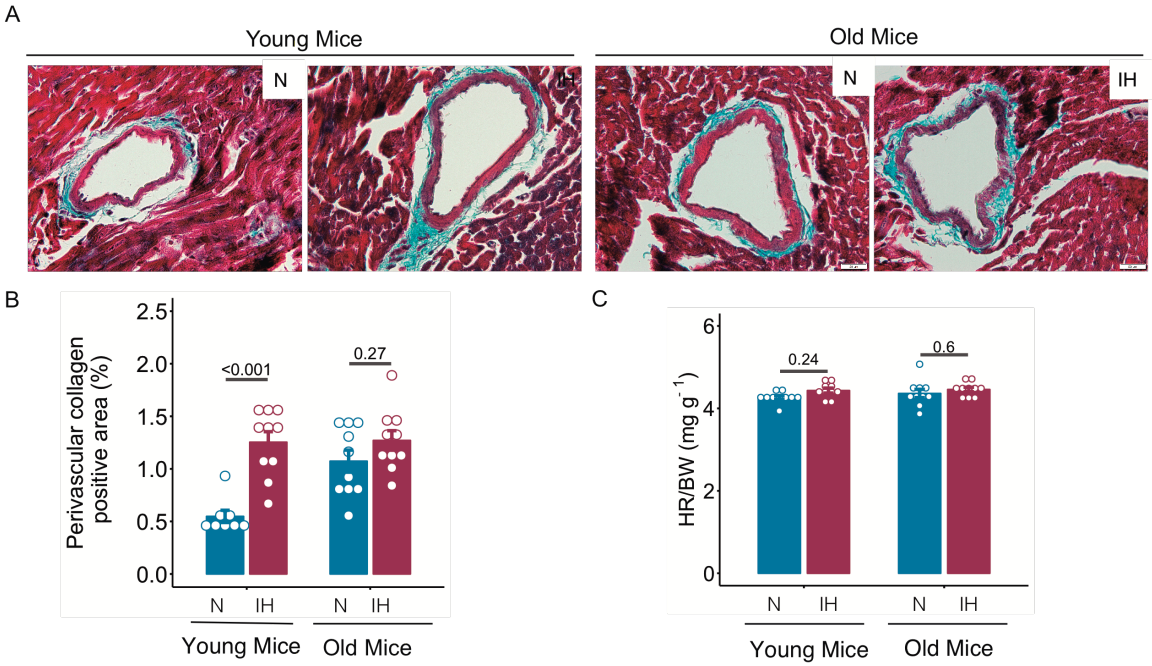
Author contributions: Conceptualization: M.S.T., I.A. A.C.G., R.F., J.M.M., M.D., D.G. Data curation: A.L.C.-G., MSG, M.T., I.B. Formal analysis: A.C.G., I.B., M.S.T., I.A., R.F., J.M.M., D.G.

Funding acquisition: M.S.T., M.D., I.A., R.F. Investigation: A.C.G., M.S.T., M.S.G., I.B., M.T., I.A., R.F., J.M.M., M.D., D.G. Methodology: A.C.G., M.S.T., M.S.G., I.B., M.T., I.A., R.F., J.M.M., M.D., D.G. Project administration: M.S.T., I.A., R.F., J.M.M. Resources: M.S.T., I.A., R.F., J.M.M. Supervision: M.S.T., I.A., R.F., J.M.M., D.G. Visualization: A.C.G., M.S.T., I.B., M.D. Writing—Original draft: A.C.G., M.S.T., M.S.G., I.B., M.T., I.A., R.F., J.M.M., M.D., D.G. Writing—review and editing: A.C.G., M.S.T., M.S.G., I.B., M.T., I.A., R.F., J.M.M., M.D., D.G.

Abbreviations: CIH, chronic IH; CV, cardiovascular; IMT, intimamedia thickness; IH, intermittent hypoxia; OSA, obstructive sleep apnoea; PBS, phosphate-buffered saline; PC, principal component; PCA, PC analysis.

FIGURES

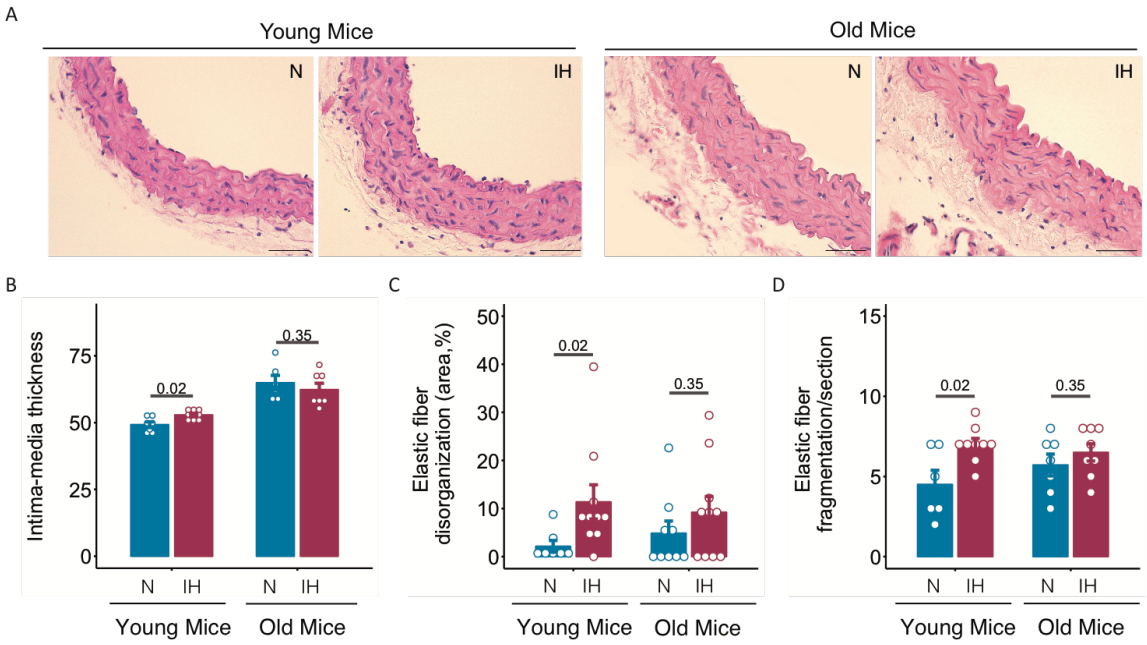
Figure 1 Effects of age and intermittent hypoxia (IH) on cardiac morphological remodelling.



Female C57BL/6 mice aged 2 months (young mice) and 18 months (old mice) were exposed to room air (normoxia, N) or IH for 8 weeks (n = 10 per group).

(A) Representative images of Gomori's trichrome stain to measure cardiac perivascular fibrosis (collagen in green; magnification: Å~400, scale bar: 50 µm). (B) Box-plot representation of the positive area of fibrotic tissue adjusted by the total area (shown as %). (C) Box-plot representation of cardiac hypertrophy measured as the heart weight-to-body weight ratio (HR/BW, expressed as mg/g). Values are the mean±SE. P-values represent intergroup comparisons between the IH (red bar) versus N (blue bar) conditions.

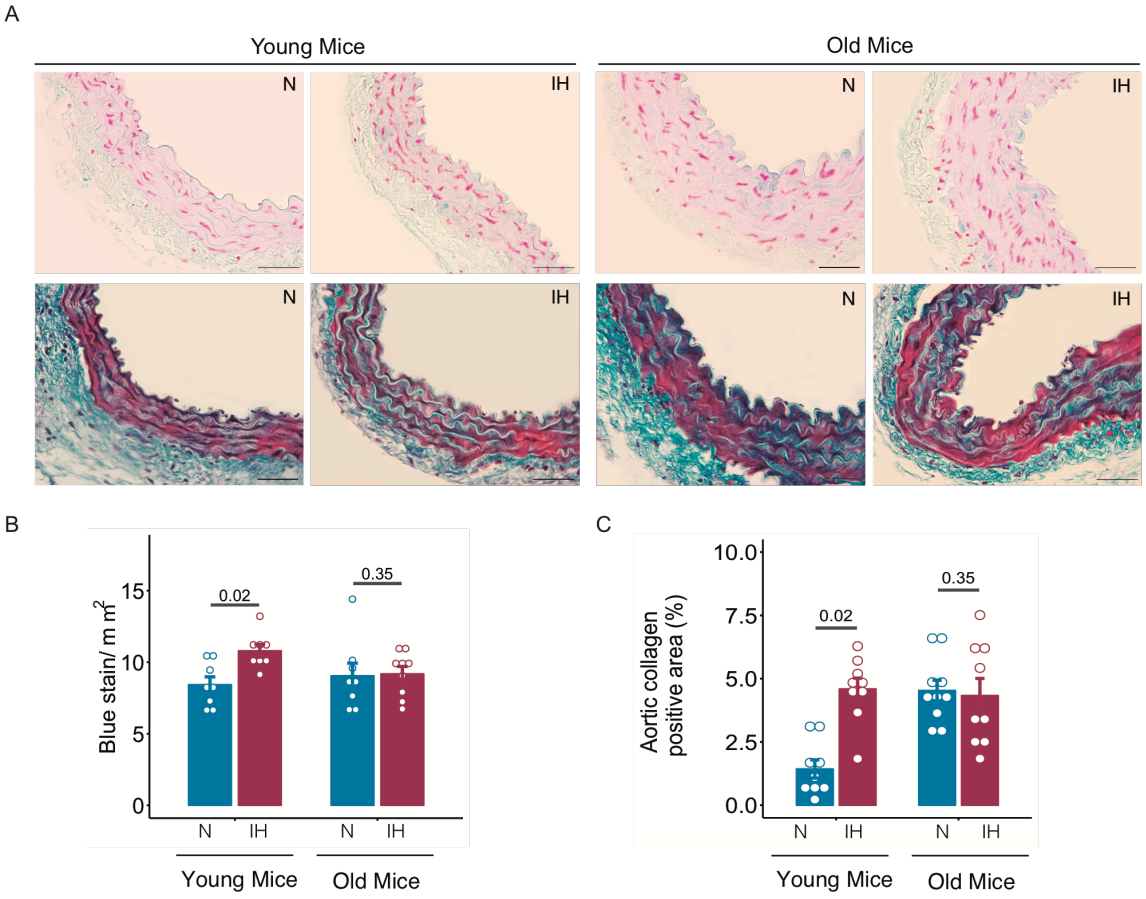
Figure 2 Effects of age and intermittent hypoxia (IH) on vascular morphological remodelling.



Female C57BL/6 mice aged 2 months (young mice) and 18 months (old mice) were exposed to room air (normoxia, N) or IH for 8 weeks (n = 10 per group).

(A) Representative images of the aortic wall with haematoxylin and eosin (HE) staining of the midthoracic aorta in each group (magnification: Å~400, scale bar: 50 µm). (B) Box-plot representation of histomorphometric analysis of the intima-media thickness (IMT, shown as µm). (C) Box-plot representation of the area of elastin fibre disorganization in the aortic wall adjusted by the total area (shown as %). (D) Box-plot representation of the number of breaks in the intima-media elastic fibre per section. The values are the mean±SE. P-values represent intergroup comparisons between the IH (red bar) versus N (blue bar) conditions.

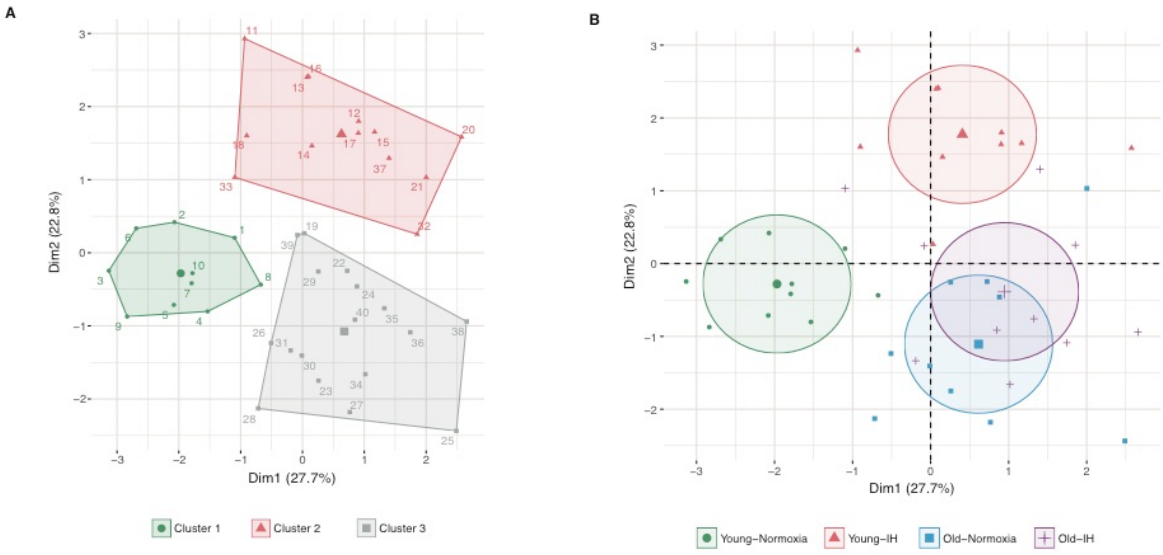
Figure 3. Effects of age and intermittent hypoxia (IH) on vascular extracellular matrix remodelling.



Female C57BL/6 mice aged 2 months (young mice) and 18 months (old mice) were exposed to room air (normoxia, N) or IH for 8 weeks (n = 10 per group).

(A, top) Representative images of Alcian blue staining from the midthoracic aorta to detect aortic wall mucoid deposition (shown in blue). (A, bottom) Representative images of the collagen-positive area of the intima-media (shown in green; magnification: Å~400, scale bar: 50 µm). (B) Box-plot representation of the total blue density to the total aortic wall area (µm2). (C) Box-plot representation of the positive area of fibrotic tissue adjusted by the total aortic wall area (shown as %). The values are the mean±SE. P-values represent intergroup comparisons between the IH (red bar) versus N (blue bar) conditions.

Figure 4. Intra-population analysis of cardiac and vascular parameters.



(A) Principal component analysis (PCA) shows the experimental groups plotted in two-dimensional space and represented by single colour-coded spheres: young mice exposed to normoxia (N) (green) and intermittent hypoxia (IH) (red), and old mice exposed to N (blue) and IH (violet). (B) Hierarchical clustering analysis revealed three different clusters based on cardiovascular characteristics: young-N (cluster 1,green), young-IH (cluster 2,red) and old-N and old-IH (cluster 3,grey).

SUPPLEMENTARY INFORMATION

TITLE:

Effect of age on the cardiovascular remodeling induced by chronic intermittent hypoxia as a murine model of sleep apnea

Authors' full names:

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Appendix S1- Methods

Histomorphological analyses

The histomorphometric analyses were performed following previously published methods (Chest. 2016 Jun;149(6):1400-8).

Intima-media thickness. The cross-sectional IMT was quantified by morphometric analysis of the H&E-stained sections (300 measurements for each animal).

Elastic fiber network analysis. The elastic fiber network analysis was performed using the autofluorescence of the elastin fibers. The elastin disruption was defined as a complete fragmentation of one elastic fiber and shown as the number of breaks. Additionally, the distance s between both ends of a fragmented fiber were quantified (adjusted by the total aortic wall area and shown as a % of space without fiber). In addition, we quantified the % of area with elastic fiber disorganization based on the inability to count the amount of organized elastic fiber and adjusted the % of disorganization by total wall area.

Figure S1. Elastin fiber disruption and fragmentation.

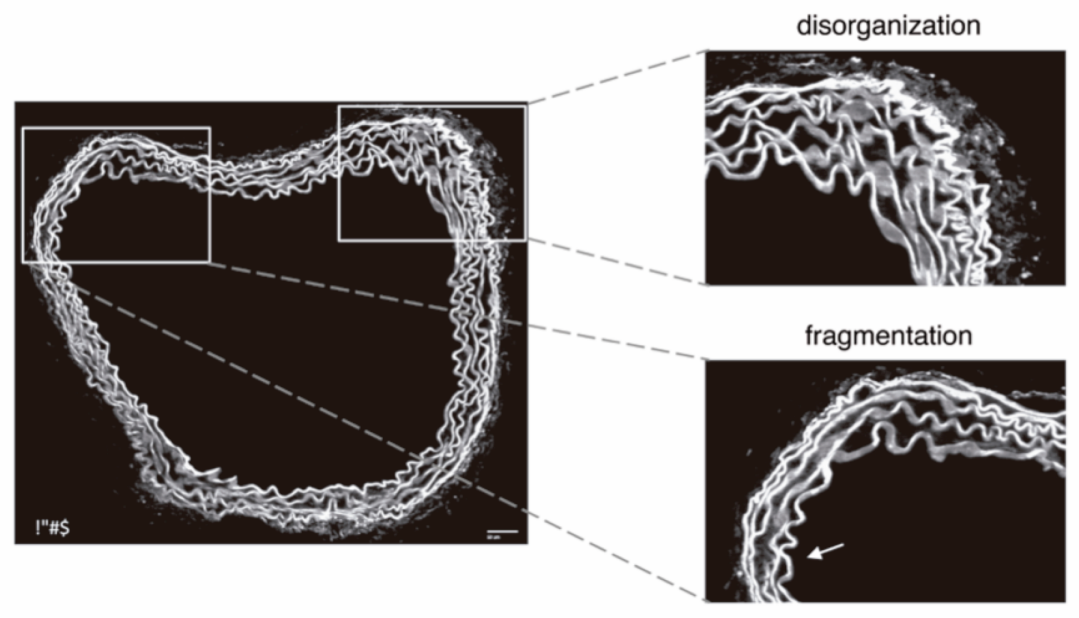

Figure legend: Representative pictures of the elastic network (original magnification _100), revealed by autofluorescence, with magnification of zoom elastic fiber disorganization (top inset) and fragmentation (bottom inset, with arrows showing fragmented elastic fiber end points).

Gomori's trichrome stain. Gomori's trichrome stain is a one-step trichrome stain that provides distinctive red muscle fibers and green collagen staining. Gomori's trichrome stain was used to detect fibrosis in the aortic wall and cardiac tissue, focusing on the perivascular fibrosis. The fibrotic tissue was quantified by measuring the positive collagen area adjusted to the total tissue area.

Alcian blue staining. The integrated density of the blue staining was quantified and adjusted

to the corresponding total wall area to detect mucoid deposition in the aortic wall.

Appendix S2- Results

Table S1. Effects of intermittent hypoxia, age, and the interaction between intermittent hypoxia and age on cardiovascular remodeling assessed by linear regression models

	Intercept			Effect of IH			Effect of age			Effect of IH and age		
Variables	Estimate	Std. Error	p value	Estimate	Std. Error	p value	Estimate	Std. Error	p value	Estimate	Std. Error	p value
Body weight, g	21	0.4	7e-34	-1.5	0.6	0.025	3.5	0.6	3.8e-06	0.6	0.9	0.51
Heart weight, mg	12	13	0.36	3.7	0.6	4.6e-07	2.5	2.5	0.31	4.2	3.1	0.19
HW/BW, $mg \cdot g^{-1}$	4.3	0.06	3.2e-38	0.17	0.1	0.087	0.1	0.1	0.31	-0.074	0.1	0.59
Cardiac F, %	0.55	0.1	6 E-06	0.71	0.1	1.1e-05	0.53	0.1	0.001	-0.51	0.2	0.011
IMT, μm^2	35	17	0.046	4.6	2.6	0.092	14	3.4	0.001	-7.3	3.8	0.063
EFD, %	2.1	3.4	0.54	9.2	4.5	0.047	2.7	4.6	0.55	-4.9	6.1	0.43
EFF/section	4.5	0.6	3.5e-07	2.5	0.8	0.007	1.2	0.9	0.19	-1.7	1.2	0.17
AWF, %	0.64	0.1	0.001	0.63	0.2	0.008	0.13	0.2	0.59	-0.42	0.3	0.18
Aortic F, %	1.4	0.4	0.005	3.2	0.6	6,00e-05	3.1	0.6	5.6e-05	-3.4	0.9	0.001
MD, %	8.4	0.6	5.1e-14	2.4	0.8	0.012	0.62	0.8	0.49	-2.2	1.2	0.08

Table legend: g, grams; mg, milligrams; HW/BG, heart rate-to-bodyweight ratio; F, fibrosis; IMT, intima-media thickness; EFD, elastin fiber disorganization; EFF, elastin fiber disorganization; AWF, area without fiber; MD, mucoid deposition.

Estudio 3

"Age modulates cardiovascular gene expression response to chronic intermittent hypoxia modeling sleep apnea"

Anabel L. Castro-Grattoni, Monique Suarez-Giron, Ivan Benítez, Marta Torres, Lourdes Tecchia, Fernando Snatamaria, Isaac Almendros, Ramon Farré, Josep M. Montserrat, Mireia Dalmases, David Gozal, Manuel Sánchez-de-la-Torre; on behalf of the Spanish Sleep Network. (Submitted)

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The effect of chronic intermittent hypoxia in cardiovascular gene expression is modulated by age in a mice model of sleep apnea

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Key words: sleep breathing disorders; obstructive sleep apnea syndrome; intermittent hypoxia; cardiovascular; age
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ABSTRACT

Study Objectives: Chronic intermittent hypoxia (CIH) is a major determinant in obstructive sleep apnea cardiovascular morbidity and this effect is influenced by age. The objective of the present study was to assess the differential molecular mechanisms at gene-level expression involved in the cardiovascular remodeling induced by CIH according to chronological age.

Methods: Two- and 18-month-old mice (N = 8 each) were subjected to CIH or normoxia for 8 weeks. Total mRNA was extracted from left ventricle myocardium and aortic arch, and gene expression of 46 intermediaries of aging, oxidative stress, and inflammation was measured by quantitative real-time PCR.

Results: Cardiac gene expression of Nrf2 (2.05-fold increase, $p < 0.001$), Sod2 (1.9-fold increase, $p = 0.035$), Igf1r (1.4-fold increase, $p = 0.028$), Mtor (1.8-fold increase, $p = 0.06$), Foxo3 (1.5-fold increase, $p = 0.020$), Sirt4, Sirt6, and Sirt7 (1.3-fold increase, $p = 0.012$; 1.1-fold change, $p = 0.031$; 1.3-fold change, $p = 0.029$) was increased after CIH in young mice, but not in old mice. In aortic tissue, eNOS was reduced in young mice ($p < 0.001$), Nrf2 was reduced in 80% ($p < 0.001$) in young mice and 45% ($p = 0.07$) in old mice, as its downstream antioxidant target Sod2 (82% reduced, $p < 0.001$). IL33

Conclusions: CIH effect in gene expression is organ-dependent, and is modulated by age. CIH increased transcriptional expression of genes involved in cardioprotection and cell survival in young, but not in old mice. In aortic tissue, CIH reduced gene expression related to an antioxidant response in both young and old mice, suggesting vascular oxidative stress and a proaging process. distinctive red muscle fibers and green collagen staining. Gomori's trichrome stain was used to detect

ABBREVIATION LIST

AngII: angiotensin II

BNP: natriuretic peptide type B

CIH: chronic intermittent hypoxia

CPAP: continuous positive air pressure

eNOS: endothelial nitric oxide synthase

Foxo3: forkhead box O3

Gdf15: growth differentiation factor 15

IH: intermittent hypoxia

Igf1r: insulin-like growth factor 1 receptor

iNOS: nitric oxide synthase 2, inducible

Mtor: mammalian target of rapamycin

Icam1: intercellular adhesion molecule

IMT: intima-media thickness

Il10: Interleukin-10

Il33: interleukine-33

Il1b: interleukin 1 beta

Mcp1: chemokine C-C motif ligand

N: normoxia

Nfkb: nuclear factor-κB

Nrf2: nuclear factor erythroid-2

OSA: obstructive sleep apnea

p53: tumor protein p5

ROS: reactive oxygen species

Sod2: superoxide dismutase 2

Tnfa: tumor necrosis factor a

Vcam1: vascular cell adhesion molecule 1

INTRODUCTION

Obstructive sleep apnea (OSA) is a highly prevalent breathing disorder that affects at least 10% of the adult population, and the prevalence increases with age [1, 2]. OSA is characterized by recurrent episodes of the complete or partial collapse of the upper airway during sleep, which results in cyclic events of arterial and tissular hypoxemia/reoxygenation of varying severity [3, 4]. The recurrence of these hypoxic and reoxygenation episodes produces a characteristic pattern of nocturnal intermittent hypoxia (IH).

Based on cross-sectional, observational, and longitudinal studies, OSA has been defined as an independent risk factor for cardiovascular and cerebrovascular diseases [5]. Moderate-tosevere OSA is associated with an increased risk for cardiovascular disease, including hypertension, atherosclerosis, coronary heart disease, stroke, and heart failure [6–10]. Moreover, sleepdisordered breathing is common in patients with cardiovascular diseases and is a significant predictor of coronary artery disease [11].

There is now ample experimental and epidemiological evidence that chronic exposure to IH (CIH) increases the cardiovascular morbidity and mortality of patients with OSA [12, 13]. Experimental animal models of IH mimicking the cyclic events of hypoxemia-reoxygenation have uncovered the mechanisms underlying the increased risk for cardiovascular pathology in OSA patients [14]. Systemic inflammation and oxidative stress induce hemodynamic alterations and maladaptive transcriptional regulation, with increased cell adhesion molecules, endothelial dysfunction, thrombotic factor activation, and vascular remodeling, all factors contributing to atherosclerotic risk and consequent cardiovascular morbidity and mortality [14, 15].

Although changes in blood oxygen saturation induced by IH is similar in young and aged mice [4], it has been shown that the effect of CIH in the cardiovascular system is modulated by age [16, 17]. Cardiovascular remodeling induced by CIH is more pronounced in younger female animals, and such changes resemble chronological age-related declines in cardiovascular structural integrity. However, the underlying mechanisms of CIH-induced cardiovascular injury are not well documented. In the present study, we assessed the differential molecular intermediates that delineate the cardiovascular

remodeling induced by CIH according to mice age, exploring specific molecules that promote or decrease oxidative stress, inflammation, and aging/ stress response.

MATERIALS AND METHODS

Study design

The study was approved by the Ethical Committee for Animal Research of the University of Barcelona and performed on C57BL/6 female mice (Charles River Laboratories, Saint Germain sur L'arbresle, France). The animals were housed in standard cages in a temperature- and light-controlled room (25°C, 12-h light/12-h dark cycles) with food and water freely available. Sixteen young (2-month-old) and 16 old (18-month-old) animals were randomly assigned to IH or normoxia (N) conditions. Each group of animals was placed in an experimental setting specially designed for normoxia or IH conditions.

Intermittent hypoxia

The system was based on a transparent methacrylate box (26 cm long, 18 cm wide, 6 cm high) flushed with air cyclically changing its oxygen content progressively (15 s) from the room air entrance (40 s) to a gas reservoir of hypoxic air at an oxygen fraction of 5% (20 s), mimicking a rate of 60 apneas/h, typical of severe OSA [18]. The mice experienced cyclic changes in oxygen saturation levels ranging from a maximum of 95.4% ± 0.1% to a minimum of 62.3% ± 3.5%, as previously described. Control mice breathing normoxic gas were placed in an identical system, but the hypoxic gas from the reservoir was replaced by room air at 21%. Both exposures were applied for 6 h/ day during the light period (10:00 am–16:00 pm, corresponding to the usual sleep/rest period of mice and mimicking OSA) for 8 weeks, with food and water being unrestricted and freely available at all times [19].

Tissue samples processing and RNA isolation

At the end of the 8 weeks exposures, the animals were anesthetized and immediately sacrificed by exsanguination through the abdominal aorta, the aortic arch of the aorta and left ventricle of the heart were removed and stored at –80°C until the time of analysis. The same amount of tissue (30 mg) was simultaneously disrupted and homogenized using the TissueLyser, following the manufactured instruction. The total RNA was purified using the AllPrep DNA/RNA/Protein Mini kit (Quiagen, Hilden, Germany), according to the manufacturer's instructions.

cDNA synthesis and RT-PCR

DNA was synthesized from 100 ug RNA using high-capacity cDNA reverse transcription kit (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Subsequently, a target-specific preamplification step was performed using a pull of the [48] TaqMan Gene Expression Assays of interest and TaqMan PreAmp Master Mix (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA; see Supplementary Material for TaqMan Gene ID). Each gene was assessed in triplicate by RT-PCR, and 384-well plates were used by means of an Applied Biosystems 7900HT thermocycler, according to the manufacturer's instructions. A total of 24 plates (384-well) were used to measure the gene expression of 48 genes in triplicate (46 target genes and the two housekeeping genes GAPDH and b-actin), in the aorta and left ventricle samples from the 32 mice. TaqMan Gene Expression Assays IDs are specified in Supplementary Table S1.

Data analysis

Gene expression data (Ct) were normalized by the mean of the endogenous controls GAPDH and β-actin (ΔCt). The differences in gene expression between groups were evaluated using ANOVA and perform pairwise comparisons adjusting for multiple testing by Tukey method (detailed data in Supplementary Tables S1 and S2). The analysis of the data was done with the R software (R Core Team 2017, Version 3.4.2, Vienna, Austria).

RESULTS

Differential gene expressions after 8 weeks of CIH exposure in the IH group compared to the normoxic-breathing group were observed for both young and old mice. CIH downregulated four genes and upregulated nine genes in left ventricle myocardium from young mice (Figure 1A). In contrast, one gene was downregulated, and one gene was upregulated after CIH exposure in cardiac tissue from old mice (Figure 1B). In the aortic tissue, CIH downregulated the expression of 13 genes in young mice (Figure 2A), whereas two genes were downregulated in old mice (Figure 2B). Therefore, the impact of CIH was more evident in young mice both in the aorta and in left ventricle myocardium and it was tissue-specific. To evaluate the effect of CIH in the cardiac and vascular tissue according to age, we compared gene expression between young and old mice. The analysis was

focused on three key processes involved in cardiovascular remodeling, oxidative stress, inflammation, and hallmark of aging and stress-response genes.

Cardiac tissue

Oxidative stress

As shown in Figure 1A, gene expression related to an antioxidant response was increased in CIH young mice, including 2.05-fold increase ($p < 0.001 = 0.002$) in the transcription factor Nrf2 (nuclear factor erythroid-2) and 1.9-fold increase ($p = 0.035 = 0.005$) in its downstream antioxidant target Sod2 (superoxide dismutase 2). Moreover, a 1.6-fold increase ($p = 0.05$) in gene expression of eNOS (endothelial nitric oxide synthase) was evident in young mice exposed to CIH.

Inflammation

CIH markedly induced Il33 (interleukin-33) expression, with a 3-fold increase ($p < 0.001$), in both young and old mice exposed to CIH. The transcription rates of the master regulator of inflammation Nfκb (nuclear factor-κB), Tnfa (tumor necrosis factor-a), Il6 (interleukin-6), Il1b (interleukin-1 beta), Mcp1 (chemokine C–C motif ligand), Il10 (interleukin-10), Icam1 (intercellular adhesión molecule), and Vcam1 (vascular cell adhesion molecule1) remained unchanged in both age groups.

Hallmark of aginf and stress-esponse genes

After 8 weeks of CIH, the cardiac gene expression rate of p21 was reduced by 70% in young mice ($p = 0.003$). CIH induced in young mice an increase in factor Foxo3 (forkhead box O3, 1.5-fold change, $p = 0.020$), Sirt4 (1.3-fold change, $p = 0.012$), Sirt6 (1.1-fold change, $p = 0.031$), and Sirt7 (1.3-fold change, $p = 0.029$). Another pathway regulated by CIH in young mice was the nutrientsignaling pathways Igf1r (insulin-like growth factor 1 receptor; 1.4-fold increase, $p = 0.028$) and its downstream Mtor (mammalian target of rapamycin; 1.8-fold increase, $p = 0.06$). In the same direction, a reduction in gene expression of AngII (angiotensin II; ~25%), BNP (natriuretic peptide type B, ~60%), and Gdf15 (growth differentiation factor 15, ~35%) was observed after CIH in both young and old mice (Figure 1).

Vascular tissue

Oxidative stress

In the aortic tissue, genes related to an antioxidant response were reduced in young (Figure 2A), but not in old mice (Figure 2B) exposed to CIH. The transcription factor Nrf2 was reduced in young mice by 80% ($p < 0.001$), but not in old mice. The antioxidant downstream Sod2 was reduced after CIH in young mice (82% reduced, $p = 0.001$) and old mice (70%, $p = 0.025$). Moreover, young mice under CIH conditions showed a decrease in eNOS and iNOS (nitric oxide synthase 2, inducible) gene expression of 85% ($p < 0.001$) and 85% ($p = 0.003$), respectively (Figure 2A).

Inflammation

As shown Figure 2A, young mice exposed to CIH showed a reduction in gene expression levels of key proinflammatory intermediaries: Il1b (90% reduced, $p = 0.028$), Il33 (84% reduced, $p = 0.001$), Mcp1 (80% reduced, $p = 0.085$), and the adhesion molecules Vcam1 (82% reduced, $p < 0.001$), and Icam1 (intercelular adhesion molecule, 85% reduced, $p < 0.001$), and Et1 (endothelin 1, 83% reduced, $p = 0.002$). However, this reduction of inflammation was not observed in old mice (Figure 2B).

Hallmark of aging and stress-response genes

Young mice exposed to CIH showed a reduction in mitocondrial Sirt3 (reduced 40%, $p = 0.04$) (Figure 2A), and VEGF (reduced %, $p = 0.02$). Old mice under CIH conditions showed a decrease in gene expression levels of p21 (78% reduced, $p = 0.001$) and p66shc (40% reduced, $p = 0.04$) (Figure 2B). A downregulation of Mef2a (myocyte enhancer factor 2A), Mtor, and Bcl2 (B cell leukemia/ lymphoma 2, $p = 0.02$) was observed in both young and old mice exposed to CIH compared to its normoxic age-matched controls.

DISCUSSION

In the present study, we showed that age modulates the molecular responses to CIH. In myocardial tissues from young mice, CIH induced upregulation of stress-response genes involved in cardioprotection and cell survival. However, this effect of CIH was not observed in aged mice. Conversely, CIH reduced genes involved in an antioxidant response and oxide nitric production in the aortic tissue from young and old mice, suggesting an increase of oxidative stress and vasoconstriction of the vasculature. Overall, our data suggest that the regulation of gene expression under CIH varies according to the organ and is modulated by age, being more evident in young mice, both in the aorta and in left ventricle myocardium. Our results suggest an adaptive response of cardioprotection under CIH conditions in young age, which is not evident in old age. Results are summarized in Figure 3.

OSA-induced IH increases reactive oxygen species (ROS) levels, leading to activation of transcription factors which, in turn, increases the expression of genes encoding proteins for hypoxia adaptation [20]. In addition, redox-sensitive transcription factors that elicit inflammatory pathways are also activated, affecting inflammatory and immune responses by the promotion of activation of endothelial cells, leukocytes, and platelets [21]. Once activated, those cells express adhesion molecules and proinflammatory cytokines that may lead to endotelial injury and dysfunction, and consequent development of cardiovascular morbidity [14, 22–25]. Prior studies have shown that pathological changes in the structural integrity of cardiovascular tissues induced by CIH are evident in young mice, but are conspicuously absent in old mice [16, 17]. Here, we assessed the differential molecular mechanisms that delineate the cardiovascular remodeling induced by CIH according to mice age, exploring intermediaries at a transcriptional level that promote or decrease aging, oxidative stress, and inflammation processes. We included both young and old mice whose ages correlate to 20 and 60–65 years old in humans, respectively [26].

It is known that the myocardial tissue has a low capacity for regeneration and repair after injury, thus it is susceptible to numerous stresses that lead to cardiomyocyte death [27]. Upon injury, the heart must adapt its function to the ever-changing workload demands. In the present study, we observed that under CIH conditions, young mice had increased gene expression of several signaling pathways that promote cardioprotection and cell survival,

which was not observed in aged mice. First, we observed an increase in gene expression levels of Nrf2, a master transcription factor with a critical function in the regulation of antioxidant defense genes, such as the enzyme Sod2 [28]. The increase in eNOS expression was also observed in young mice under CIH, an enzyme involved in coronary vasodilation by nitric oxide production and a promoter of several antistress, antioxidant, and antiapoptotic adaptations. These results are consistent with previous findings showing an antioxidant response to ROS generation under CIH stress [29–31]. Other targets that were upregulated by CIH in cardiac tissue from young mice were the transcription factor Foxo3, nuclear sirtuin6 and sirtuin7, and the mitochondrial sirtuin4. Both Foxo3 and sirtuins are involved in adaptive responses to the cellular environment; promoting the transcription of genes involved in DNA repair and stability, stress resistance, antioxidant, antiapoptotic, and antiaging actions [32–35]. The reduction in p21 levels suggests a decrease of senescence cell process, promoting cell survival. Moreover, we observed an increase in the Igf1r/Mtor pathway, which has antiapoptotic and prosurvival properties and mediates physiological heart growth, the normal growth of the heart from birth to early adulthood [36]. This is important for the maintenance of cardiac bioenergetics during stress conditions, promoting mitochondrial metabolism, and ATP production by increasing mitochondrial Ca2+ uptake and respiration [37]. The physiological growth as an adaptive response to the CIH stress was also linked with reduced levels of AngII and Gdf15 in young mice, and reduced expression of BNP in young and old mice, molecular intermediaries that promote pathological cardiac hypertrophy [38]. This is in agreement with previous findings that show a reduction in BNP and ANP gene expression in lean healthy mice exposed to 4-week IH (ref). The authors found an increased left ventricle contractibility and suggested adaptive or compensatory physiological changes promoted by IH. Finally, IL33 was increased by CIH in young and old mice, interleukin with cardioprotective effects mediated by its anti-hypertrophic and antiapoptotic properties [39]. These cardioprotective responses to CIH observed in the present study may explain, at least in part, the preconditioning-like effect that has been associated with CIH to protect the heart against ischemia/ reperfusion or hypoxia/reoxygenation-induced injury [40–42].

The cardiovascular morphological, cellular, and molecular responses to CIH are dependent on severity, frequency, and duration of IH stimulus, generally going from adaptation to maladaptation. Here, we studied a long-term severe pattern of IH (modeling severe OSA) and we observed a modulation by age and a specific response according to the tissue. Although we observed an adaptive and cardioprotective response to CIH at the myocardial level, our results showed an opposite effect at the vascular level. CIH reduced gene expression of intermediaries involved in antioxidant response and nitric oxide production in both young and old mice. Impairments in antioxidant capacity may further exacerbate oxidative stress, and suggest the occurrence of mitochondrial dysfunction, one of the hallmarks of aging [43]. A long-term oxidative stress state promotes cumulative damage of proteins, DNA, and macromolecules, which is the principal responsible for the process of aging and atherosclerotic risk [44]. In fact, several studies have suggested that OSA induces oxidative stress, compromise intra- and intercellular communication, impair nutrient sensing, induce DNA damage, and cellular senescence [43]. In addition, it was observed that proatherogenic remodeling induced by 8 weeks of CIH is not reversible with usual OSA treatment [45]. Although we did not observe morphological vascular remodeling induced by CIH in old mice in previous work by our group, here we confirm that aged mice are as susceptible to ROS generation as young mice at the molecular level. Overall, cardiac response to CIH is higher in young mice and the vascular tissue is susceptible to CIH in both young and aged mice. This corroborates previous findings on the highest susceptibility of aortic tissue to CIH, suggesting that the pathological damage induced by CIH is tissue-specific [46].

Past studies had demonstrated that circulating proinflammatory cytokines were higher in sleep apnea patients and were consistently correlated with the severity of OSA [47–54]. It is also has been shown that sleep apneic men appear to have a more severe inflammatory profile compared to women [55]. The dimorphic gender effect could explain in part the lack of significantly elevated expression of inflammatory mediators at the cardiovascular tissue level in the present study. Our findings indicate a need for further insightful studies to address potential sex differences in cardiovascular biomarker responses to OSA-related stresses, identifying mechanisms at the structural, cellular, and molecular levels. Our study has some limitations. First, our model is based on the application of IH and may not

represent the pathology of OSA completely. However, it allows the study of isolated outcomes after IH that improve the understanding of the possible molecular events at the tissue. Second, we investigated gene expression at the transcriptional level, but further studies are necessary to investigate protein expression within the tissues, considering post-transcriptional and posttranslational modifications, e.g. epigenetic modifications mediated by miRNAs. Third, we used female mice rather than male mice due to the absence of relevant studies in female mice and the late emergence of sexually dimorphic cardiovascular outcomes with increased susceptibility in women. Finally, we only mimicked severe OSA, and it will be necessary to explore the cardiovascular-related gene expression after mild or moderate OSA in the future. The main strengths of our work include the use of older mice that were 18 months of age and the use of a conventional mouse strain, which allowed us to assess the cardiovascular impact induced by IH per se, while avoiding the presence of other risk factors or comorbidities, such as high-fat, high-sugar diet or genetically induced cardiovascular disease propensity.

In conclusion, our findings suggest that CIH induces upregulation of stress-response genes involved in antioxidant, antiapoptotic, and antistress responses mediating cardioprotection and cell survival in young mice, but not in old mice. These cardioprotective responses could partially explain the ischemic preconditioning process associated with sleep apnea. The vascular reduction of genes involved in antioxidant responses could lead to cumulative oxidative damage of intracellular components suggesting a vascular aging and atherosclerotic risk induced by CIH independent of age. These biomarkers offer insight into the physiopathology of the disease and are of potential diagnostic, prognostic, and therapeutic utility in OSA.

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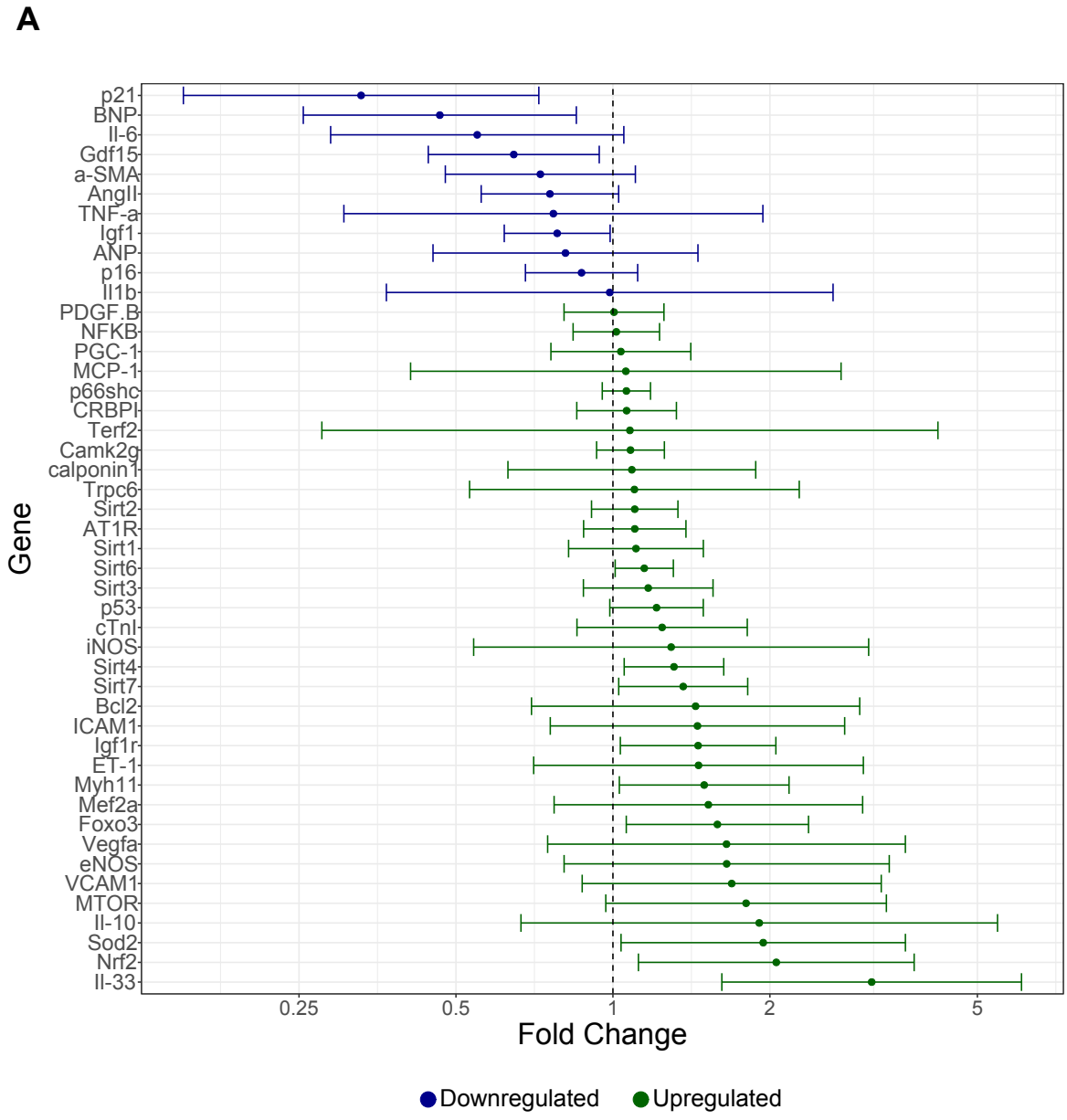
FIGURE LEGENDS

Figure 1. Differential gene expression in oxidative stress, inflammation, aging hallmarks, and stress response pathways after chronic intermittent hypoxia in cardiac tissue. (A) Female C57BL/6 mice aged 2 months old (young mice) and (B) female C57BL/6 mice aged 18 months old (old mice) were exposed to room air (normoxia) or to CIH for 8 weeks (n = 8 per group). The mRNA values of each gene measured by RT-PCR are shown as fold change between mice exposed to CIH versus normoxia. Bars in blue correspond to the genes with decreased expression; bars in green correspond to genes with increased impressio



B

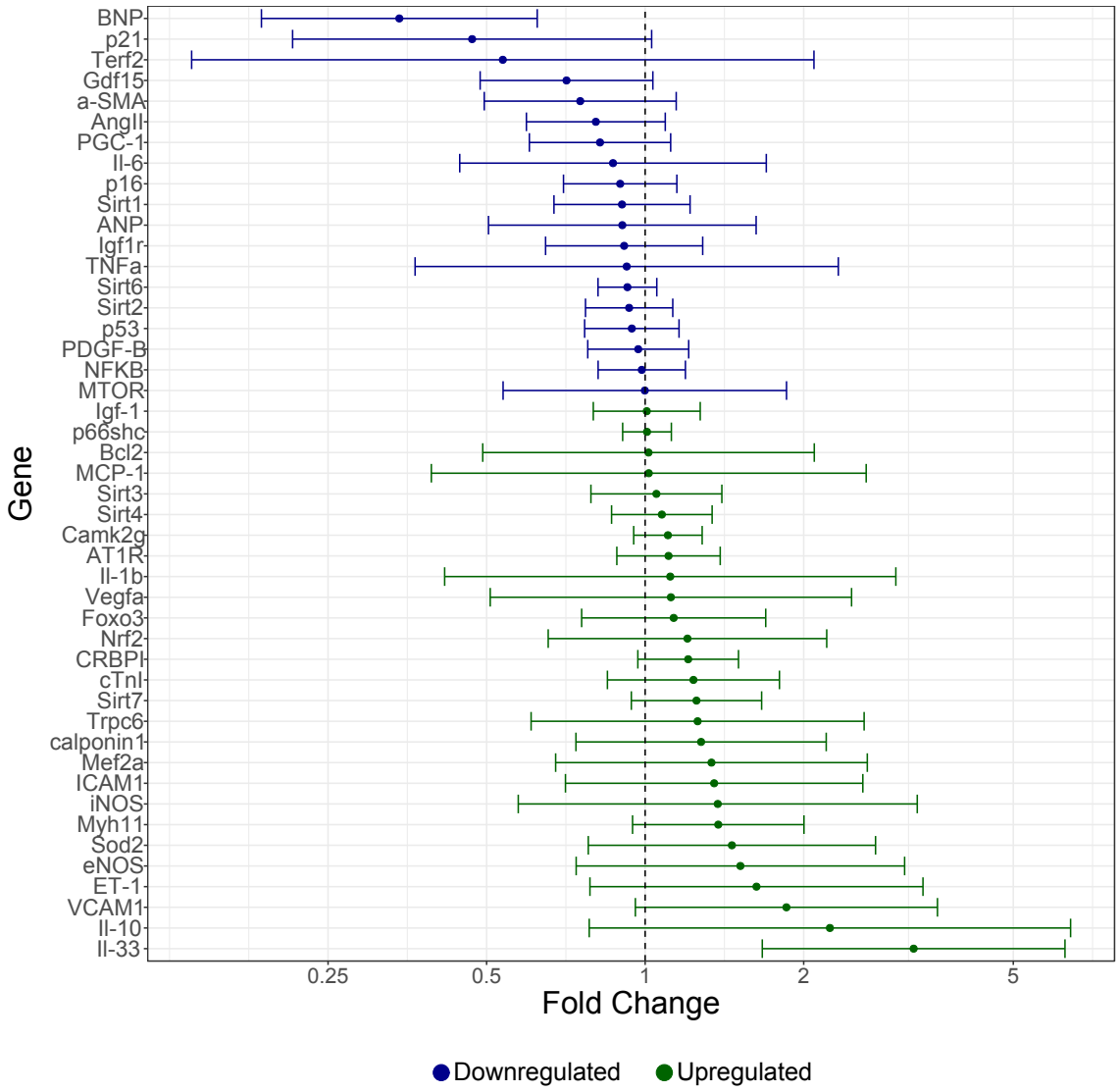
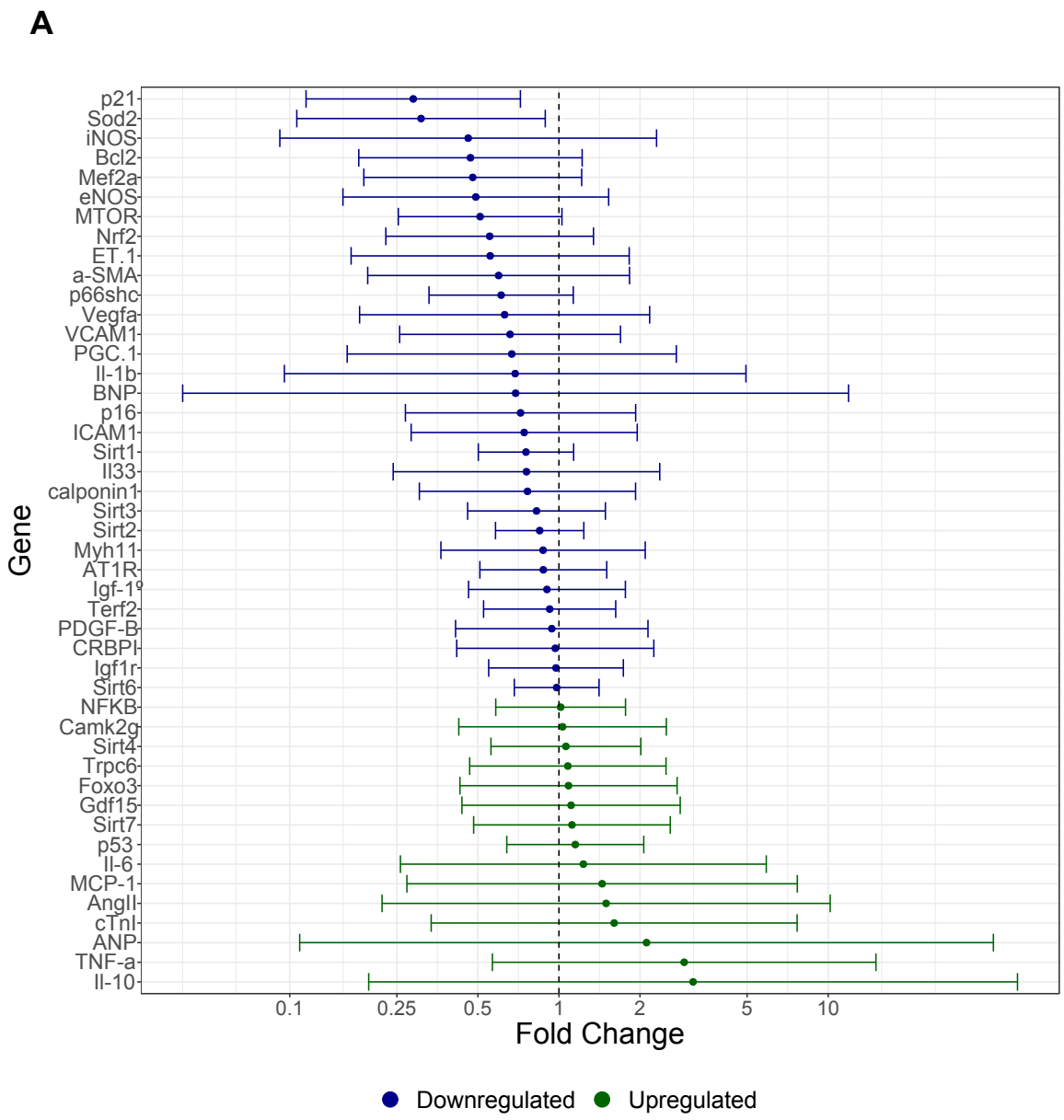
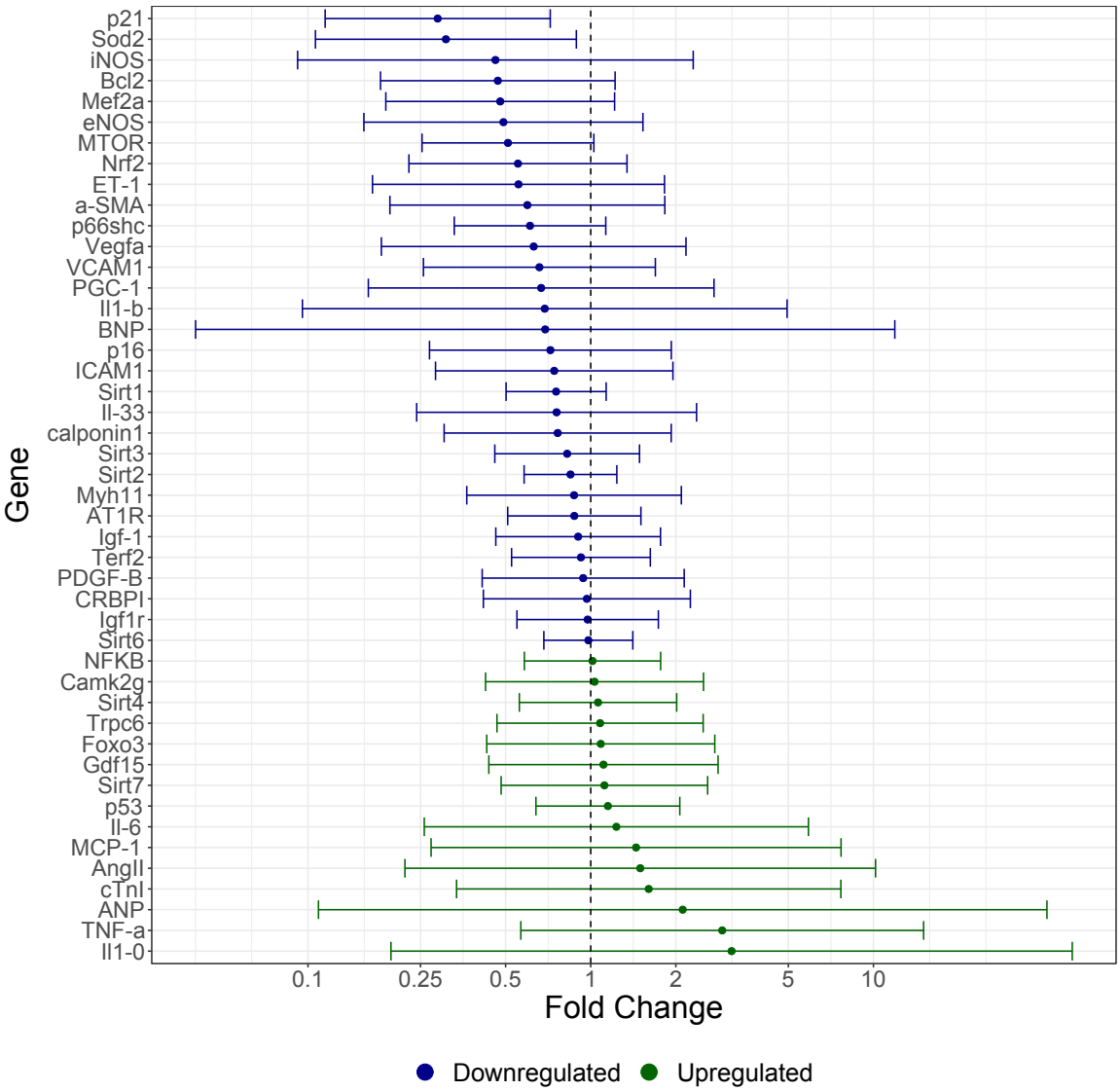

BNP
p21
Terf2
Gdf15
a-SMA
AngII
PGC-1
Il-6
p16
Sirt1
ANP
Igf1r
TNFa
Sirt6
Sirt2
p53
PDGF-B
NFKB
MTOR
Igf-1
p66shc
Bcl2
MCP-1
Sirt3
Sirt4
Camk2g
AT1R
Il-1b
Vegfa
Foxo3
Nrf2
CRBPI
cTnI
Sirt7
Trpc6
calponin1
Mef2a
ICAM1
iNOS
Myh11
Sod2
eNOS
ET-1
VCAM1
Il-10
Il-33
Gene
0.25
0.5
1
2
5
Fold Change
Downregulated
Upregulated

Figure 2. Differential gene expression in oxidative stress, inflammation, aging hallmarks, and stress response pathways after chronic intermittent hypoxia in aortic tissue. (A) Female C57BL/6 mice aged 2 months old (young mice) and (B) female C57BL/6 mice aged 18 months old (old mice) were exposed to room air (normoxia) or to CIH for 8 weeks (n = 8 per group). The mRNA values of each gene measured by RT-PCR are shown as fold change between mice exposed to CIH versus normoxia. Bars in blue correspond to the genes with decreased expression; bars in green correspond to genes with increased impression.



B


p21
Sod2
iNOS
Bcl2
Mef2a
eNOS
MTOR
Nrf2
ET-1
a-SMA
p66shc
Vegfa
VCAM1
PGC-1
Il1-b
BNP
p16
ICAM1
Sirt1
Il-33
calponin1
Sirt3
Sirt2
Myh11
AT1R
Igf-1
Terf2
PDGF-B
CRBPI
Igf1r
Sirt6
NFKB
Camk2g
Sirt4
Trpc6
Foxo3
Gdf15
Sirt7
p53
Il-6
MCP-1
AngII
cTnI
ANP
TNF-a
Il1-0
Gene
0.1
0.25
0.5
1
2
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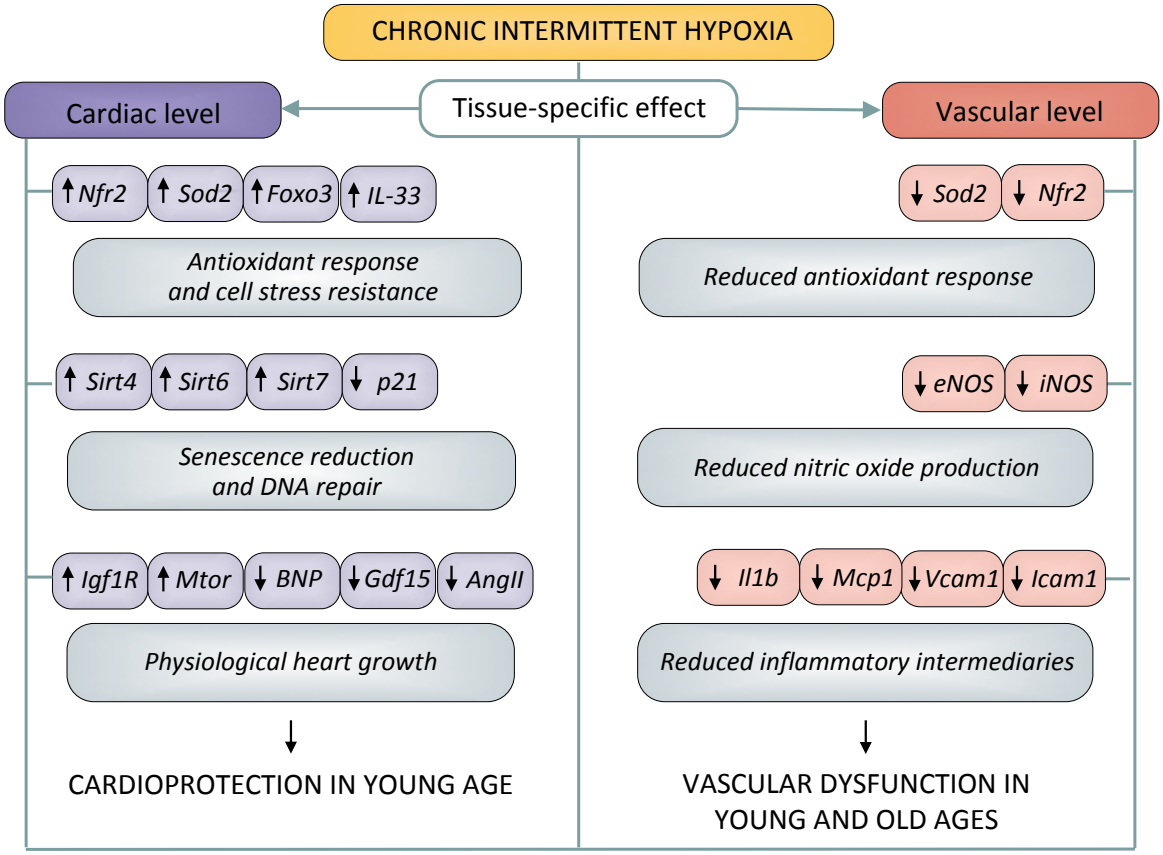
10
Fold Change
Downregulated
Upregulated

Figure 3. Schematic representation of the principal changes found in the expression of genes in cardiac and aortic tissue in mice exposed to chronic intermittent hypoxia. Increased and decreased expressions are represented by up and down arrows.



SUPPLEMENTARY INFORMATION

TITLE: The effect of chronic intermittent hypoxia in cardiovascular gene expression is modulated by age in a mice model of sleep apnea

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Table S1. List of genes

Table legend: TaqMan Gene ID, Gene Abbreviation and Gene Name used to assess several genes gene expression by RT-PCR.

Table S2. Genes assessed in left ventricle cardiac tissue

Table S3. Genes assessed in aortic arch

Table legend: TaqMan Gene Expression Assays used to assessed several genes gene expression by RT-PCR. Gene expression data (Ct, expressed as median [confidence interval]) were normalized by the mean of the endogenous controls GAPDH and β-actin (ΔCt). The differences in gene expression between groups were evaluated using ANOVA (shown as p value overall) and performs pairwise comparisons adjusting for multipletesting by Tukey method. Groups: Young mice exposed to normoxia (Y-N); young mice exposed to intermittent hipoxia (Y-IH); old mice exposed to normoxia (O-N); old mice exposed to intermittent hipoxia (O-IH).

ID assay	Gene	Gane name
Mm01320970_m1	VCAM1	vascular cell adhesion molecule 1
Mm01318991_m1	MEF2a	myocyte enhancer factor 2A
Mm01313000_m1	SOD2	superoxide dismutase 2
Mm01288386_m1	IL-10	Interleukin 10
Mm01271860_m1	p53BP1	protein 53 binding protein 1
Mm01255770_g1	BNP	natriuretic peptide type B
Mm01255747_g1	ANP	natriuretic peptide type A
Mm01253555_m1	TERF2	telomeric repeat binding factor 2
Mm01248607_m1	SIRT7	sirtuin 7
Mm01242613_m1	MTOR	regulatory associated protein of, complex 1
Mm01208835_m1	PGC-1	peroxisome proliferator-activated 1
Mm01201915_m1	SIRT4	sirtuin 4
Mm01185722_m1	FOXO3	forkhead box O3
Mm01176083_m1	TRPC6	transient receptor potential cation channel, C, 6
Mm01168521_m1	SIRT1	sirtuin 1
Mm01149204_m1	SIRT2	sirtuin 2
Mm01149042_m1	SIRT6	sirtuin 6
Mm00802831_m1	IGF-1R	insulin-like growth factor I receptor
Mm00725412_s1	a-SMA	actin, alpha 2, smooth muscle, aorta
Mm00618054_m1	CAMK2g	calcium/calmodulin-dependent protein kinase II
Mm00599662_m1	ANGII	angiotensinogen
Mm00516023_m1	ICAM1	intercellular adhesion molecule
Mm00507771_m1	AT1R	angiotensin II, type I receptor-associated protein
Mm00505403_m1	IL-33	interleukin 33
Mm00494449_m1	p16	cyclin-dependent kinase inhibitor 2A
Mm00487032_m1	CALP 1	calponin 1
Mm00482418_m1	NFKB	NFKB
Mm00477784_m1	NRF2	nuclear factor, erythroid derived 2, like 2
Mm00477631_m1	BCL2	B cell leukemia/lymphoma 2
Mm00468942_g1	p66shc	src 2 domain-containing transforming protein C1
Mm00452131_m1	SIRT3	sirtuin 3
Mm00446190_m1	IL-6	interleukin 6
Mm00443258_m1	TNF-alpha	tumor necrosis factor
Mm00443013_m1	MYH11	myosin, heavy polypeptide 11, smooth muscle
Mm00442228_m1	GDF15	growth differentiation factor 15
Mm00441242_m1	MCP-1	chemokine (C-C motif) ligand
Mm00441119_m1	CRBPI	retinol binding protein 1, cellular
Mm00440677_m1	PDGF-B	platelet derived growth factor, B polypeptide
Mm00440502_m1	iNOS	nitric oxide synthase 2, inducible
Mm00439560_m1	IGF-I	insulin-like growth factor 1
Mm00438656_m1	ET-1	endothelin 1
Mm00437306_m1	VEGFa	vascular endothelial growth factor A
Mm00437164_m1	cTNI	troponin I
Mm00435217_m1	eNOS	nitric oxide synthase 3, endothelial cell
Mm00434228_m1	IL-1b	interleukin 1 beta
Mm00432448_m1	p21	cyclin-dependent kinase inhibitor 1A (P21)

Gene	Y-N N=8	Y-IH N=8	O-N N=8	O-IH N=8	P value overall	p. Y-IH vs Y-N	p. O-IH vs O-N	p. O-N vs Y-N	p. O-N vs Y-IH	p. O-IH vs Y-N	p. O-IH vs Y-IH
VCAM1	7.10 [6.3;7.8]	]6.35 [6.00;6.7]	6.81 [6.7;7.46]	5.92 [5.4;6.3]	0.011	0.157	0.073	0.839	0.547	0.011	0.622
MEF2a	3.75 [2.9;4.5]	3.15 [2.8;3.4]	3.84 [3.1;4.5]	3.42 [2.9;3.9]	0.219	0.348	0.655	0.995	0.240	0.792	0.870
SOD2	1.01 [0.2;1.7]	0.05 [-0.1;0.2]	0.82 [0.1;1.4]	0.28 [-0.1;0.7]	0.024	0.035	0.369	0.943	0.116	0.146	0.905
IL-10	18.1 [17.1;19.1]	17.2 [16;17.7]	17.6 [16;18.6]	16.5 [15;17.5]	0.040	0.355	0.179	0.823	0.848	0.030	0.580
p53BP1	8.89 [8.7;9.0]	8.61 [8.4;8.8]	8.68 [8.5;8.8]	8.76 [8.5;8.9]	0.089	0.867	0.252	0.915	0.676	0.498	0.074
BNP	1.57 [1.1;1.9]	2.67 [1.9;3.4]	1.27 [0.6;1.8]	2.82 [2.6;3.0]	<0.001	0.009	<0.001	0.780	0.001	0.003	0.967
ANP	0.20 [-0.2;0.6]	0.51 [-0.02;1]	0.47 [-0.0;0.9]	0.61 [0.06;1.1]	0.598	0.763	0.966	0.824	0.999	0.554	0.985
TERF2	6.22 [6.07;6.3]	6.12 [5.96;6.2]	6.20 [6.10;6.3]	7.10 [4.71;9.4]	0.492	0.999	0.602	1.000	0.999	0.622	0.530
SIRT7	7.31 [6.9;7.7]	6.86 [6.7;6.9]	7.05 [6.8;7.2]	6.73 [6.5;6.9]	0.004	0.029	0.162	0.334	0.597	0.003	0.808
MTOR	8.11 [7.2;8.9]	7.26 [6.9;7.5]	7.73 [7.3;8.1]	7.73 [7.2;8.2]	0.104	0.068	1.000	0.658	0.658	0.489	0.662
PGC-1	2.98 [2.6;3.3]	2.93 [2.7;3.1]	2.91 [2.5;3.2]	3.19 [3.02;3.3]	0.306	0.990	0.321	0.968	0.999	0.577	0.396
SIRT4	7.70 [7.5;7.8]	7.31 [7.1;7.4]	7.66 [7.4;7.8]	7.55 [7.3;7.8]	0.011	0.012	0.801	0.982	0.029	0.584	0.188
FOXO3	4.77 [4.2;5.2]	4.10 [3.7;4.4]	4.35 [3.9;4.7]	4.17 [4.02;4.3]	0.018	0.020	0.832	0.224	0.655	0.041	0.989
TRPC6	12.9 [12;13.2]	12.8 [11;13.9]	12.9 [12;13.2]	12.6 [12;12.9]	0.809	0.825	0.825	1.000	0.981	0.837	0.963
SIRT1	7.02 [6.7;7.2]	6.88 [6.7;7.0]	7.18 [6.8;7.5]	7.33 [7.06;7.6]	0.042	0.787	0.790	0.740	0.230	0.232	0.036
SIRT2	3.33 [3.1;3.5]	3.19 [3.0;3.3]	3.19 [2.9;3.3]	3.29 [3.1;3.4]	0.409	0.523	0.749	0.510	1.000	0.978	0.762
SIRT6	6.91 [6.8;7.0]	6.71 [6.5;6.8]	6.85 [6.7;6.9]	6.96 [6.8;7.0]	0.007	0.031	0.369	0.754	0.232	0.913	0.006
IGF-1R	6.48 [6.2;6.6]	5.94 [5.4;6.4]	6.35 [6.08;6.6]	6.48 [6.2;6.6]	0.017	0.028	0.884	0.889	0.129	1.000	0.027
a-SMA	7.77 [7.4;8.1]	8.23 [7.9;8.5]	8.40 [8.04;8.7]	8.81 [8.3;9.2]	0.001	0.183	0.274	0.038	0.867	<0.001	0.064
CAMK2g	4.33 [4.1;4.5]	4.22 [4.1;4.3]	4.42 [4.3;4.5]	4.27 [4.1;4.4]	0.101	0.502	0.287	0.703	0.082	0.883	0.904
ANGII	6.82 [6.5;7.0]	7.22 [7.01;7.4]	6.82 [6.4;7.2]	7.13 [6.9;7.2]	0.032	0.081	0.235	1.000	0.083	0.232	0.944
ICAM1	8.04 [7.2;8.8	7.50 [7.2;7.7]	7.47 [6.9;8.04]	7.04 [6.5;7.5]	0.055	0.412	0.591	0.366	1.000	0.033	0.538

AT1R	5.54 [5.3;5.7]	5.40 [5.1;5.6]	5.48 [5.2;5.6]	5.33 [5.1;5.4]	0.334	0.653	0.611	0.948	0.923	0.306	0.928
IL-33	8.03 [7.2;8.8]	6.38 [5.9;6.8]	7.16 [6.5;7.7]	5.47 [5.1;5.9]	<0.001	<0.001	<0.001	0.084	0.141	<0.001	0.064
p16	12.9 [12;13]	13.1 [12;13]	10.5 [10;10]	10.7 [10;10]	<0.001	0.436	0.630	0.000	0.000	0.000	0.000
CALP 1	8.02 [7.3;8.7]	7.90 [7.36;8]	8.14 [7.8;8.4]	7.79 [7.5;8.0]	0.649	0.975	0.619	0.974	0.835	0.855	0.981
NFKB	5.88 [5.6;6.7]	5.86 [5.6;64]	5.88 [5.7;6.5]	5.90 [5.7;6.2]	0.981	0.996	0.997	1.000	0.997	0.997	0.975
NRF2	4.35 [3.5;5.1]	3.31 [2.9;3.6]	4.08 [3.6;4.5]	3.81 [3.3;4.3]	0.021	0.016	0.840	0.828	0.104	0.350	0.422
BCL2	8.79 [7.8;9.6]	8.26 [7.9;8.5]	8.32 [7.6;8.9]	8.30 [7.7;8.8]	0.484	0.525	1.000	0.621	0.999	0.587	1.000
p66shc	4.78 [4.6;4.9]	4.69 [4.5;4.8]	4.72 [4.6;4.7]	4.71 [4.6;4.7]	0.456	0.438	0.997	0.716	0.966	0.590	0.994
SIRT3	4.87 [4.6;5.1]	4.64 [4.4;4.8]	4.82 [4.5;5.1]	4.75 [4.4;5.0]	0.486	0.459	0.965	0.989	0.651	0.860	0.896
IL-6	12.7 [12;13]	13.6 [12;14]	12.6 [12;13]	14.7 [10;19]	0.408	0.925	0.441	1.000	0.905	0.474	0.834
TNF-a	12.9 [12;13]	13.3 [12.5;1]	12.5 [11;13]	12.6 [11;13]	0.415	0.865	0.995	0.854	0.408	0.942	0.547
MYH11	5.97 [5.5;6.3]	5.39 [4.9;5.8]	5.80 [5.5;6.0]	5.34 [5.1;5.5]	0.008	0.031	0.115	0.841	0.175	0.019	0.996
GDF15	9.21 [8.9;9.5]	9.85 [9;10.2]	8.37 [7.8;8.8]	8.86 [8.7;8.9]	<0.001	0.018	0.084	0.001	<0.001	0.316	<0.001
MCP-1	9.82 [8.6;1]	9.74 [9.1;10]	9.07 [8.2;9.8]	9.05 [8.3;9.7]	0.276	0.998	1.000	0.461	0.559	0.435	0.532
CRBPI	6.64 [6.4;6.4]	6.55 [6.3;6.7]	6.67 [6.4;6.8]	6.40 [6.3;6.]	0.116	0.876	0.114	0.989	0.713	0.206	0.590
PDGF-B	4.01 [3.8;4.1]	4.01 [3.8;4.1]	3.96 [3.7;4.2]	4.01 [3.8;4.1]	0.968	1.000	0.982	0.979	1.000	1.000	0.982
iNOS	7.74 [6.8;8.6]	7.36 [6.9;7.7]	8.11 [7.1;9.0]	7.65 [6.9;8.3]	0.458	0.852	0.754	0.847	0.385	0.998	0.923
IGF-I	6.54 [6.4;6.6]	6.90 [6.78;7]	7.08 [6.8;7.3]	7.07 [6.8;7.3]	<0.001	0.036	1.000	0.001	0.483	0.001	0.532
ET-1	9.07 [8.2;9.9]	8.52 [8.2;8.7]	8.86 [8.1;9.5]	8.16 [7.6;8.7]	0.117	0.499	0.949	0.815	0.108	0.781	0.283
VEGFa	3.42 [2.4;4.4]	2.69 [2.4;2.9]	3.13 [2.3;3.8]	2.97 [2.3;3.5]	0.385	0.326	0.980	0.904	0.720	0.711	0.909
cTNI	2.11 [2.4;1.7]	2.42 [2.7;2.1]	2.1 [-2.4;1]	2.45 [2.7;2.1]	0.205	0.407	0.432	0.998	0.516	0.333	0.999
eNOS	7.25 [6.4;8.0]	6.52 [6.2;6.7]	7.37 [6.6;8.1]	6.77 [6.2;7.2]	0.108	0.247	0.247	0.986	0.135	0.604	0.909
IL-1b	12.7 [11;14]	12.7 [12;13]	12.3 [11;13]	12.1 [11;12]	0.587	1.000	0.990	0.856	0.838	0.693	0.670
p21	4.74 [4.3;5.1]	6.34 [5.3;7.3]	5.69 [5.3;6.0]	6.78 [6.0;7.5]	<0.001	0.003	0.062	0.126	0.404	<0.001	0.723

Gene	Y-N N=8	Y-IH N=8	O-N N=8	O-IH N=8	P value overall	p. Y-IH vs Y-N	p. O-IH vs O-N	p. O-N vs Y-N	p. O-N vs Y-IH	p. O-IH vs Y-N	p. O-IH vs Y-IH
VCAM1	4.70 [3.9;5.4]	7.14 [6.2;8.0]	6.27 [5.4;7]	6.87 [6;7.6]	<0.001	<0.001	0.627	0.019	0.327	0.001	0.953
MEF2a	2.44 [1.6;3.1]	4.84 [3.9;5.7]	4.59 [3.8;5.3]	5.65 [4.8;6.5]	<0.001	<0.001	0.159	0.001	0.955	<0.001	0.371
SOD2	1.55 [1;2.01]	4.03 [2.7;5.2]	1.39 [0.7;2.01]	3.09 [1.9;4.2]	<0.001	0.001	0.025	0.992	<0.001	0.048	0.363
IL-10	18.6 [16;21]	20.9 [18;23]	18.4 [16;20]	16.7 [14;19]	0.067	0.438	0.675	0.998	0.351	0.577	0.042
p53BP1	8.08 [7.2;8.8]	7.59 [7.2;7.8]	8.64 [8.1;9.14]	8.43 [8.11;8]	0.012	0.400	0.914	0.296	0.011	0.665	0.049
BNP	12.0 [10;14]	13.9 [12;15]	16.5 [13;19.4]	17.1 [13;20]	0.008	0.596	0.984	0.028	0.330	0.012	0.184
ANP	7.70 [5.6;9.7]	7.98 [4.4;11]	9.86 [6;12.7]	8.78 [7.1;10]	0.523	0.998	0.900	0.520	0.633	0.899	0.956
TERF2	4.44 [3.7;5]	4.69 [4.2;5.0]	5.21 [4.7;5.6]	5.32 [4.8;5.7]	0.019	0.847	0.980	0.073	0.322	0.031	0.170
SIRT7	3.08 [2;4.1]	3.75 [3.2;4]	3.94 [3.3;4.5]	3.78 [3.1;4.4]	0.236	0.438	0.984	0.229	0.972	0.399	1.000
MTOR	5.66 [4.9;6.3]	7.39 [6.8;7.9]	7.39 [6.8;7.9]	7.99 [7.2;8.7]	<0.001	<0.001	0.062	0.005	0.744	<0.001	0.384
PGC-1	4.2 [3.2;5.2]	5.43 [4.2;6.6]	3.84 [2.5;5.1]	4.43 [3.0;5.8]	0.200	0.395	0.862	0.949	0.166	0.995	0.538
SIRT4	7.30 [6.5;8]	7.30 [6.8;7]	7.47 [6.9;7.9]	7.39 [6.8;7.9]	0.948	1.000	0.994	0.957	0.956	0.994	0.994
FOXO3	3.41 [2.3;4.5]	4.28 [3.4;5.0]	5.00 [4.3;5.6]	4.88 [4.2;5.5]	0.012	0.303	0.995	0.016	0.478	0.027	0.624
TRPC6	8.81 [8.3;9]	9.80 [8.6;10]	8.81 [8.3;9.2]	8.70 [7.9;9.4]	0.065	0.142	0.995	1.000	0.140	0.994	0.086
SIRT1	6.19 [5.8;6.5]	6.15 [5.8;6.4]	6.83 [6.4;7.2]	7.23 [6.8;7.5]	<0.001	0.997	0.256	0.031	0.019	<0.001	<0.001
SIRT2	3.10 [2.8;3]	2.87 [2.6;3]	2.74 [2.3;3.1]	2.67 [2.2;3]	0.163	0.978	0.637	0.747	0.928	0.148	0.293
SIRT6	6.45 [6.2;6.6]	6.52 [6.2;6.8]	6.89 [6.5;7.2]	6.91 [6.5;7.3]	0.038	0.984	0.999	0.127	0.242	0.095	0.188
IGF-1R	5.14 [4.7;5.5]	4.95 [4.4;5.4]	6.30 [5.9;6.6]	6.34 [5.6;7]	<0.001	0.923	0.999	0.004	0.001	0.003	<0.001
a-SMA	2.50 [1.7;3]	2.17 [1.4;2.8]	5.09 [4;6.1]	5.84 [4.5;7.1]	<0.001	0.940	0.596	0.001	<0.001	<0.001	<0.001
CAMK2 g	2.43 [1.2;3.6]	2.76 [2.2;3.2]	3.86 [3.2;4.4]	3.82 [3.1;4.4]	0.008	0.897	1.000	0.024	0.110	0.030	0.133
ANGII	10.9 [9.7;12]	11.5 [10;12]	12.0 [8.9;15]	11.4 [10;11]	0.783	0.953	0.939	0.731	0.956	0.967	1.000
ICAM1	5.55 [4.8;6]	8.23 [7.2;9.1]	7.19 [6.31;8]	7.62 [6.7;8.4]	<0.001	<0.001	<0.001	0.835	0.016	0.200	0.002
AT1R	5.57 [4.8;6.2]	5.89 [5.3;6.4]	6.10 [5.7;6.4]	6.29 [6;6.55]	0.102	0.687	0.906	0.285	0.891	0.083	0.519
IL-33	3.92 [3;4.7]	6.53 [5.3;7.6]	5.36 [4.3;6.4]	5.76 [4.8;6.7]	0.002	0.001	0.909	0.103	0.235	0.024	0.587
p16	10.7 [9.6;11]	10.6 [10;11]	8.02 [7;8.9]	8.49 [7.6;9.3]	<0.001	0.996	0.799	<0.001	<0.001	0.001	0.002
CALP 1	0.38 [1.2;0.5]	0.28 [0.8;0.3]	2.01 [1.2;2.7]	2.40 [1.4;3.3]	<0.001	0.997	0.856	<0.001	<0.001	<0.001	<0.001
NFKB	5.86 [5;6.67]	5.56 [5.2;5.9]	5.73 [5.5;5.8]	5.71 [5.3;6.1]	0.786	0.735	1.000	0.970	0.937	0.954	0.956
NRF2	2.85 [2.4;3.3]	5.22 [4.2;6.1]	4.45 [3.7;5.1]	5.30 [4.4;6.1]	<0.001	<0.001	0.799	0.011	<0.001	0.001	0.002
BCL2	7.58	6.49	6.70	4.60	<0.001	0.002	0.159	0.004	0.976	<0.001	0.318

	[6.5;8.6]	[5.7;7.2]	[5.9;7.4]	[3.7;5.4]							
p66shc	1.26 [0.5;1.9]	1.26 [0.8;1.6]	1.37 [0.8;1.9]	2.08 [1.5;2.6]	0.049	1.000	0.151	0.986	0.986	0.076	0.077
SIRT3	3.22 [2.4;3.9]	3.89 [3.4;4.3]	3.21 [2.9;3.4]	3.49 [2.96;4]	0.119	0.159	0.810	1.000	0.148	0.830	0.564
IL-6	12.9 [10;15]	12.6 [11;13]	11.6 [11;12]	11.3 [10;11]	0.214	0.992	0.991	0.428	0.595	0.302	0.443
TNF-a	10.6 [9.1;12]	11.7 [11;12]	11.8 [9.6;14]	10.3 [9.3;11.]	0.199	0.552	0.302	0.472	0.999	0.988	0.368
MYH11	1.65 [0.7;2.5]	1.62 [1.;2.2]	0.31 [0.3;0.9]	0.51 [0.3;1.3]	<0.001	1.000	0.974	0.001	0.001	<0.001	<0.001
GDF15	8.44 [7.4;9.4]	9.22 [8.1;10]	8.85 [8.5;9.1]	8.70 [8.1;9.3]	0.470	0.408	0.990	0.841	0.841	0.952	0.721
MCP-1	10.0 [8.1;12]	9.74 [9.11;10 .4]	12.2 [10.8;13]	9.05 [8.3;9.7]	0.065	0.085	0.930	0.912	0.285	1.000	0.095
CRBPI	2.53 [1.8;3.2]	2.32 [1.7;2.9]	3.78 [3.1;4.4]	3.83 [2.8;4.8]	0.002	0.965	1.000	0.041	0.014	0.033	0.011
PDGF-B	2.52 [1.7;3.3]	3.57 [2.8;4.3]	2.82 [2.1;3.56]	2.91 [2.2;3.5]	0.129	0.099	0.997	0.898	0.337	0.805	0.446
iNOS	7.68 [6.7;8.5]	10.4 [8.5;12]	8.43 [7.2;9.6]	9.55 [7.9;11]	0.019	0.019	0.561	0.812	0.131	0.147	0.778
IGF-I	4.23 [3.4;5]	5.04 [4.5;5]	5.30 [4.8;5.7]	5.45 [4.8;6.0]	0.008	0.121	0.975	0.025	0.882	0.009	0.661
ET-1	9.07 [8.2;9.9]	8.52 [8.2;8.7]	8.86 [8.1;9.5]	8.16 [7.6;8.7]	<0.001	0.002	0.540	0.009	0.923	<0.001	0.886
VEGFa	2.85 [2.4;3.2]	5.46 [4;6.86]	3.63 [2.83;4]	4.30 [2.8;5.7]	0.004	0.002	0.632	0.044	0.142	0.311	0.738
cTNI	6.56 [4.8;8.2]	7.61 [6.1;9.1]	8.69 [7.4;9.9]	8.01 [7;8.95]	0.096	0.588	0.843	0.069	0.565	0.315	0.962
eNOS	7.25 [6.4;8.0]	6.52 [6.2;6.7]	7.37 [6.6;8.1]	6.77 [6.2;7.2]	<0.001	<0.001	0.338	0.024	0.403	<0.001	0.999
IL-1b	11.1 [8.1;14]	14.2 [13;15]	12.0 [10;13]	12.5 [11;13]	0.039	0.028	0.954	0.855	0.153	0.561	0.360
p21	2.81 [1.8;3.7]	3.62 [2.9;4.2]	1.55 [0.5;2.5]	3.35 [2.8;3.8]	0.001	0.363	0.005	0.065	0.001	0.690	0.944

Estudio 4

"Blood pressure response to CPAP treatment in subjects with obstructive sleep apnoea: the predictive value of 24-h ambulatory blood pressure monitoring"

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Blood pressure response to CPAP treatment in subjects with obstructive sleep apnoea: the predictive value of 24-h ambulatory blood pressure monitoring

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ABSTRACT

The reduction in blood pressure (BP) with continuous positive airway pressure (CPAP) is modest and highly variable. In this study, we identified the variables that predict BP response to CPAP.

24-h ambulatory BP monitoring (ABPM), C-reactive protein (CRP), leptin, adiponectin and 24-h urinary catecholamine were measured before and after 6 months of CPAP in obstructive sleep apnoea (OSA) patients.

Overall, 88 middle-aged, obese male patients with severe OSA (median apnoea–hypopnoea index 42 events·h−1) were included; 28.4% had hypertension. 62 patients finished the study, and 60 were analysed. The daytime diastolic BP (−2 mmHg) and norepinephrine (−109.5 nmol·day-1) were reduced after CPAP, but no changes in the 24-h BP, night-time BP, dopamine, epinephrine, CRP, leptin or adiponectin were detected. The nocturnal normotension was associated with an increased night-time-BP (+4 mmHg) after CPAP, whereas nocturnal hypertension was associated with a reduction of 24-h BP (−3 mmHg). A multivariate linear regression model showed differential night-time BP changes after CPAP. Specifically, low night-time heart rate (<68 bpm) and BP dipper profile were associated with increased night-time BP and new diagnosis of nocturnal hypertension.

Our results suggest that nocturnal hypertension, circadian BP pattern and night-time heart rate could be clinical predictors of BP response to CPAP and support the usefulness of 24-h ABPM for OSA patients before treatment initiation. These results need to be confirmed in further studies.

INTRODUCTION

Obstructive sleep apnoea (OSA) has been linked to a number of cardiovascular diseases, including hypertension, acute coronary syndrome, arrhythmia, coronary heart disease, stroke and increased mortality [1, 2]. The pathogenesis of this association is probably multifactorial, involving sympathetic nervous system overactivation, oxidative stress, inflammation, metabolic and hormonal deregulation and the impairment of endothelial and cardiac function [3]. As a consequence of heightened sympathetic activity, OSA patients at all levels of severity experience a marked increase in blood pressure (BP) during sleep and wakefulness [4, 5]. The night-time BP increase results in the lack of a circadian BP pattern and a higher incidence of nocturnal hypertension [6, 7], which are associated with target organ damage and worsened cardiovascular outcomes [7–9].

According to several meta-analyses, continuous positive airway pressure (CPAP) treatment reduces BP in normotensive and hypertensive patients with OSA [10–13]. However, the impact of CPAP treatment on BP is not regular. In minimally symptomatic patients, CPAP has a neutral effect on BP [14], whereas in subjects with resistant hypertension, CPAP can decrease the systolic BP by 5–7 mmHg [15]. Additionally, although the effect of CPAP treatment on BP is related to treatment compliance, there is individual variability that could be related to epigenetic factors, at least in part [16].

According to these data, beyond the variable effect of CPAP on BP in OSA patients, the identification of the clinical and biological profiles that best predict the BP response to this treatment is necessary. Interest should be focused on night-time BP effects, based on evidence that night-time BP is considered to be a better predictor of cardiovascular morbi-mortality [17]. To address this issue, we designed a pre–post study to identify clinical characteristics at baseline, including 24-h ambulatory blood pressure monitoring (ABPM), a sleep study and cardiovascular biomarkers, which could allow us to discriminate patients who

would benefit from CPAP treatment from those who would not, with regard to BP.

METHODS

Study design and patients

The present study was an observational, multicentre, pre–post study that aimed to assess changes in BP after 6 months of CPAP treatment in patients who were newly diagnosed with severe OSA. Patients were consecutively recruited from the sleep units of University Hospital Arnau de Vilanova and Santa Maria (Lleida, Spain) and Araba Hospital (Vitoria, Spain). Eligible patients were males and females aged 30–80 years, with or without a prior history of hypertension (defined as taking antihypertensive medication or a blood pressure >140/90 mmHg) and with an apnoea–hypopnea index (AHI) ⩾15. We excluded subjects with CPAP treatment, psychological or physical incapacities, drug or alcohol addiction or chronic intake of hypnotics, or who refused to participate in the study. The study was approved by the ethics committee (ID number 710), and the patients provided signed informed consent.

Follow-up

The patients answered a detailed questionnaire that included comorbidities, toxic habits, current medications, anthropometric data and OSA clinical history at baseline. Follow-up visits were performed at 1, 3 and 6 months. Daytime sleepiness was assessed at each visit using the Spanish version of the Epworth Sleepiness Scale (ESS) [18].

Procedures

Sleep study and CPAP treatment

The OSA diagnosis was obtained via a conventional polysomnographic (EMBLA S7000; Embla Systems, Broomfield, CO, USA) or cardiorespiratory sleep study (Embletta; ResMed, Sydney, Australia), according to international recommendations [19] as previously described [18] and as specified in the online supplementary material. CPAP titration was performed using an autoCPAP device (Auto-set-T; ResMed), following a validated protocol according to Spanish guidelines. The optimal pressure was determined from the raw data, and the patients were provided a CPAP machine for home use. At each visit, CPAP compliance was objectively measured as the hours of CPAP use per day according to the internal clock on the CPAP device, and patient compliance was defined as ⩾4 h of use.

Biochemical measurements

Blood and 24-h urine samples were collected at baseline and after 6 months of treatment. Haematological parameters and leptin, adiponectin and high-sensitivity C-reactive protein (hsCRP) levels were measured from blood samples. The levels of dopamine, epinephrine and norepinephrine were quantified in the 24-h urine samples (methodology is detailed in the online supplementary material).

24-h ABPM

The patients were subjected to 24-h ABPM at baseline and after 6 months of CPAP treatment (Spacelabs monitor 90207; OSI Systems, Hawthorne, CA, USA), and BP levels and heart rate (HR) levels were measured every 20 and 30 min during the daytime and night-time periods, respectively. The 24-h ABPM procedure, diagnoses of hypertension and nocturnal hypertension and assessment of circadian pattern were performed according to international recommendations [20] (online supplementary material).

Statistical analyses

Quantitative variables with a normal distribution are presented as mean±SD and the remaining variables are presented as median (interquartile intervals). Qualitative variables are presented as the absolute and relative frequencies. Post-CPAP changes in quantitative variables were assessed as differences from baseline and described as median (interquartile intervals). The Chi-squared or Fisher's exact test were used to compare qualitative variables. The bivariate analysis of the associations between changes in the night-time mean BP and baseline was based on correlations (Pearson and Spearman) for quantitative variables and mean differences for qualitative variables. We applied a multivariate linear regression model to assess changes in the night-time mean BP, and all significant covariates were included. We recoded quantitative variables that, according to their median or cut-off value, improved the coefficient of determination. We performed a post hoc analysis of the statistical power to assess whether a group of four variables could significantly predict the post-CPAP changes in the night-time mean BP by fitting a linear multiple regression model. We defined the null hypothesis in terms of the determination coefficient of the model and assessed the statistical significance of its deviation from the zero value. Thus, having fixed a type I error of 0.05 and after recruiting 88 patients and

losing 32% of patients to follow-up, we maintained 80% power to detect a significant coefficient of determination >0.18 (that is, an effect size of 0.22).

RESULTS

The 88 included patients were middle-aged, obese males with severe OSA and an ESS of 10.7±5.02 (table 1). Of the entire sample, 28.4% had previously reported hypertension, 34.1% exhibited a nondipper circadian pattern and 50% had nocturnal hypertension.

62 patients completed the follow-up, and 60 were included in the post-CPAP analysis and in the multivariate model (figure 1). Table 1 shows the baseline characteristics of this subgroup of patients. After 6 months of CPAP treatment, there was a reduction in ESS, red blood cells and haemoglobin and norepinephrine urine levels (−109.5 nmol·day-1; p<0.001), suggesting a decline in sympathetic activity (online supplementary table S2). No other significant changes were observed in the other tested biomarkers (dopamine, epinephrine, hsCRP, adiponectin and leptin). Despite the marked reduction in the norepinephrine level, no significant changes were found for the 24-h BP (mean, systolic (SBP) and diastolic (DBP)) or night-time BP. Only the daytime DBP was significantly reduced by −2 mmHg (p=0.018) after treatment. We observed an additional benefit in compliant patients who experienced a significant reduction (−2 mmHg) in daytime SBP (p=0.047), daytime DBP (p=0.0014) and 24-h DBP (p=0.026) (online supplementary table S3).

The analysis of the changes after CPAP treatment in patients with or without hypertension did not reveal any differences (data not shown). Notably, the assessment of these changes in patients with or without nocturnal hypertension showed a marked differential BP response (figure 2). After CPAP treatment (online supplementary table S2), nocturnal normotensive patients showed increases in the night-time mean BP (median increase of +4 mmHg; p=0.008), night-time SBP (median increase of +5 mmHg; p=0.014) and night-time DBP (median increase of +3 mmHg; p=0.008). In contrast, patients with nocturnal hypertension showed a decrease in the 24-h mean BP (median decrease of −3 mmHg; p=0.011), 24-h SBP (median decrease of −4 mmHg; p=0.015) and 24-h DBP (median decrease of −2 mmHg; p=0.017) after CPAP treatment (figure 2).

Differential night-time BP response to CPAP treatment predicted by haemodynamic biomarkers

A linear multiple regression model was used to identify the clinical and biological variables at baseline that could predict post-CPAP changes in night-time mean BP. The adjusted model explained 33.4% of the variability in the changes in the night-time mean BP after CPAP treatment (table 2) and included a significant interaction between dipping status and mean night-time HR (<68 bpm versus ⩾68 bpm). The contribution of CPAP compliance to the model approached significance. Therefore, the relevance of CPAP compliance on the impact of CPAP treatment on BP was included. The interaction between dipping status and night-time mean HR levels defined four OSA phenotypes. The baseline characteristics are presented in online supplementary table S4, and the post-CPAP changes are presented in table 3 and figure 3.

First, dipper patients with low night-time HR, particularly noncompliant patients (noncompliers +9.7 mmHg, p=0.0013; compliers +5.4 mmHg, p=0.0007), exhibited a marked increase in the night-time mean BP after CPAP treatment (table 2). Consequently, 33.3% of these patients were newly diagnosed with nocturnal hypertension, and 41.67% showed a change from a dipper pattern at baseline to a nondipper pattern after 6 months of CPAP treatment. In addition, the night-time HR significantly increased after treatment.

A neutral change in BP after CPAP treatment was observed in dipper and nondipper patients with a high night-time HR (⩾68 bpm). Finally, nondipper patients with a low night-time HR showed an important beneficial change after CPAP treatment in decreasing the night-time BP (median decrease of −6.2 mmHg, p<0.01) (table 3). After considering the association with CPAP adherence, compliant patients exhibited the greatest night-time BP decrease of −7.1 mmHg (p=0.0014) (table 3).

DISCUSSION

The main contribution of this study is the possible identification, via 24-h ABPM, of OSA patients who will show a favourable decrease in BP after CPAP treatment. Reduced BP after CPAP treatment was observed in patients with nocturnal hypertension and in nondipper patients with ⩾4 h of CPAP use per night. However, increased BP was observed in nocturnal normotensive patients and in dipper patients with a low HR, even among CPAP compliers. The haemodynamic biomarkers at baseline, specifically the circadian BP pattern and night-time HR, facilitated the establishment of a predictive model of CPAP treatment responses. The identification of patients who could be adversely affected by CPAP treatment might prevent adverse increases in cardiovascular haemodynamic parameters and related negative long-term cardiovascular consequences, particularly in asymptomatic patients. According to our results, 24-h ABPM should be performed for the clinical management of OSA before initiating CPAP treatment.

Patients with OSA show increased BP and a higher incidence of hypertension [21–24]. Due to repetitive hypoxaemia and sympathetic excitation, OSA patients exhibit a rise in the nocturnal BP with a consequent absence of a decrease in the nocturnal BP (nondipper circadian pattern) and night-time hypertension [6, 7]. Clinically, both elevated night-time BP and the nondipper pattern are considered important predictors of advanced target organ damage and future fatal and nonfatal cardiovascular events after adjustment for traditional risk factors in both hypertensive patients and the general population [25–28]. Accordingly, current therapeutic strategies for OSA patients are directed at decreasing the arterial BP and subsequently reducing the cardiovascular risk.

The beneficial effects of CPAP treatment have been documented in multiple studies and include reduced BP levels in patients with OSA (affected by patient adherence, age and hypertensive status) [13, 14, 29–33]. Specific OSA phenotypes, such as patients with symptomatic or resistant hypertension, clearly benefit from CPAP treatment [34]. In the current study, nocturnal hypertension was found to affect the outcome of CPAP treatment. We observed a favourable change in BP after CPAP treatment in nocturnal hypertensive patients, whereas nocturnal normotensive subjects experienced an unfavourable response (increased night-time BP levels).

Previous studies have suggested a possible detrimental effect of CPAP treatment, even when adherence to the use of CPAP is documented [18, 33, 34]. In the BARBÉ et al. [18] and MARTINEZ-GARCIA et al. [34] randomised controlled trials, 25–30% of patients who used CPAP for ⩾4 h per day showed no change or increased BP. Similarly, BRATTON et al. [14] performed a meta-analysis in which CPAP treatment was associated with increased BP levels in minimally symptomatic patients (patients without excessive daytime sleepiness) and patients with low CPAP adherence. The inconsistency between these studies suggests the need to identify specific subsets of patients who are more likely to benefit from CPAP treatment and those who may be adversely affected. The characterisation of OSA phenotypes and the impact of CPAP treatment on the spectrum of OSA constitute the first step for the accurate application of precisión medicine [35]. Moreover, diagnostic and personalised therapeutic decision-making tools are needed to manage sleep apnoea and to effectively predict responses to adherent CPAP use [16].

Several studies have reported that night-time BP is the BP measure with the best predictive value of cardiovascular risk [17, 25, 36]. A 10-mmHg increase in the mean night-time SBP is associated with a 21% increase in cardiovascular mortality [25]. Based on this consideration, we analysed the baseline characteristics to identify those variables with a predictive value for the night-time BP response to CPAP treatment. The circadian BP pattern and night-time HR can be used to distinguish two opposite phenotypes (with clinical implications) based on the night-time BP responses to CPAP treatment. Nondipper patients with a low HR (<68 bpm) experienced a night-time BP decrease after CPAP treatment. However, CPAP treatment was associated with increases in the night-time BP and HR in patients with “normal” haemodynamic characteristics at baseline (dipper pattern and low HR). This unfavourable change after CPAP treatment might worsen long-term cardiovascular outcomes. A possible explanation for these results is that these patients have adapted to OSA stress, counterbalancing the sympathetic hyperactivity, preserving the physiological circadian BP pattern and maintaining a lower HR at night.

Current international guidelines recognise the prognostic value of 24-h ABPM in some patients, specifically in patients with resistant hypertension, episodic hypertension and white-coat hypertension [20]. However, in the clinical management of OSA patients, 24-h ABPM is not recommended. Our findings reinforce the clinical value of 24-h ABPM for

patients with OSA to effectively predict the response to CPAP treatment on cardiovascular haemodynamic outcomes.

The strengths of the present study include its multicentre design, the high adherence to CPAP treatment and the use of 24-h ABPM to detect changes in night-time and daytime BP, which is considered the gold standard for BP measurements [37]. Nevertheless, this study has several potential limitations. First, the small size of the study population indicates that the present study is an exploratory work that needs to be confirmed and validated in further studies. This issue was addressed by performing a post hoc analysis of the statistical power, as detailed in the methodology section. Second, although polysomnography and cardiorespiratory sleep studies were used to diagnose OSA, the agreement level of diagnostic efficacy between the two techniques is close to 90% [38]. Third, the study design does not make it possible to assess the changes that could occur in the absence of CPAP treatment for ethical reasons, and in consequence, the natural evolution of BP cannot be accounted. Nonetheless, such changes are unlikely to be observed in the absence of treatment during the relatively short follow-up time. Fourth, the study population includes a wide range of ages. Although this represents a posible limitation, the present study aims to apply the proposed model to the whole spectrum of OSA patients.

CONCLUSIONS

Ours results suggest that nocturnal hypertension, the circadian BP pattern and the night-time HR at baseline could be important clinical predictors of the BP response to CPAP treatment in patients with severe OSA. Our findings further support the usefulness of 24-h ABPM for OSA patients prior to initiation of CPAP treatment for the prediction of treatment response and the avoidance of an unnecessary increase in cardiovascular risk. The results of the present study need to be confirmed and validated in further studies.

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TABLES AND FIGURES

TABLE 1 Subject characteristics at baseline

	Analysed	Incomplete follow-up	p-value	All patients
Patients	60	28		88
Demographic and clinical characteristics				
Age years	52.3±9.56	49.0±11.7	0.205	51.2±10.3
Sex male	48 (80.0)	23 (82.1)	1.000	71 (80.7)
Hypertension	20 (33.3)	5 (17.9)	0.213	25 (28.4)
Diabetes mellitus	8 (13.3)	0 (0.00)	0.051	8 (9.1)
Dyslipidaemia	6 (10.0)	2 (7.14)	1.000	8 (9.1)
Ischaemic heart disease	4 (6.67)	0 (0.00)	0.302	4 (4.6)
Arrhythmia	3 (5.00)	0 (0.00)	0.548	3 (3.4)
Tobacco use	21 (35.0)	11 (39.3)	0.880	32 (36.4)
Alcohol	11 (18.3)	4 (14.3)	0.766	15 (17)
Anthropometric characteristics				
BMI kg·m^{-2}	30.6 (28.0–33.5)	29.4 (27.4–33.3)	0.610	30.2 (27.9–33.4)
Neck circumference cm	41.7±3.35	41.2±3.06	0.517	41.6±3.26
OSA characteristics				
ESS score range (0–24)	10.8±4.74	10.4±5.67	0.743	10.7±5.02
AHI events·h^{-1}	46.0 (32.5–60.8)	33.7 (26.7–57.0)	0.120	42.5 (29.9–58.9)
Min oxygen saturation %	81.0 (77.0–85.0)	80.5 (70.2–84.5)	0.564	81 (76–85)
Mean oxygen saturation %	93.3 (92.0–95.0)	92.5 (92.0–95.2)	0.793	93 (92–95)
t_{Sat90} %	4.03 (1.19–10.4)	4.32 (0.85–13.6)	0.690	4.1 (1.04–12.5)
Central apnoeas events·h^{-1}	1.00 (0.00–12.0)	0.00 (0.00–7.50)	0.351	1 (0–9.8)
Blood cells				
Lymphocytes n 10^9 L	2.30 (1.95–2.72)	2.40 (2.09–2.84)	0.540	2.3 (2–2.7)
RBCs 1012 L	4.97 (4.67–5.25)	5.21 (4.84–5.46)	0.094	5 (4.7–5.3)
Haemoglobin g·dL^{-1}	15.4 (14.2–16.4)	15.8 (15.1–16.2)	0.317	15.6 (14.6–16.4)
Platelets 109 L	214 (186–252)	213 (198–229)	0.470	213 (188–248)
Catecholamines and cardiovascular risk biomarkers				
Dopamine nmol·day^{-1}	1358 (1071–1920)	1306 (952–2034)	0.743	1358 (986–1946)
Epinephrine nmol·day^{-1}	30.0 (16.4–42.4)	21.8 (16.0–27.3)	0.044	22.0 (16.4–38.2)
Norepinephrine nmol·day^{-1}	361 (225–479)	361 (277–468)	0.597	361 (236–479)
hsCRP mg·L^{-1}	1.64 (1.12–4.08)	2.93 (1.79–6.48)	0.225	2.2 (1.2–5.3)
Leptin ng·mL^{-1}	8.28 (4.85–10.7)	5.92 (3.29–11.5)	0.420	8 (4.5–10.9)
Adiponectin µg·mL^{-1}	5.37 (3.08–7.63)	5.14 (4.18–7.10)	0.908	5.3 (3.6–7.5)
24-h ABPM				
24-h mean BP mmHg	92.5±8.41	92.5±8.08	0.991	92.5±8.3
24-h SBP mmHg	123 (116–129)	124 (117–129)	0.510	123 (116–129)
24-h DBP mmHg	77.2±7.66	76.4±8.73	0.690	77±8
24-h HR bpm	73.5±10.9	76.0±10.1	0.314	74.3±10.7
Daytime mean BP mmHg	96.4±8.71	95.6±8.65	0.694	96.1±8.7
Daytime SBP mmHg	127 (119–133)	126 (118–135)	0.803	127 (119–134)
Daytime DBP mmHg	81.3±8.24	79.4±9.40	0.391	80.7±8.6
Daytime HR bpm	76.7±11.6	77.9±10.5	0.631	77±11.2
Night-time median BP mmHg	83.0 (78.8–89.0)	83.0 (80.0–91.0)	0.533	83 (80–89)
Night-time SBP mmHg	112 (106–119)	117 (110–124)	0.125	114 (107–120)
Night-time DBP mmHg	68.5 (64.0–73.0)	67.0 (65.0–73.0)	0.981	68 (64–73)
Night-time HR bpm	66.0 (61.0–70.2)	71.5 (63.2–78.8)	0.049	67 (61.2–73)
Nondipper pattern DR ⩾0.9	19 (31.7)	10 (40.0)	0.626	29 (34.1)
Medication				
β-Blockers	5 (8.33)	1 (3.57)	0.660	6 (6.82)
ACE inhibitors	7 (11.7)	3 (10.7)	1.000	10 (11.4)
Angiotensin receptor blocker	6 (10.0)	0 (0.00)	0.171	6 (6.82)
Calcium channel blocker	6 (10.0)	0 (0.00)	0.171	6 (6.82)
Diuretic	7 (11.7)	4 (14.3)	0.738	11 (12.5)
α-Adrenergic blockers	5 (8.33)	2 (7.14)	1.000	7 (7.95)
Antiplatelet	2 (3.33)	0 (0.00)	1.000	2 (2.27)
Anticoagulants	0 (0.00)	4 (14.3)	0.009	4 (4.55)
Hypolipidaemics	12 (20.0)	2 (7.14)	0.210	14 (15.9)
Antacids	13 (21.7)	2 (7.14)	0.130	15 (17.0)
Antiarrhythmics	1 (1.67)	1 (3.57)	0.538	2 (2.27)

Oral antidiabetics	6 (10.0)	0 (0.00)	0.171	6 (6.82)

Data are presented as n, mean±SD, n (%) or median (intequartile interval). BMI: body mass index; OSA: obstructive sleep apnoea; ESS: Epworth Sleepiness Scale; AHI: apnoea–hypopnea index; t_{Sat90}: time spent at night with an oxygen saturation <90%; RBCs: red blood cells; hsCRP: high-sensitivity C-reactive protein; ABPM: ambulatory blood pressure monitoring; BP: blood pressure; DBP: diastolic blood pressure; HR: heart rate; SBP: systolic blood pressure; DR: dipping ratio; ACE: angiotensin-converting enzyme.

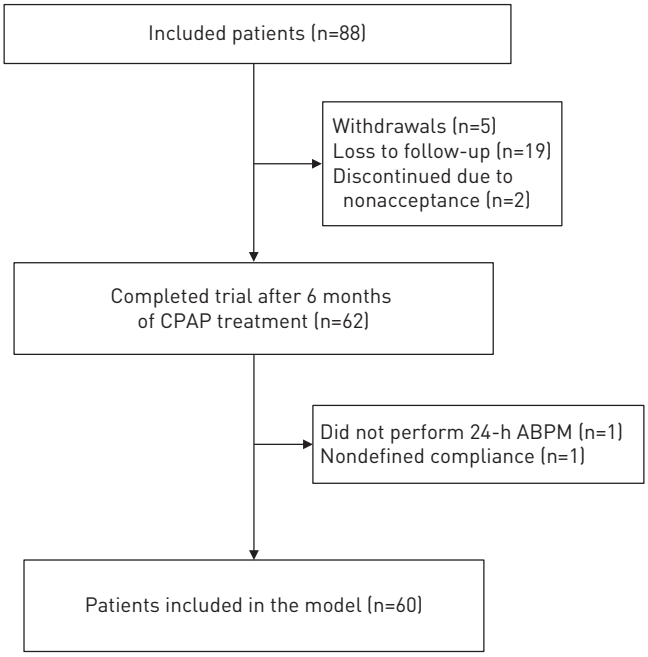

FIGURE 1 Study flowchart. 88 patients (34 from Lleida hospital and 54 from Vitoria hospital) were included. 26 did not finish the study: withdrawals n=5 (n=3 from Lleida, n=2 from Vitoria); lost to follow-up n=19 (n=7 from Lleida, n=12 from Vitoria); discontinued due to nonacceptance n=2 (from Lleida). 62 patients completed the 6-month follow-up (n=22 from Lleida and n=40 from Vitoria), and 61 performed the 24-h ambulatory blood pressure monitoring (ABPM) (n=22 from Lleida, n=39 from Vitoria). CPAP: continuous positive airway pressure.

TABLE 2 Adjusted model and predicted post-continuous positive airway pressure (CPAP) mean change in night-time blood pressure

	Estimate	Pr (>|t|)	Noncompliers	Compliers
Multivariate linear regression model (A)				
(Intercept)	+9.7±2.85	0.001		
Nondipper	−12.6±2.55	<0.001		
Mean HR (68–105 bpm)	−6.6±2.39	0.008		
CPAP complier	−4.2±2.64	0.114		
Nondipper mean night-time HR (68–105 bpm)	+10.4±4.45	0.023		
Multiple R-squared 0.3335				
Estimated mean change according to A				
Dipper/low HR			+9.7**	+5.4***
Dipper/high HR			+3.1	−1.2
Nondipper/low HR			−2.9	−7.1**
Nondipper/high HR			+0.9	−3.3

Data are presented as mean±SD. CPAP compliance is defined as ⩾4 h·day^{-1}. HR: heart rate. **: p⩽0.01; ***: p⩽0.001.

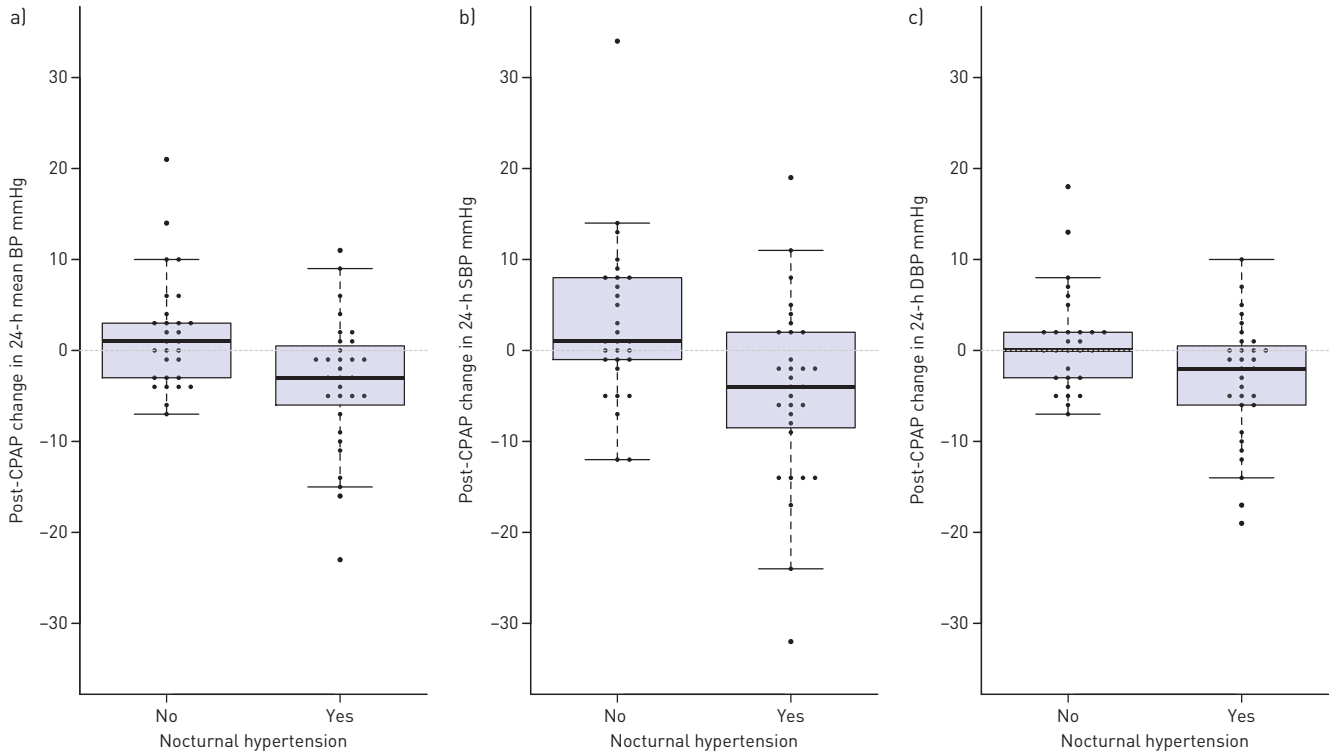

FIGURE 2 Change in blood pressure after continuous positive airway pressure (CPAP) treatment in patients with and without nocturnal hypertension. Blood pressure (BP) was assessed *via* 24-h ambulatory BP monitoring before and after 6 months of CPAP treatment in obstructive sleep apnoea patients with or without nocturnal hypertension. The bars represent the medians and interquartile intervals of the a) 24-h mean BP, b) 24-h systolic blood pressure (SBP) and c) 24-h diastolic blood pressure (DBP) changes assessed from baseline. The changes were different between groups; all p<0.01.

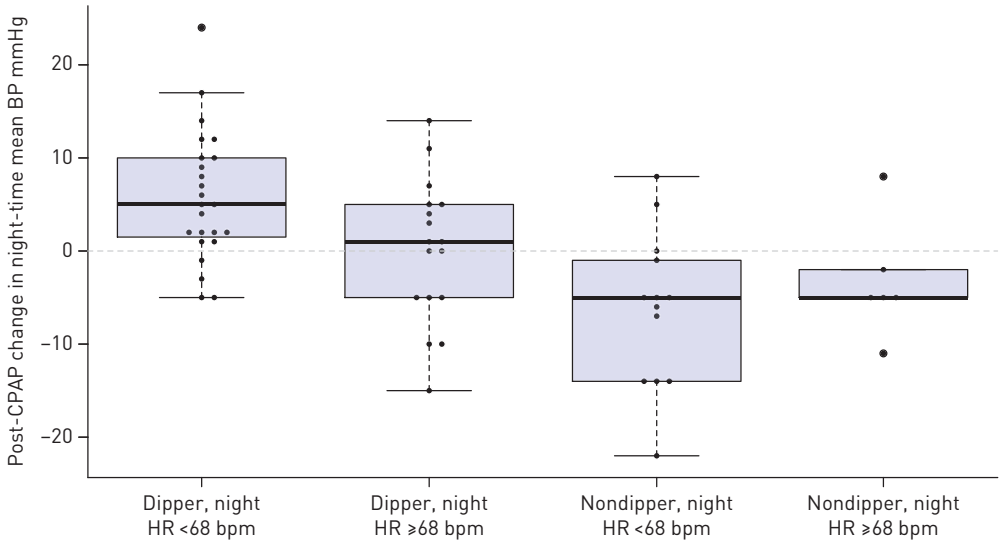

FIGURE 3 Change in night-time blood pressure (BP) after continuous positive airway pressure (CPAP) treatment in patients stratified by circadian BP pattern and night-time heart rate at baseline. The bars represent the medians and interquartile intervals for the night-time BP after 6 months of CPAP treatment (changes from baseline). The night-time BP was assessed *via* 24-h ambulatory BP monitoring. The groups were stratified by the circadian BP pattern (dipper pattern: dipping ratio (night-time/daytime BP) <0.9; nondipper pattern: dipping ratio ⩾0.9) and the mean night-time heart rate (HR) (low defined as <68 bpm and high as ⩾68 bpm). The changes were different between groups; p<0.001.

DISCUSIÓN

El SAOS es considerado un importante problema de salud pública debido a su asociación con un aumento de morbilidad y mortalidad cardiovascular.[4,21,129,169,170] El SAOS se ha asociado con un aumento de hipertensión, enfermedad coronaria, arritmia, accidente cerebrovascular, y de hecho pacientes SAOS no tratados muestran cinco veces mayor riesgo de mortalidad por casusa cardiovascular.[171] Aunque distintos componentes provocados por la apnea del sueño, como son los cambios en la presión intratorácica negativa, la fragmentación del sueño y aumento de la actividad simpática durante el sueño contribuyen a la fisiopatología asociada al SAOS, la evidencia disponible señala un papel principal de los eventos de hipoxia y reoxigenación secundarios a las obstrucciones de la vía aérea, como el mayor mediador y promotor del desarrollo de enfermedad cardiovascular.[172]

Con el fin de mejorar la comprensión del vínculo fisiopatológico entre la SAOS y la enfermedad cardiovascular, se han desarrollado diferentes modelos experimentales durante los últimos 25 años.[173] Los modelos experimentales han permitido evaluar cada componente de la enfermedad humana, sin factores desconocidos y/o de confusión (duración de la enfermedad, cronología de eventos, contribución variable de los diferentes componentes del SAOS, factores genéticos y ambientales). Algunos modelos animales han intentado reproducir las obstrucciones repetidas de las vías respiratorias superiores.[174–177] Sin embargo, basándose en la hipótesis de que la HI es el componente más importante del SAOS con respecto al sistema cardiovascular, la mayoría de las investigaciones experimentales en este campo han utilizado los modelos animales de HI en los que los animales respiran aire de forma cíclica con baja concentración de oxígeno durante el período de sueño, imitando el patrón de hipoxemia que experimentan los pacientes con SAOS. [172,178–180]

De esta manera se ha observado que las complicaciones cardiovasculares inducidas por la HI son el resultado de múltiples interacciones entre mecanismos intermedios incluidos el estrés oxidativo, la inflamación tisular y sistémica, la desregulación metabólica, sobreactivación simpática, disfunción endotelial, aumento de la presión arterial y frecuencia cardíaca.[181] Dichas alteraciones contribuyen al desarrollo de una remodelación vascular preaterosclerótica y cardíaca que son el punto de inicio y progresión de las complicaciones cardiovasculares asociadas a la HI.[173]

Las consecuencias cardiovasculares asociadas a la HI dependen del patrón de exposición, frecuencia, severidad y tiempo de exposición. Además de la evidencia disponible donde la HI causa efectos adversos a nivel cardiovascular,[182] otros estudios han demostrado efectos beneficiosos de la HI tanto en modelos animales como en pacientes SAOS.[183] Los efectos opuestos inducidos por la HI dependen principalmente del tiempo experimental, donde una exposición prolongada (4-8 semanas) es requerida para causar efectos perjudiciales.[184] En el primer estudio del presente trabajo de tesis doctoral, evaluamos diferentes cambios cardiovasculares a nivel morfológico resultantes del efecto directo de 6 semanas de HI, un paradigma experimental común para imitar SAOS grave en pacientes. Sin embargo, no se habían desarrollado estudios dirigidos a evaluar la reversibilidad de las consecuencias cardiovasculares asociadas a la HI.

Para afrontar esta necesidad, llevamos a cabo el primer estudio de la tesis, dirigido a evaluar el grado de reversibilidad de las consecuencias cardiovasculares a nivel morfológico promovidas por la exposición a hipoxia intermitente cónica (HIC). Planteamos un modelo murino de recuperación ("Recovery model") que simulaba el tratamiento con CPAP u otro tratamiento efectivo de los pacientes SAOS, tal y como sucede en la clínica en aquellos pacientes que cumplen el tratamiento (tratamiento efectivo 4 horas o más). Nuestros resultados confirmaron la hipótesis de que la restauración de la respiración normal en condiciones de normoxia, eliminando el estrés de la HI, es suficiente para la recuperación cardiovascular homeostática.

Encontramos que la HI promovía una remodelación preaterosclerótica a nivel vascular, como se había evidenciado en estudios previos.[45,154,155,185,186] Esta remodelación consistía en un aumento del grosor intima-media, parámetro previamente establecido como indicador de riesgo cardiovascular en estadios tempranos de la enfermedad aterosclerótica,[187] que correlaciona con el grado de desaturación de O_2 e inflamación en pacientes SAOS.[72] Además, se observó una marcada remodelación de diferentes componentes de la matriz extracelular. En primer lugar se visualizó una alteración de las láminas de elastina que conforman un elemento clave en la arquitectura de la pared vascular. En segundo lugar se encontró un aumento en los niveles de tejido fibrótico y mucopolisacáridos en el espacio interlaminar de la pared vascular promovido por el estrés generado durante el período de HIC. Cambios en los componentes de la matriz

extracelular han sido implicados en la patogénesis aterosclerótica y juegan un papel importante en la comunicación intercelular, llevando a una respuesta fibroproliferativa y promoviendo la unión de los lípidos a la pared vascular y la formación de células espumosas.[188,189] Tanto el grosor intima-media como el equilibrio estructural de los componentes extracelulares elastina y colágeno son parámetros claves en definir la elasticidad de la pared vascular. Por lo tanto, estos resultados sugirieron que la HI estaría induciendo un aumento de la rigidez arterial que provocaría a su vez un incremento de la disfunción vascular y resistencia vascular sistémica promoviendo un aumento de la presión arterial crónica.

Aunque en nuestro estudio no se evaluaron los cambios en la presión arterial, dos estudios similares han descrito que la misma cepa de ratones C57BL6 exhiben un incremento de la presión arterial después de 14 y 90 días de exposición a HI.[129,184] Los aumentos de la presión arterial (10 a 20 mmHg) en modelos de roedores de HI son comparables con otros modelos experimentales de animales con hipertensión.[179] Por lo tanto, podríamos especular que los ratones expuestos a HI exhiben aumentos en la presión arterial que pueden inducir a su vez cambios funcionales, mecánicos y estructurales en la pared aórtica en respuesta al estrés hemodinámico y biomecánico.

Con una importante implicación clínica y aportando una valiosa información a la literatura, hallamos que una vez cesa la exposición a los ciclo de hipoxia-reoxigenación, las alteraciones morfológicas vasculares son revertidas. Al igual que sucede en pacientes SAOS tratados con CPAP, donde distintos estudios han evidenciado una reducción de la presión arterial[190], una mejora en la función endotelial[85,86,191] y vasodilatación[192], así como una reducción de la rigidez arterial y grosor intima-media.[62,193]

En cuanto a la remodelación del ventrículo izquierdo, nuestros resultados no mostraron hipertrofia de los cardiomiocitos, pero si un aumento de la fibrosis perivascular sin presencia de fibrosis intersticial, como diferentes autores ya habían descrito.[194] Un aumento de la fibrosis perivascular se ha asociado con un deterioro del flujo sanguíneo coronario y participa en la progresión de la insuficiencia cardíaca.[195] Debido a las asociaciones independientes significativas entre el SAOS y la insuficiencia cardíaca, muchos estudios han evaluado el efecto de la CPAP como tratamiento para los pacientes

con insuficiencia cardíaca.[196,197] En el presente estudio, observamos una normalización de la fibrosis perivascular coronaria después de la recuperación en condiciones normóxicas. La restauración de la normoxia fue suficiente para reducir la fibrosis perivascular, probablemente por la reducción de la respuesta inflamatoria y estrés oxidativo en el tejido miocárdico. Este hallazgo tiene relevancia clínica y sugiere el beneficio de la normalización respiratoria efectiva en pacientes con SAOS con enfermedades cardíacas, probablemente al mejorar el flujo sanguíneo coronario.

Desde un punto de vista traslacional, nuestros resultados sugirieron que una detección precoz de los pacientes SAOS y la posterior intervención terapéutica para normalizar la respiración, podría permitir una reversión de las alteraciones cardiovasculares tempranas que hayan podido experimentar y alterar el curso natural de las enfermedades cardiovasculares.

El segundo estudio de la tesis estuvo enfocado a resolver otro factor importante dentro del estudio de las consecuencias cardiovasculares asociadas a la HI inducida por el SAOS. La gran mayoría de investigación a nivel experimental se ha llevado a cabo en ratones jóvenes que corresponde a una edad de 20-30 años en humanos. Este punto abre una problemática en dos sentidos. Por un lado la prevalencia del SAOS es claramente mayor en edad adulta y en edad avanzada, aumentando a partir de 60 años.[198,199] Y por otro lado, el daño generado por la falta de oxígeno de forma intermitente a nivel tisular podría no ser el mismo en un tejido joven que está en crecimiento, que en un tejido longevo. Por esta razón, llevamos a cabo el segundo estudio con el fin de evaluar el rol de la edad en las consecuencias cardiovasculares a nivel morfológico. Específicamente ratones de 2 meses de edad (correlación 20-30 años en humanos), y ratones de 18 meses (correlación 60-70 años en humanos) fueron expuestos a HI durante 8 semanas.[200] Los resultados revelaron que el efecto de la HI a nivel cardiovascular es modulado por la edad. Al igual que el primer estudio de la tesis, que fue realizado en ratones jóvenes, observamos un aumento del grosor intima-media, de la desorganización y fragmentación de las láminas de elastina, así como un aumento de fibrosis y mucopolisacáridos en la pared vascular.[201] Esta remodelación vascular supone una pérdida del equilibrio de la elasticidad en la pared aórtica, y por lo tanto promueve rigidez arterial, aumento de la presión arterial, y remodelación aterogénica.[202,203] A nivel de tejido cardíaco, se observó un incremento de la

hipertrofia del ventrículo izquierdo, así como un aumento de la fibrosis perivascular en ratones jóvenes expuestos a HI, como habíamos evidenciado en el primer estudio.[204] Sin embargo, dicha remodelación aórtica y cardíaca no fue evidente en ratones viejos.

Por lo tanto evidenciamos que la susceptibilidad del tejido cardíaco y aórtico a un patrón grave de HI no es igual en diferentes edades. Estos hallazgos coinciden con los publicados por Quintero et al. [205], quienes muestran que el envejecimiento juega un papel protector contra los efectos deletéreos de la HIC. De hecho, estos investigadores informaron que la edad avanzada se asoció con un mejor estado redox y respuestas antioxidante a la HIC en ratas longevas en comparación con los animales jóvenes, una observación que puede explicar la protección relativa observada aquí contra los déficits vasculares. En el contexto de la extrapolación de los hallazgos actuales a la enfermedad humana, postulamos que la ausencia de remodelación cardiovascular causada por la HIC en ratones viejos puede explicar, al menos en parte, la menor mortalidad observada en pacientes con SAOS de más de 50 años de edad. [206–208] y las tasas de mortalidad más altas informadas en sujetos más jóvenes con SAOS grave en comparación con la población general.[116]

En este segundo estudio realizamos un análisis de componente principales y la clasificación jerárquica ascendente de los individuos permitió agrupaciones diferenciales de los grupos experimentales de acuerdo con las características cardiovasculares morfológicas. Dicho análisis separó tres clusters diferentes entre los ratones analizados. El cluster 1 correspondió a los ratones jóvenes expuestos a condiciones de normoxia, el cluster 2 separaba claramente a los ratones jóvenes expuestos a HI y finalmente el tercer cluster agrupaba a los ratones de edad avanzada que habían estado tanto en normoxia como en condiciones de HIC. Dichos resultados sugirieron en primer lugar que el impacto de la HI era atenuado por la edad avanzada, ya que no fue evidente un daño cardiovascular asociado a la HI en ratones longevos. En segundo lugar, el cluster 2 correspondiente a los ratones jóvenes se encontraba desplazado en la dimensión hacia el mismo plano que el cluster 3, correspondiente a ambos grupos de ratones de edad avanzada. Por lo tanto, nuestro resultados sugirieron que la HIC podría estar promoviendo un envejecimiento cardiovascular precoz en edades tempranas.

Con el objetivo de profundizar en los mecanismos moleculares involucrados en la remodelación cardiovascular morfológica estudiada en el Estudio 2 de la tesis, llevamos a cabo el Estudio 3. En dicho estudio evaluamos el rol de la edad en el impacto de la HIC en regular la expresión génica a nivel cardíaco y vascular. Para ello nos centramos en estudiar intermediarios moleculares que promueven o atenúan los procesos celulares de estrés oxidativo, inflamación, así como envejecimiento y respuesta a estrés celular.

Los resultados obtenidos mostraron que el efecto de la HIC en la expresión génica es modulado por la edad y es específica de tejido. La activación de vías de señalización involucradas en promover supervivencia celular de los cardiomiocitos es fundamental dentro de los mecanismos de respuesta adaptativa ante un estímulo estresor en el tejido de miocardio, ya que además de ser susceptible al estrés, muestra una baja capacidad de regeneración y reparación después de ser dañando. A nivel de tejido cardíaco, la HIC indujo la expresión de genes involucrados en promover cardioprotección, siendo evidente en ratones jóvenes, pero no en ratones longevos. Se observó una respuesta antioxidante mediada por Nrf2, principal factor de transcripción de genes que median distintas reacciones que neutralizan ROS y reducen su producción, como es la enzima Sod2, también aumentada su expresión en ratones jóvenes expuestos a HIC. Un incremento en la expresión de eNOS sugiere un aumento de la producción de óxido nítrico a nivel del endotelio de los vasos cardíacos, importante para la vasodilatación y neutralización de ROS. Además, se observó un aumento de la expresión génica del factor de transcripción FoxO3 y de las sirtuinas, ambos promueven supervivencia celular mediando adaptaciones anti-estrés, anti-oxidante y anti-apoptóticas. Estos resultados son consistentes con hallazgos previos que muestran una respuesta antioxidante frente a la generación de estrés oxidativo inducido por la HIC[209–211]. Otro de los mecanismos de cardioprotección observado en los ratones jóvenes expuestos a HIC es la sobreregulación de la vía IGF-1R/mTORC que media una respuesta adaptativa de hipertrofia fisiológica acompañado de acciones anti-apoptóticas y de supervivencia celular. De hecho, esta respuesta adaptativa de hipertrofia cardiaca fisiológica se encontró acompañada con una reducción de los niveles de angiotensina II, BNP and Gdf-15 inducida por la HI. [212]

Los mecanismos moleculares encontrados pueden explicar en parte el precondicionamiento isquémico asociado a la HI, en donde diversos estudios han

demostrado que la exposición a HI reduce el daño cardíaco asociado a futuros eventos isquémicos.[213,214215] Sin embargo, una exposición prolongada a HI puede pasar de ser una respuesta adaptativa, como puede ser la hipertrofia, una maladaptación que comprometa la función cardiaca.[180] Nuestros resultados muestran un aumento de la expresión de p53 en ratones jóvenes expuestos a HI y supone un nuevo mecanismo molecular asociado a la transición de una respuesta adaptativa a una respuesta maladaptativa a la HI. Un reciente estudio muestra que antes un estímulo estresor, los cardiomiocitos presentan una remodelación morfológica adaptativa y activan un módulo de respuesta a estrés oxidativo mediado principalmente por el factor de transcripción Nrf2, tal y como hemos observado en los ratones jóvenes expuestos a HIC. De esta manera los cardiomiocitos pueden regular la biogénesis mitocondrial para producir más ATP, esencial para afrontar los cambios morfológicos asociados a la hipertrofia. Sin embargo, un estímulo sostenido induce un aumento del daño oxidativo en el DNA, aumentando la expresión de p53. Un aumento de p53 se ha asociado con un aumento de la heterogeneidad transcripcional e induce la expresión de programas de genes patogénicos que promueven la transición de un modulo adaptativo de hipertrofia hacia un modulo de fallo cardíaco.[216]

A pesar de la repuesta cardioprotectora observada a nivel de tejido cardiaco el impacto de la HI a nivel vascular es diferente, sugiriendo que es específico de tejido. De forma independiente de la edad, la HIC redujo el sistema antioxidante y la producción de óxido nítrico, suponiendo un aumento del estrés oxidativo y la vasoconstricción de la vasculatura. Un incremento persistente del estrés oxidativo podría inducir estrés oxidativo crónico y causar daño en estructuras intracelulares, siendo uno de los principales mecanismos responsables de envejecimiento y senescencia vascular, así como riesgo aterosclerótico.[217] El daño oxidativo en el tejido vascular puede pasar a ser irreversible incluso si el estrés hipóxico es resuelto, como se ha visto previamente que la remodelación proaterogénica inducida por 8 semanas de HIC no es reversible tras un período en condiciones de normoxia simulando el tratamiento efectivo del SAOS [218], pudiendo suceder tanto en edades jóvenes como en adultos de edad avanzada. Aunque en el Estudio 2 de la tesis observamos que la remodelación morfológica vascular inducida por la HI no era evidente en ratones de edad avanzada, en el Estudio 3 confirmamos que a

nivel molecular, los ratones de edad avanzada también son susceptibles a la generación de ROS, tal y como sucede en ratones jóvenes.

Finalmente, el Estudio 4 se focalizó en evaluar el efecto del tratamiento con CPAP en pacientes con SAOS moderado-grave de nuevo diagnóstico. Como consecuencia del aumento de la presión arterial nocturna y diurna en pacientes con SAOS, muestran una alteración en el patrón circadiano de presión arterial y una mayor incidencia de hipertensión nocturna[105,106], que está asociada con daño en el órgano diana y el empeoramiento de los resultados cardiovasculares[106–108]

Según varios metanálisis, el tratamiento con CPAP reduce la presión arterial en pacientes normotensos e hipertensos con SAOS[109–112]. Sin embargo, el impacto del tratamiento con CPAP en la presión arterial no es regular. En pacientes mínimamente sintomáticos, la CPAP tiene un efecto neutral sobre la presión arterial[112], mientras que en sujetos con hipertensión resistente, la CPAP puede disminuir la presión arterial sistólica en 5–7 mmHg[113]. Además, aunque el efecto del tratamiento con CPAP sobre la presión arterial está relacionado con el cumplimiento del tratamiento, existe una variabilidad individual que podría estar relacionada con los factores epigenéticos, al menos en parte[114].

Según estos datos, más allá del efecto variable de la CPAP sobre la presión arterial en pacientes con SAOS, es necesaria la identificación de los perfiles clínicos y biológicos que mejor predicen la respuesta de la presión arterial. El interés debe centrarse en los efectos de la presión arterial nocturna, dado su potente valor predictor de morbimortalidad cardiovascular[219]. Para abordar este problema, diseñamos un estudio pre-post para identificar las características clínicas al inicio del estudio que podrían permitirnos discriminar a los pacientes que se beneficiarían de la CPAP de aquellos pacientes que no se beneficiarían del tratamiento con CPAP.

La principal contribución del estudio fue la posible identificación, a través de un MAPA-24hs, de pacientes SAOS que tras el tratamiento con CPAP mostrarán una disminución favorable en la presión arterial. Se observó una reducción de la presión arterial con el tratamiento con CPAP en pacientes con hipertensión nocturna y en pacientes no-dippers que cumplían el tratamiento (4h≥CPAP). Sin embargo, se observó un aumento de la

presión arterial en pacientes normotensos nocturnos y en pacientes con frecuencia cardíaca baja, incluso entre los que cumplen el tratamiento con CPAP.

Por lo tano, biomarcadores hemodinámicos a nivel basal, específicamente la hipertensión nocturna, el patrón circadiano de la presión arterial y la frecuencia cardíaca nocturna, podrían ser importantes factores clínicos predictivos de la respuesta de la presión arterial al tratamiento con CPAP en pacientes con SAOS grave. La identificación de pacientes que podrían verse afectados negativamente por el tratamiento con CPAP podría prevenir aumentos adversos en los parámetros hemodinámicos cardiovasculares y consecuencias cardiovasculares negativas relacionadas a largo plazo, particularmente en pacientes asintomáticos. De acuerdo con los resultados obtenidos, incorporar en el manejo clínico de los pacientes SAOS la realización de un MAPA-24h podría representar una herramienta de carácter predictivo de respuesta previa al inicio del tratamiento con CPAP. Este hallazgo evidencia la necesidad de disponer de información a nivel de cada perfil de pacientes con SAOS para poder estimar adecuadamente el efecto de la intervención con CPAP para el tratamiento del SAOS y su impacto en distintas variables cardiovasculares.

En resumen, el tratamiento del SAOS debe ser integral y personalizado para hacer frente a la complejidad fisiopatológica del individuo, sumando fuerzas para reducir además de la sintomatología clínica asociada al SAOS, el riesgo cardiovascular y prevenir complicaciones posteriores. Es incuestionable la importancia del diagnóstico precoz de los pacientes SAOS, la educación en salud del sueño y la activación de medidas terapéuticas integrales y personalizadas para reducir al máximo el riesgo ligado a las alteraciones a nivel genómico, epigenético, proteómico y metabolómico que se desarrollan secundarias a la cronicidad de los eventos hipóxicos.

CONCLUSIONES

Las conclusiones de la tesis son:

1). La hipoxia intermitente crónica induce una remodelación pre-aterosclerótica y una remodelación cardíaca inicial en un modelo murino de apnea del sueño. Dicha remodelación cardiovascular es normalizada tras un periodo en condiciones de normoxia, simulando el tratamiento efectivo del SAOS:

2). La edad modula la remodelación cardiovascular inducida por hipoxia intermitente crónica simulando apnea del sueño grave, en donde la edad avanzada es menos suceptible al efecto deletéreo de la hipoxia intermitnete. Los ratones jóvenes mostraron alteraciones cardiovasculares a nivel estructural similares a los ratones longevos, sugiriendo un posible envejecimiento cardiovascular prematuro asociado al SAOS.

3) El impacto en la expresión génica de marcadores a nivel cardiovascular inducido por la hipoxia intermitente crónica es modulado por la edad y es específico de órgano. La hipoxia intermitente activa vías de cardioprotección en ratones jóvenes, pero no en ratones longevos, mediado principalmente por un aumento de la respuesta antioxidante y respuesta a estrés. A nivel vascular, la hipoxia intermitente induce un aumento del estrés oxidativo tanto en ratones jóvenes como longevos.

4) La hipertensión nocturna, el patrón circadiano de la presión arterial y la frecuencia cardíaca nocturna son varibales hemodinámicas que pueden ser importantes predictores clínicos del tratamiento con CPAP en términos de presión arterial nocturna en pacientes con SAOS.

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