Cardiac consequences of intermittent hypoxia: a matter of dose? A systematic review and meta-analysis in rodents

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Shareable abstract (@ERSpublications)
This meta-analysis shows that IH induces cardiac remodelling and contractile dysfunction in rodents, independently of IH characteristics. Conversely, the dual response to myocardial ischaemia–reperfusion seems to be related to IH intensity and duration. https://bit.ly/3rdnR32

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Abstract

Aim Intermittent hypoxia (IH) is considered to be a major contributor to obstructive sleep apnoea-related cardiovascular consequences. The present meta-analysis aimed to assess the effects of IH on cardiac remodelling, function and infarct size after myocardial ischaemia across different rodent species and IH severities.

Methods and results Relevant articles from PubMed, Embase and Web of Science were screened. We performed a random effect meta-analysis to assess the effect of IH on myocardium in rodents by using standardised mean difference (SMD). Studies using rodents exposed to IH and outcomes related to cardiac remodelling, contractile function and response to myocardial ischaemia–reperfusion were included. 5217 articles were screened and 92 were included, demonstrating that IH exposure induced cardiac remodelling, characterised by cardiomyocyte hypertrophy (cross-sectional area: SMD=2.90, CI (0.82–4.98), I^2=94.2%), left ventricular (LV) dilation (LV diameter: SMD=0.64, CI (0.18–1.10), I^2=88.04%), interstitial fibrosis (SMD=5.37, CI (3.22–7.53), I^2=94.8) and apoptosis (terminal deoxynucleotidyl transferase dUTP nick end labelling: SMD=6.70, CI (2.96–10.44), I^2=95.9). These structural changes were accompanied by a decrease in LV ejection fraction (SMD=−1.82, CI (−2.52–−1.12), I^2=94.22%). Importantly, most of the utilised IH protocols mimicked extremely severe hypoxic disease. Concerning infarct size, meta-regression analyses highlighted an ambivalent role of IH, depending on its severity. Indeed, IH exposure with inspiratory oxygen fraction (F_{IO_2}) <7% was associated with an increase in infarct size, whereas a reduced infarct size was reported for F_{IO_2} levels above 10%. Heterogeneity between studies, small study effect and poor reporting of methods in included articles limited the robustness of the meta-analysis findings.

Conclusion This meta-analysis demonstrated that severe IH systematically induces cardiac remodelling and contractile dysfunction in rodents, which might trigger or aggravate chronic heart failure. Interestingly, this meta-analysis showed that, depending on stimulus severity, IH exhibits both protective and aggravating effects on infarct size after experimental ischaemia–reperfusion procedures.

Introduction

Obstructive sleep apnoea (OSA), the most common sleep-disordered breathing (SDB) disorder, is one of the most frequent chronic diseases, affecting nearly one billion people worldwide [1]. OSA is recognised as an independent risk factor for incident and prevalent cardiovascular diseases, including hypertension, arrhythmia, stroke and coronary heart disease [2, 3]. OSA is characterised by the repetitive occurrence of partial or complete upper airway obstruction during sleep, which leads to consequences that include: 1) sleep fragmentation, due to the occurrence of micro-arousal at the end of each apnoea/hypopnoea, 2) intra-thoracic pressure swings and 3) repetitive cycles of arterial oxygen desaturation and reoxygenation, namely intermittent hypoxia (IH), with underlying cardiovascular complications.

During the past two decades, numerous experimental and clinical reports have considered IH to be the main contributor to OSA-associated cardiovascular complications and mortality [4–6]. Systematic reviews and meta-analyses have reported that OSA patients exhibit increased left ventricular (LV) hypertrophy, dilation and subclinical markers of LV diastolic dysfunction in accordance with the severity of IH [7–9]. OSA-associated cardiac remodelling results in impaired LV systolic function (*i.e.* decreased LV ejection fraction (EF)), which also correlates with OSA severity. In patients hospitalised for acute coronary syndrome (ACS), SDB is associated with higher peak troponin levels in plasma, which might reflect increased myocardial insults [10]. Buchner *et al.* [11] demonstrated that patients with SDB exhibit a larger infarct size and impairment of ventricular remodelling after myocardial infarction compared to patients without SDB. These clinical studies highlight the potential deleterious impact of IH on both cardiac remodelling and susceptibility to myocardial ischaemia. In contrast, several studies did not report such detrimental effects of OSA on cardiovascular outcomes. For example, the recent ISAACC study failed to demonstrate that OSA patients with ACS exhibit a higher recurrence of cardiovascular events compared with patients without OSA [12]. In addition, large clinical trials did not evidence any beneficial effects of continuous positive airway pressure against cardiovascular events incidence (SAVE [13] and ISAAC [12, 14]), questioning the exact contribution of OSA to cardiovascular morbi-mortality.

Currently, OSA severity is essentially based on the apnoea–hypopnoea index, a metric that poorly reflects the severity of IH. More and more studies are suggesting that the burden of nocturnal hypoxaemia might represent the key predictor of cardiovascular risk in OSA patients [15–17]. Animal models of IH exposure in rodents have been developed in order to dissect the specific impact of IH on the cardiovascular system, due to the absence of any of the confounding factors usually present in clinical trials. These experimental studies aim to understand the mechanisms involved in different IH-controlled conditions (cycles, intensity, duration). These preclinical studies also allow easy access to tissue samples, facilitating the characterisation of cardiac remodelling at cellular and tissue level.

This systematic review and meta-analysis aimed to assess the effects of IH on cardiac remodelling, function and response to myocardial ischaemia across all rodent species. This might help to identify whether specific IH patterns would have different impacts on the myocardium.

Methods

The methodology was described and preregistered in PROSPERO (www.crd.york.ac.uk/prospero/, CRD42020170266, 24 April 2020) and a Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement is given in supplementary file S1. The review questions were: 1) does IH induce cardiac remodelling, contractile dysfunction and alter the response to myocardial ischaemia–reperfusion in rodent models? and 2) does the hypoxic pattern (cycles, intensity, duration per day and total duration) influence cardiac responses?

Study search and selection

Articles published between 1980 and May 2021 were searched using the PubMed, Embase and Web of Science databases. A first selection was conducted to select all studies that used IH in rodents, with the following terms: (Intermittent[All Fields] AND (“hypoxia”[MeSH Terms] OR “hypoxia”[All Fields])) AND ((“rodentia”[MeSH Terms] OR “rodentia”[All Fields] OR “rodent”[All Fields]) OR (“mice”[MeSH Terms] OR “mice”[All Fields] OR “mouse”[All Fields]) OR (“rats”[MeSH Terms] OR “rats”[All Fields] OR “rat”[All Fields])). Two investigators (OH, CA, EB, ABM or GF) independently screened all references for inclusion, and discrepancies were resolved by discussion among the team. Eligibility was considered if they were written in English and addressed myocardial outcomes after IH exposure in rodents. The full texts of all studies referring to myocardium were reviewed for final inclusion by two independent investigators (CA and EB).

Animals and IH protocols

We included studies using rodents (male, female, young, aged, lean, obese, various strains) exposed to IH and reporting outcomes related to cardiac remodelling, contractile function and response to myocardial ischaemia–reperfusion (infarct size). We defined IH as one period of hypoxia, followed by one period of reoxygenation, repeated several times during the same day. We included all studies in which IH was compared to a control normoxic group.

Exclusion criteria

We excluded studies that were not conducted on rodents (*i.e.* cell culture models), studies in which hypoxia was applied continuously and repeated for several days, studies applying IH exposure in prenatal or perinatal periods, studies without a separate control group, and studies without cardiac outcomes.

Outcome extraction

A standardised Excel file was used to extract data. Data from graphs were extracted using ImageJ tools.

For each study, we extracted bibliographic details (first author, journal, PubMed identifier, year), rodent characteristics (species, strain, sex, initial body weight, age), as well as IH pattern description (inspiratory oxygen fraction (F_{IO_2}) (%), hypoxia duration (seconds), reoxygenation duration (seconds), duration of IH protocol per day (hours), total duration of IH exposure (days)).

As main outcomes, we selected the most often used parameters to characterise haemodynamic, cardiac contractile function, cardiac remodelling and response to myocardial ischaemia–reperfusion. An exhaustive list of extracted parameters is provided in supplementary file S2. For haemodynamic evaluation, we extracted mean arterial pressure (MAP) and heart rate (HR) data. Cardiac function was assessed through analysis of LV EF and LV diameter in diastole (LVDd) obtained from echocardiography. Cardiac remodelling was documented by analysis of hypertrophy (heart weight-to-tibia length and cardiomyocyte cross-sectional area (CSA)), interstitial fibrosis (Sirius red and Masson trichrome staining and connective tissue growth factor (CTGF) expression) and apoptosis (terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) staining and caspase 3 (Casp3) expression). Finally, infarct size was used to evaluate the response to myocardial ischaemia–reperfusion. For all outcomes, data were extracted as number of animals per group, mean and standard deviation (SD). Each protocol from one study was extracted as a separate group.

Risk of bias assessment

Risk of bias and study quality were assessed by two investigators (CA and EB). Risk of bias was evaluated in accordance with the SYstematic Review Center for Laboratory animal Experimentation (SYRCLE) recommendations [18] and, accordingly, bias was classified into selection bias (sequence generation, baseline characteristics, allocation concealment), performance bias (randomised housing of animals, blinding of investigators), detection bias (random outcome assessment, blinding of outcome assessor), attrition bias (incomplete outcome data) and reporting bias (selective outcome reporting). Each risk of bias was scored as high, low or unclear.

Statistical analysis

For each outcome, data were abstracted and analysed using SMD, SMD=(Mc−Me)/SD, where Mc is the mean of the outcome measure in the control group, Me is the mean of the outcome measure in the experimental groups and SD is the pooled standard deviation of the two groups [19]. An effect size of 0.8 was considered large, 0.5 moderate and 0.2 small [20]. In cases of missing SD values, we tried to calculate or estimate them from confidence intervals, standard errors, t-values, p-values or F-values. The remaining SD values were imputed using the mean outcome-specific SD from the other included studies.

Given the high anticipated heterogeneity in the included studies, we performed random effect meta-analysis through the restricted maximum-likelihood estimator method. To account for correlation among different groups (n, IH, different patterns, *etc*.), we used a hierarchical model with a random intercept between groups and studies.

All results were represented using the orchard plot, an innovative data visualisation tool used for displaying the results of a large number of outcomes [21].

We explored sources of heterogeneity through prespecified subgroup analyses and meta-regressions according to population (species, strain, sex, body weight, year of publication, age) and IH protocols (F_{IO_2} (%), hypoxia duration (seconds), reoxygenation duration (seconds), duration of IH protocol per day (hours), total duration of IH exposure (days)).

First, we performed univariate meta-regressions on study and animal characteristics. Then, we added the significant predictors ($p<0.2$) in the meta-regression model for IH protocol parameters.

Funnel plot asymmetry was explored using Egger's regression test, with $p<0.05$ suggesting publication bias [22]. Trim and fill analysis was performed to assess the robustness of the results [23]. All statistical analyses were performed using R statistical software with the Meta and Metafor packages (version 3.6.1)

Results

Search and study selection

Our initial searches identified 2583 articles from PubMed, 3387 from Embase and 4603 from Web of Science. After duplicate removal, 5127 articles were screened based on titles and abstracts. 227 articles

were eligible for full-text assessment and, among them, 135 were excluded as they did not reach all the prespecified criteria (*i.e.* inappropriate protocols, outcome out of interest, peri- or pre-natal studies). Finally, 92 articles were included (figure 1 and supplementary file S3).

Study characteristics

Characteristics of the 92 studies included in the analysis are summarised in table 1. 87 studies used male animals, one study used a female animal and four did not report animal sex. 35 studies (40%) were conducted in mice and 57 (60%) in rats. In the mice studies, C57bl6J mice were used in 28 studies, FVB and Swiss×129 mice in seven studies, and ob/ob mice in two studies. In the rat studies, 38 studies used Sprague–Dawley rats, 16 used Wistar rats, two used spontaneously hypertensive rats and Wistar Kyoto rats, and two studies did not report the rat strain used.

IH characteristics are described in figure 2. The median values of F_{IO_2} during hypoxia periods were 5.5% (min–max 4–10%), with hypoxia duration of 30 s (min–max 10–360 s), followed by 45 s (min–max 20–360 s) of reoxygenation. Cycles were repeated in average for 8 $h \cdot day^{-1}$ (min–max 0.5–12 $h \cdot day^{-1}$) for a median duration of 28 days (min–max 1–95 days).

Risk of bias and study quality

Risk of bias and study quality were assessed using the SYRCLE risk of bias tool for animal studies [18] and the results are reported in figure 3 (overall score) and supplementary file S4 (individual study quality).

Risk of bias was lowest for risks related to selection, such as sequence generation, baseline characteristics and allocation concealment (figure 3). Of note, risk of bias was poorly described across the majority of the studies we analysed and were therefore reported as "unclear" risk (figure 3 and supplementary file S4).

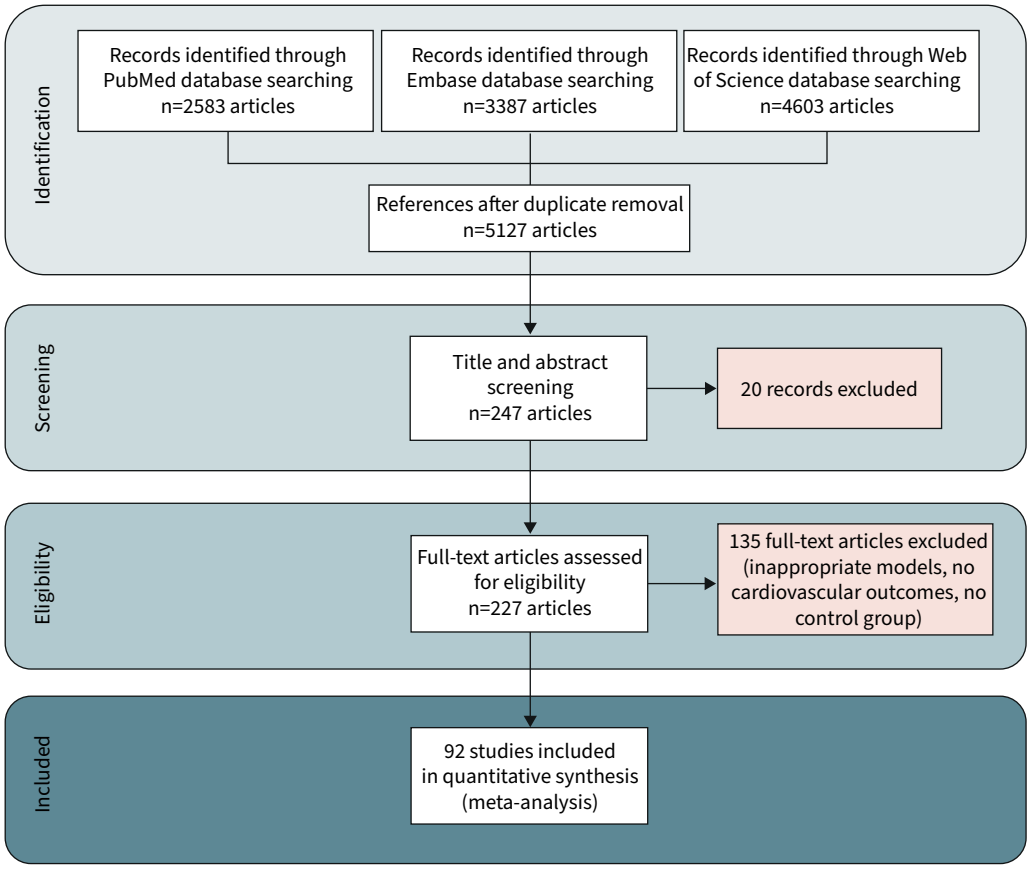

FIGURE 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) study flowchart.

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TABLE 1 Study characteristics

Article[#]	Animal characteristics					IH protocol characteristics					Haemodynamic parameter	Cardiac function	Cardiac remodelling							Response to ischaemia
	Species	Strain	Sex	Body weight (g)	Age (weeks)	F_{IO_2} (%)	Hypoxia/ Reox (s)	IH duration (h per day)	Protocol duration (day)	MAP	HR	Ejection fraction	Structure	Hypertrophy		Fibrosis		Apoptosis		Infarct size
													LVDd	HW/TL	CSA	PVF	IF	Casp3	TUNEL	
Bao *et al.* 2020 [S1]	Mice	C57Bl6J	M	ND	9	5	30/30	12	28			x		x	x		x		x	
Béguin *et al.* 2005 [S2]	Rat	Wistar	M	350	ND	5–10	40/20	0.5–4.0	1											x
Béguin *et al.* 2007 [S3]	Rat	Wistar	M	350	ND	10	40/20	4	1											x
Belaidi *et al.* 2008 [S4]	Rat	Wistar	M	340	ND	10	40/20	4	1											x
Belaidi *et al.* 2009 [S5]	Rat	SHR and WKY	M	ND	9	5	40/20	8	14	x										x
Belaidi *et al.* 2016 [S6]	Mice	S129 and C57Bl6J	M	ND	8	5	30/30	8	14											x
Bober *et al.* 2018 [S7]	Rat	SD	M	340	ND	6.5	80/120	8	7											
Bourdier *et al.* 2016 [S8]	Rat	Wistar	M	325	8	5	30/30	8	21	x	x							x		x
Cai *et al.* 2003 [S9]	Mice	ND	M	25	ND	6	360/360	1	1											x
Castro-Grattoni *et al.* 2016 [S10]	Mice	C57Bl6J	M	ND	6	5	20/40	6	42						x	x				
Castro-Grattoni *et al.* 2019 [S11]	Mice	C57Bl6J	F	ND	8–72	5	20/40	6	56							x				
Chen *et al.* 2005 [S12]	Rat	SD	M	250	ND	5	30/30	8	35	x	x	x	x							
Chen *et al.* 2008 [S13]	Rat	SD	M	185	ND	5	20/100	8	42	x	x							x	x	
Chen *et al.* 2010 [S14]	Mice	C57Bl6J	M	ND	26	4.5	45	10	56	x	x	x	x		x		x		x	
Chen *et al.* 2011 [S15]	Rat	SD	M	290	9	4	30/45	6	12									x		
Chen *et al.* 2011 [S16]	Rat	WKY and SHR	M	255	9	4	30/45	6	10–30									x		
Chen *et al.* 2015 [S17]	Rat	SD	M	325	9	6	30/45	6	12			x	x							
Chen *et al.* 2016 [S18]	Rat	SD	M	ND	9	4	30/45	8	14			x	x							
Chen *et al.* 2016 [S19]	Rat	SD	M	ND	16	4	30/45	8	14								x			
Del Rio *et al.* 2016 [S20]	Rat	SD	M	250	ND	5	ND	8	28	x	x						x			

Continued

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TABLE 1 Continued

Article[#]	Animal characteristics					IH protocol characteristics					Haemodynamic parameter	Cardiac function	Cardiac remodelling							Response to ischaemia
	Species	Strain	Sex	Body weight (g)	Age (weeks)	F_{IO_2} (%)	Hypoxia/ Reox (s)	IH duration (h per day)	Protocol duration (day)	MAP	HR	Ejection fraction	Structure	Hypertrophy		Fibrosis		Apoptosis		Infarct size
													LVDd	HW/TL	CSA	PVF	IF	Casp3	TUNEL	
Détrait *et al.* 2020 [S21]	Mice	C57Bl6J	M	ND	19	5	30/30	8	28–42		x	x	x	x	x		x			
Ding *et al.* 2014 [S22]	Rat	Wistar	M	215	8	5.5	60/60	8	35		x	x	x					x	x	
Ding *et al.* 2014 [S23]	Rat	Wistar	M	ND	8	5.5	20/100	ND	35								x			
Du *et al.* 2019 [S24]	Mice	C57Bl6J	M	ND	4	5.5	120/60	12	56			x								
Farré *et al.* 2018 [S25]	Mice	C57Bl6J	ND	ND	8–72	6	20/40	6	42											
Fu *et al.* 2016 [S26]	Rat	SD	M	225	9	4.5	120/120	ND	21–56			x	x						x	
Guan *et al.* 2019 [S27]	Rat	SD	M	205	ND	9	15/90	8	35			x			x		x		x	
Guo *et al.* 2015 [S28]	Rat	Wistar	M	210	8	7	120/120	8	7–28	x										
Han *et al.* 2014 [S29]	Rat	SD	M	151	ND	10	240/120	6	14–28									x		
Han *et al.* 2018 [S30]	Rat	SD	M	165	ND	10	240/120	8	42			x	x				x	x	x	
Hayashi *et al.* 2008 [S31]	Mice	C57Bl6J	M	ND	10	5	30/30	8	10	x		x	x				x			
Hayashi *et al.* 2011 [S32]	Mice	C57Bl6J	M	ND	8	5	60/60	8	7						x	x				
Imano *et al.* 2018 [S33]	Mice	C57Bl6J	M	ND	8	5	90/300	8	28			x	x		x	x				
Inamoto *et al.* 2010 [S34]	Mice	C57Bl6J	M	ND	8	5	30/30	8	10							x				
Jiang *et al.* 2020 [S35]	Rat	SD	M	225	ND	5	90/90	8	28			x	x					x		
Joyeux-Faure *et al.* 2005 [S36]	Rat	Wistar	M	230	ND	5	40/20	8	35	x										x
Lai *et al.* 2015 [S37]	Mice	C57Bl6J	ND	ND	22	7	60/60	8	56				x	x					x	
Lai *et al.* 2015 [S38]	Mice	C57Bl6J	M	ND	22	7	60/60	8	56				x	x				x	x	
Li *et al.* 2016 [S39]	Rat	Wistar	M	ND	16	5	30/90	8	28			x								
Li *et al.* 2017 [S40]	Rat	SD	M	225	ND	9	240/350	8	42						x		x		x	
Lu *et al.* 2020 [S41]	Rat	SD	M	200	6	7.5	90/90	8	42							x				

Continued

TABLE 1 Continued

Article[#]	Animal characteristics					IH protocol characteristics					Haemodynamic parameter	Cardiac function	Cardiac remodelling							Response to ischaemia
	Species	Strain	Sex	Body weight (g)	Age (weeks)	F_{IO_2} (%)	Hypoxia/ Reox (s)	IH duration (h per day)	Protocol duration (day)	MAP	HR	Ejection fraction	Structure	Hypertrophy		Fibrosis		Apoptosis		Infarct size
													LVDd	HW/TL	CSA	PVF	IF	Casp3	TUNEL	
Lucking *et al.* 2014 [S42]	Rat	Wistar	M	225	ND	5	90/210	8	14	x	x		x							
Ma *et al.* 2020 [S43]	Rat	SD	M	190	8	8	210/90	6	42	x		x	x				x			
Maeda *et al.* 2013 [S44]	Rat	SD	M	ND	7	4	ND	8	21		x									
Matsumoto *et al.* 2009 [S45]	Mice	C57Bl6J	M	ND	10	5	30/30	8	10							x				
Milano *et al.* 2013 [S46]	Mice	C57Bl6J	M	ND	9	7	180/120	1.7	14											x
Morand *et al.* 2018 [S47]	Rat	Wistar	M	340	8	5	30/30	8	14	x	x									
Moreau *et al.* 2015 [S48]	Rat	SD	M	ND	ND	6.5	80/120	8	7–95	x	x									
Moulin *et al.* 2020 [S49]	Mice	Swiss×S129	M	ND	ND	5	30/30	8	21										x	x
Moulin *et al.* 2020 [S50]	Mice	Swiss×S129	M	ND	10	5	30/30	8	21										x	x
Naghshin *et al.* 2009 [S51]	Mice	C57Bl6J	M	23	11	5	30/30	12	28		x	x	x							
Naghshin *et al.* 2012 [S52]	Mice	FVB	M	ND	11	5.5	30/30	12	28		x	x								
Nakagawa *et al.* 2019 [S53]	Mice	ND	M	ND	ND	5	60/300	8	14											
Nishioka *et al.* 2013 [S54]	Mice	C57Bl6J	M	ND	8	5	30/30	8	10		x					x				
Pai *et al.* 2016 [S55]	Rat	SD	M	ND	20	5	40/40	8	28		x		x	x					x	
Park *et al.* 2007 [S56]	Mice	C57Bl6J	M	ND	10	6	120/120	8	7–28	x	x									x
Ramirez *et al.* 2012 [S57]	Rat	SD	M	ND	ND	10	180/180	8	7						x	x				
Ramond *et al.* 2007 [S58]	Rat	Wistar	M	300	ND	5	40/20	4	1											x
Ramond *et al.* 2013 [S59]	Rat	Wistar	M	300	ND	5	30/30	8	14	x										x
Ray *et al.* 2015 [S60]	Rat	Wistar	M	ND	ND	5	90/330	8	14	x	x				x					
Rodriguez *et al.* 2014 [S61]	Mice	C57Bl6J and ob/ob	M	ND	10	5.5	30/30	12	28		x		x							
Sun *et al.* 2020 [S62]	Rat	SD	M	220	ND	9	15/30	8	35			x							x	

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TABLE 1 Continued

Article[#]	Animal characteristics					IH protocol characteristics					Haemodynamic parameter	Cardiac function	Cardiac remodelling							Response to ischaemia
	Species	Strain	Sex	Body weight (g)	Age (weeks)	F_{IO_2} (%)	Hypoxia/ Reox (s)	IH duration (h per day)	Protocol duration (day)	MAP	HR	Ejection fraction	Structure	Hypertrophy		Fibrosis		Apoptosis		Infarct size
													LVDd	HW/TL	CSA	PVF	IF	Casp3	TUNEL	
Tao *et al.* 2019 [S63]	Mice	C57Bl6J	M	ND	8	5	30/30	8	28			x					x	x	x	
Tong *et al.* 2019 [S64]	Mice	C57Bl6J	M	22	6	5	20/40	8	13							x				
Totoson *et al.* 2013 [S65]	Rat	Wistar	M	290	ND	5	30/30	8	14–28											x
Wang *et al.* 2013 [S66]	Rat	Wistar	M	250	ND	7.6	30/30	4	1–28										x	x
Wang *et al.* 2017 [S67]	Rat	SD	ND	260	ND	9	90/90	8	21											
Wang *et al.* 2017 [S68]	Rat	SD	M	200	ND	9	90/90	8	21		x		x							
Wang *et al.* 2018 [S69]	Mice	C57Bl6J	M	23	ND	5.5	60/60	8	84										x	
Wang *et al.* 2018 [S70]	Rat	SD	M	225	9	8	90/210	5	28			x	x				x			
Wang *et al.* 2020 [S71]	Mice	C57Bl6J	M	ND	8	8	20/40	12	56			x	x	x			x			
Wei *et al.* 2017 [S72]	Rat	SD	M	250	10	7.6	30/30	8	35			x	x							
Williams *et al.* 2010 [S73]	Rat	SD	M	180	ND	5	30/30	8	10		x		x						x	
Xie *et al.* 2015 [S74]	Rat	SD	M	200	ND	8	240/350	8	42						x		x		x	
Xu *et al.* 2015 [S75]	Rat	SD	M	200	ND	8.5	10/80	8	35	x										
Yang *et al.* 2011 [S76]	Mice	C57Bl6J and ob/ob	M	ND	10	5	30/30	12	28			x	x	x						
Yang *et al.* 2018 [S77]	Rat	SD	M	200	8	6	120	8	42			x	x					x	x	
Yang *et al.* 2019 [S78]	Rat	SD	M	200	9	6	80/40	8	42											
Yeung *et al.* 2015 [S79]	Rat	SD	M	70	4	5	15/45	8	28								x			x
Yin *et al.* 2014 [S80]	Mice	FVB and 129S1	M	ND	9	8	20/100	12	7–56			x	x	x						
Yuan *et al.* 2014 [S81]	Rat	Wistar	M	200	ND	5	30/30	8	42											
Yuan *et al.* 2015 [S82]	Rat	SD	M	235	ND	8	240/350	8	42										x	
Zhang *et al.* 2018 [S83]	Rat	SD	M	300	ND	8	ND	6	30			x	x				x			

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TABLE 1 Continued

Article[#]	Animal characteristics					IH protocol characteristics					Haemodynamic parameter	Cardiac function	Cardiac remodelling							Response to ischaemia
	Species	Strain	Sex	Body weight (g)	Age (weeks)	F_{IO_2} (%)	Hypoxia/ Reox (s)	IH duration (h per day)	Protocol duration (day)	MAP	HR	Ejection fraction	Structure	Hypertrophy		Fibrosis		Apoptosis		Infarct size
													LVDd	HW/TL	CSA	PVF	IF	Casp3	TUNEL	
Zhang *et al.* 2017 [S84]	Mice	C57Bl6J	M	22	6	5	20/40	8	84			x	x			x				
Zhang *et al.* 2018 [S85]	Rat	SD	M	ND	ND	8	ND	6	30			x	x							
Zhang *et al.* 2018 [S86]	Rat	SD	M	ND	ND	8	ND	6	30			x	x				x			
Zhao *et al.* 2019 [S87]	Rat	SD	M	205	ND	9	90/90	8	35			x	x							
Zhou *et al.* 2014 [S88]	Mice	129S1 and FVB	M	ND	9	8	60/60	ND	21–28									x	x	
Zhou *et al.* 2017 [S89]	Mice	C57Bl6J and FVB	M	ND	ND	8	20/100	12	3–28			x	x							
Zhou *et al.* 2018 [S90]	Mice	129s1 and C57Bl6J	ND	ND	ND	8	30/30	12	28			x	x				x			
Zhou *et al.* 2018 [S91]	Rat	SD	M	165	ND	4.5	30/60	8	21			x	x					x	x	
Zhu *et al.* 2020 [S92]	Rat	SD	M	190	ND	10	30/60	8	35			x								

[#]: For full details of the studies included in the meta-analysis see supplementary file S3, references listed in this table refer to this file. Casp3: caspase 3; CSA: cross-sectional area; F: female; F_{IO_2}: inspired oxygen fraction (in percentage); HR: heart rate; Hypoxia/Reox (s): duration of hypoxia and reoxygenation (in seconds); HW/TL: heart weight to tibia length ratio; IF: interstitial fibrosis; IH: intermittent hypoxia; LVDd: left ventricular diameter in diastole; M: male; MAP: mean arterial pressure; ND: no data; PVF: perivascular fibrosis; SD: Sprague–Dawley; SHR: spontaneously hypertensive rat; TUNEL: terminal deoxynucleotidyl transferase dUTP nick end labelling; WKY: Wistar Kyoto.

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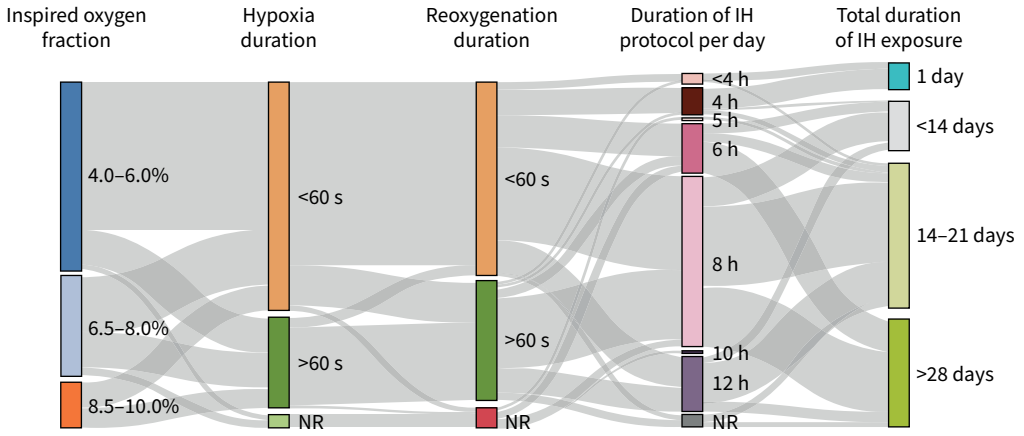

FIGURE 2 Characteristics of intermittent hypoxia (IH) protocols. NR: not reported.

Meta-analysis and meta-regression

Impact of IH on systemic haemodynamic and cardiac parameters

We confirmed our recent meta-analysis on the vascular impact of IH [24] and demonstrated that IH induces a significant increase in MAP (SMD=1.19; CI (0.85–1.52); I^2=68.5%) (figure 4a) and HR (SMD=0.34; CI (0.06–0.62); I^2=48.9%) (figure 4b). Most importantly, IH significantly reduces EF (SMD=−1.82; CI (−2.52–−1.12); I^2=94.2%) (figure 4c). IH also significantly affects cardiac structure, significantly increasing cardiomyocyte CSA (SMD=2.90; CI (0.82–4.98)) and LVDd (SMD=0.64; CI (0.18–1.10)) (figure 5a), with high heterogeneity between studies (94.2 and 88.0% for CSA and LVDd, respectively). IH induces cardiac fibrotic remodelling, characterised by perivascular fibrosis (SMD=2.29; CI (1.25–3.33); I^2=79.5), interstitial fibrosis (SMD=5.37; CI (3.22–7.53)); I^2=94.8) and CTGF expression (SMD=2.22; CI (0.66–3.78); I^2=90.6) (figure 5b). Finally, IH also induces cardiac apoptosis (Casp3: SMD=7.89; CI (1.03–14.7); I^2=99.3; TUNEL: SMD=6.70; CI (2.96–10.44); I^2=95.9) (figure 5c).

Univariate meta-regressions were performed according to population characteristics (strain, species, sex, body weight, year of publication, age). While strain seems to exert an effect on MAP (p=0.04), subgroup analysis does not demonstrate any effect of species, sex, year of publication and age. After adjustment for significant confounders in univariate meta-regressions (mainly strain), we performed meta-regressions on IH pattern specificities (F_{IO_2} (%), hypoxia duration (seconds), reoxygenation duration (seconds), duration of IH per day (hours) and total duration of IH exposure (protocol duration, in days)). The meta-regression

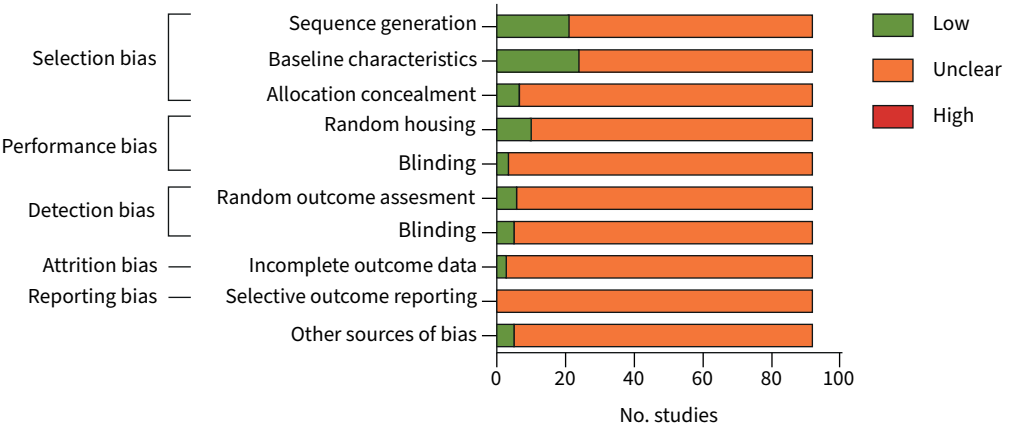

FIGURE 3 Risk of bias assessment according to the SYstematic Review Center for Laboratory animal Experimentation (SYRCLE) (overall score).

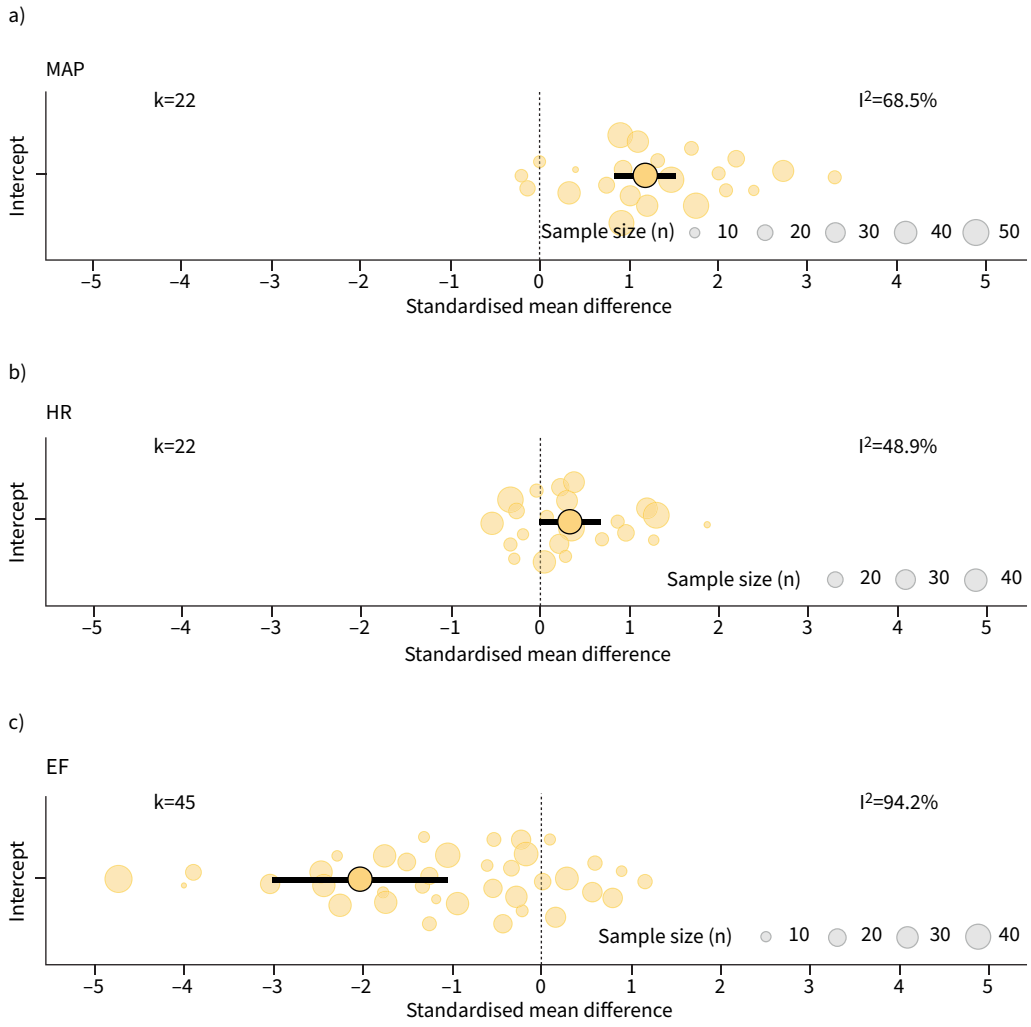

FIGURE 4 Orchard plots of the effect of intermittent hypoxia (IH) on a) mean arterial blood pressure (MAP); b) heart rate (HR); and c) ejection fraction (EF). Data were extracted in IH and control groups from primary studies and expressed as standardised mean differences. The pooled estimate (yellow) indicates an increase in MAP and HR and a reduction in EF upon IH exposure compared to control normoxic groups.

analyses are summarised in supplementary file S5. The IH protocol characteristics did not significantly influence the analysed parameters, apart from Casp3 level which is significantly associated with protocol duration (supplementary file S5).

Impact of IH on myocardial response to ischaemia–reperfusion: infarct size

Finally, 17 studies with a total of 24 protocols were included for analysis of myocardial response to ischaemia–reperfusion. The meta-analysis did not reveal any global impact of IH on infarct size (SMD=0.20; CI (−0.58–0.99); I^2=86.4). Nevertheless, figure 6a reveals a high heterogeneity between studies (I^2=86.4%), with two distinct effects of IH that we explored in depth using meta-regressions analysis.

IH protocol significantly influences the response to myocardial ischaemia and, particularly, the univariate model highlighted specific effects of F_{IO_2} levels and duration of IH exposure per day. Indeed, most of the studies using F_{IO_2}<7% demonstrate an increase in infarct size, whereas a decrease is shown for F_{IO_2} levels of at least 10% (figure 6b). Interestingly, a similar association is found between IH duration per day, with a reduction in infarct size for short-term exposure to IH (<4 h per day), whereas longer exposure induces an increase in infarct size (figure 6c). The multivariate meta-regression model revealed that only F_{IO_2} levels are associated with IH-induced increase in infarct size (supplementary file S5).

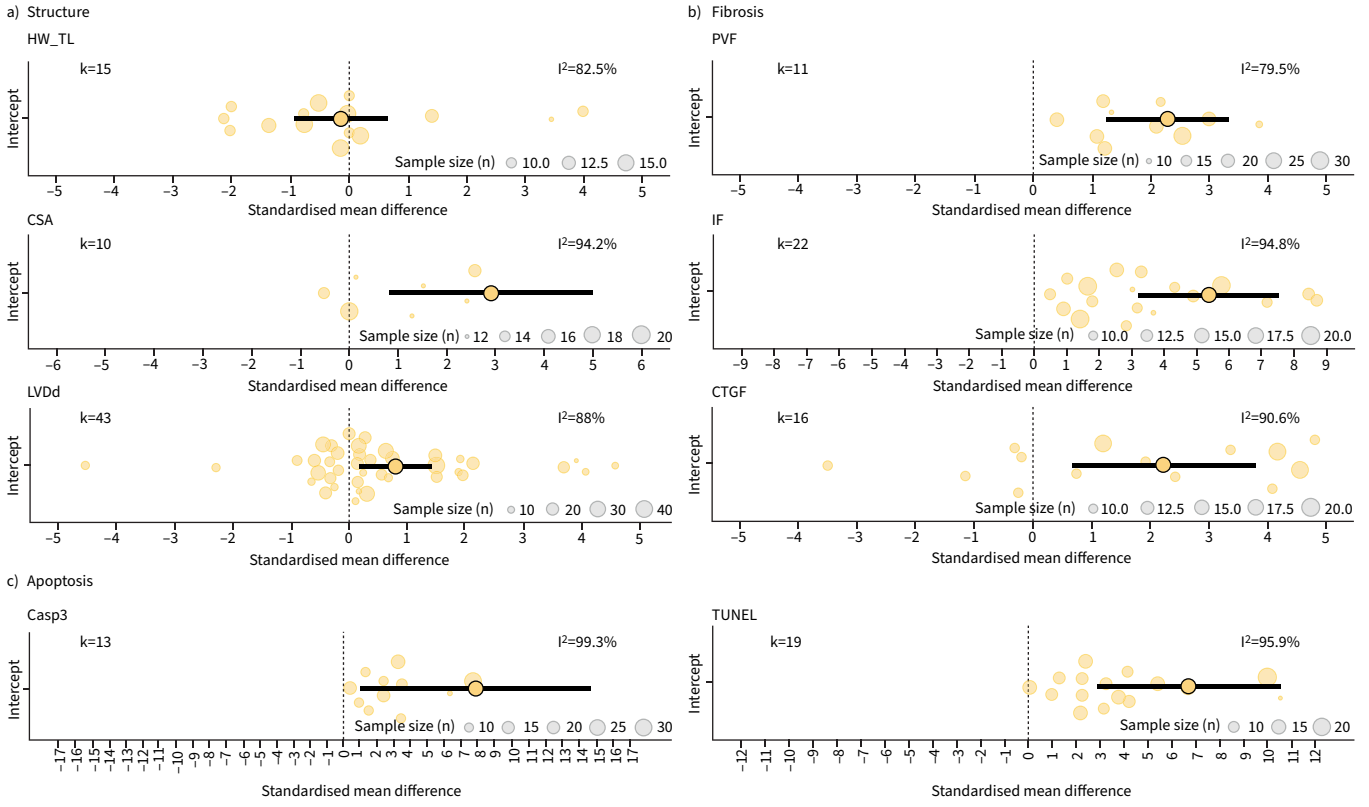

FIGURE 5 Orchard plots of the effect of intermittent hypoxia (IH) on cardiac remodelling. a) cardiac dimensions; b) fibrosis and c) apoptosis. Data were extracted in IH and control groups from primary studies and expressed as standardised mean differences. The pooled estimate (yellow) indicates that IH induces an overall cardiac hypertrophy (HW_TL: heart weight to tibia length; CSA: cross-sectional area) and dilation (LVDd: left ventricular diameter in diastole), associated with increased perivascular (PVF) and interstitial fibrosis (IF) and apoptosis (Casp3: caspase 3). CTGF: connective tissue growth factor; TUNEL: terminal deoxynucleotidyl transferase dUTP nick end labelling.

Publication bias

The results of Funnel plot and Egger regression tests showed significant asymmetry, suggesting that small-study effects were probably induced by publication bias for all outcomes (supplementary file S6). However, the SMD remained significant for all outcomes after correcting for missing studies through trim and fill analysis, suggesting a consistent effect of IH on the outcomes despite publication bias.

Discussion

Main findings

The finding of this study is original as this is the first meta-analysis evaluating the cardiac consequences of rodents exposed to IH. After reviewing more than 5400 experimental studies, we finally exploited 92 studies including a total of 1412 animals exposed to various regimens of IH *versus* control conditions. We found that IH induces cardiac contractile dysfunction, remodelling, and protective or impaired response to myocardial ischaemia–reperfusion depending on the burden of IH.

Elevation of arterial blood pressure

Our analysis confirmed that IH significantly increases arterial blood pressure in rodents. These results are consistent with studies describing an elevation of MAP in healthy volunteers exposed to IH for 14 nights [25, 26] and support the role of IH as the major risk factor for hypertension in patients with OSA [27]. Although clinical studies suggest that severe oxygen desaturation enhances an increased risk of hypertension [28], our meta-regression does not allow us to demonstrate any specific effect of hypoxia protocol characteristics (*i.e.* pattern, duration, intensity).

Robust and innovative methodology in the field

Most previous studies addressing the possible link between sleep apnoea and heart structure and function were observational, lacking a comparison group and limited by small sample sizes being unadjusted for

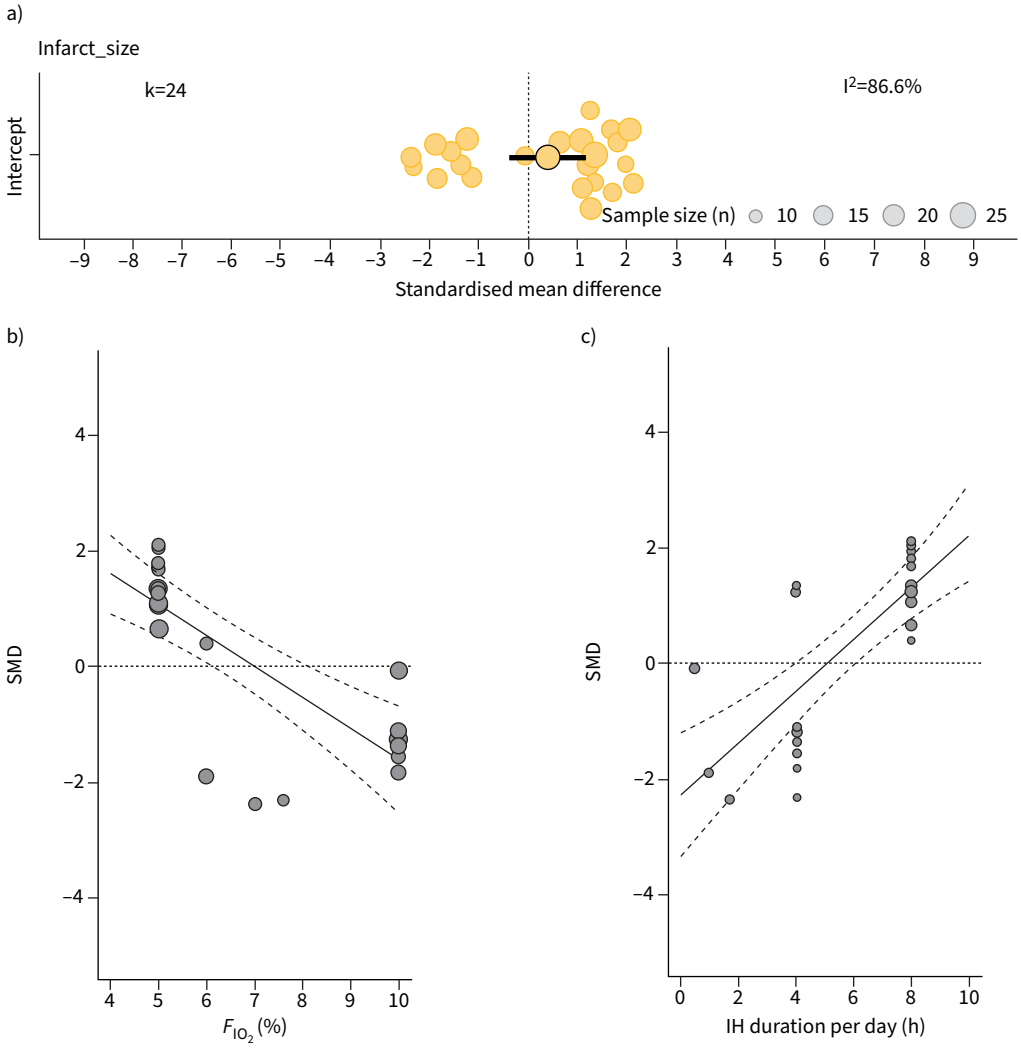

FIGURE 6 Impact of intermittent hypoxia (IH) on myocardial infarct size. a) Orchard plot indicates a high heterogeneity of IH response to ischaemia and reveals two distinct groups, one exhibiting a reduction in infarct size and the other an increase. Data were extracted in IH and control groups from primary studies and expressed as standardised mean differences (SMDs). Univariate analysis demonstrated that b) inspired oxygen fraction (F_{IO_2}) and c) IH duration per day were key parameters influencing myocardial infarct size.

confounders. OSA patients are exposed not only to IH but also to large swings in negative intra-thoracic pressure, due to pharyngeal obstructions affecting preload and afterload, and to sleep fragmentation triggered by micro-arousals accompanying desaturations. These three major consequences of OSA contribute concomitantly to associated cardiovascular dysfunctions [4] and the aim of the present analysis was to isolate the effect of IH alone. Indeed, the distinctiveness of experimental studies of exposure to IH in rodents is that they provide a true control group, the severity of hypoxia can be varied and there is easy access to heart tissue samples. By conducting a meta-analysis of these studies, we have further increased the robustness of the data by combining information gained from major groups involved in the field and achieving a sample size of more than 1400 animals. Such meta-analyses in rodents could represent very useful tools for evaluating mechanistic hypotheses, guide translational research and address new hypotheses, as clearly described by Farré *et al.* [29].

Cardiac remodelling and dysfunction

We demonstrated that, in rodents, chronic IH induces cardiac remodelling, characterised by hypertrophy (increased cardiomyocytes size), LV dilation (increased LVDd), and both myocardial fibrosis and apoptosis. These experimental studies in rodents are very valuable as they are in accordance with the few

studies in humans describing the association between SDB and both LV hypertrophy [30–32] and myocardial fibrosis [33, 34], but generally conducted in small samples, with no comparison group. Finally, this IH-induced cardiac remodelling could initiate, or at least contribute to, the reduced EF we evidenced in our meta-analysis and is in accordance with that reported in OSA patients [8, 9, 35]. The deleterious role of chronic IH was consistent across all measured outcomes. However, despite the large number of studies included, the *post hoc* subgroup analysis did not reveal any dose-response relationship between specific IH pattern (hypoxia intensity, hypoxia cycle duration, total duration of IH protocol) and structural and functional abnormalities. A limitation is that most of the IH protocols used were quite similar, with nadir F_{IO_2} around 6%, 1 min hypoxia–reoxygenation cycles and long-duration protocols (*i.e.* at least 3 weeks) (figure 2), and did not reflect the heterogeneity of hypoxia encountered in OSA patients. Of note, the nadir SaO_2, which is rarely reported in the literature, was around 60% [36], mimicking extremely severe sleep apnoea [37]. Accordingly, recent clinical studies demonstrated cardiac remodelling and incident heart failure only in severely hypoxic patients [8, 9, 17]. Also, in the "research agenda", preclinical studies should now include the complexity of OSA phenotypes for developing animal models of OSA with comorbid conditions (*i.e.* obesity, type 2 diabetes, hypertension, pre-existing coronary disease, *etc.*) reflecting routine practice. In that way, two recent studies demonstrated that IH, when applied during the time course evolution of ischaemic cardiomyopathy, exacerbated pathological remodelling and precipitated contractile dysfunction in rodents [38, 39]. This could ultimately reflect the specific impact of IH in different patient phenotypes [40].

Myocardial response to ischaemia–reperfusion

In this meta-analysis, we also investigated the impact of IH on myocardial susceptibility to ischaemia–reperfusion, assessed by infarct size. A strength of our meta-analysis was to reveal the high heterogeneity between studies, with a meta-regression allowing identification of two distinct responses to IH. Some studies demonstrated that IH reduces infarct size following ischaemia–reperfusion, whereas others reported an increase in infarct size. F_{IO_2} significantly affects infarct size, with an increase in infarct size for an F_{IO_2}<7% and an opposite decrease for an F_{IO_2}>10%. The second key exposure parameter was the duration of IH per day, with a reduction in infarct size for short-term exposure to IH (<4 h per day), whereas longer exposure increased infarct size. These findings strongly suggest that the severity of oxygen desaturation might represent predictors of individual response to myocardial infarction in OSA patients. These results are in accordance with clinical studies suggesting that patients with severe OSA exhibit an increase in infarct size following an acute coronary event [11], whereas other reports suggest that moderate OSA may confer protection against ischaemia–reperfusion injury [41–43]. From these observations emerged the concept of a dual response to IH that could result in cardioprotection, namely hypoxic conditioning [44], and/or maladaptive responses resulting in cardiac damage [45]. Using specific patterns of IH (F_{IO_2}, duration per day, *etc.*), rodent studies have contributed to understanding of the mechanisms responsible for either beneficial or detrimental responses to myocardial infarction. Among them, many works focused on hypoxia-inducible factor-1 (HIF-1), a key regulator of oxygen homeostasis. Of note, HIF-1 induces the transcription of more than 150 genes that drive both adaptive and maladaptive responses [46]. For example, it has been clearly demonstrated that moderate IH confers cardioprotection through HIF-1-dependant nitric oxide synthase (NOS) gene transcription [47], whereas severe IH contributes to increased ischaemic insults through HIF-1-dependant endothelin-1 gene transcription [48] or pro-apoptotic pathway activation (*i.e.* HIF-1-activating transcription factor 4 (ATF4)-C/EBP homologous binding protein (CHOP)) [49]. Thus, a better characterisation of the hypoxemic burden (*i.e.* hypoxia duration and intensity) associated with the identification of specific biomarkers of beneficial (*i.e.* HIF-1-NOS) or detrimental (*i.e.* HIF-1-ET-1/ATF4-CHOP) effects of IH is mandatory to better stratify cardiovascular risk in OSA patients and improve personalised therapeutic strategies [3].

Limitations of the study

Besides a significant pooled effect of chronic IH, heterogeneity between studies was high (I^2>50% for all the parameters) and probably multifactorial. Differences in population characteristics (animal species, strain, age, sex, body weight), IH protocols and a lack of precision regarding period of IH exposure (during sleep or wakefulness) can explain a part of this heterogeneity, while multivariate meta-regressions showed inconsistent results among studied outcomes and should be interpreted cautiously due to the limited number of studies in some subgroups. For example, as similarly reported in our recent meta-analysis investigating the vascular impact of IH in rodents [24], among the 92 studies included in our analysis, only one used female animals, whereas 87 used male animals, and four did not report animal sex. This represents an important limitation of preclinical studies in regards to recent literature highlighting the effect of sex on OSA-associated cardiovascular dysfunctions [50]. Along the same lines, most of the animal studies included in our meta-analysis were conducted in healthy and young rodents, in the absence of any comorbidities. As the OSA population is highly heterogeneous, affecting both young and elder patients,

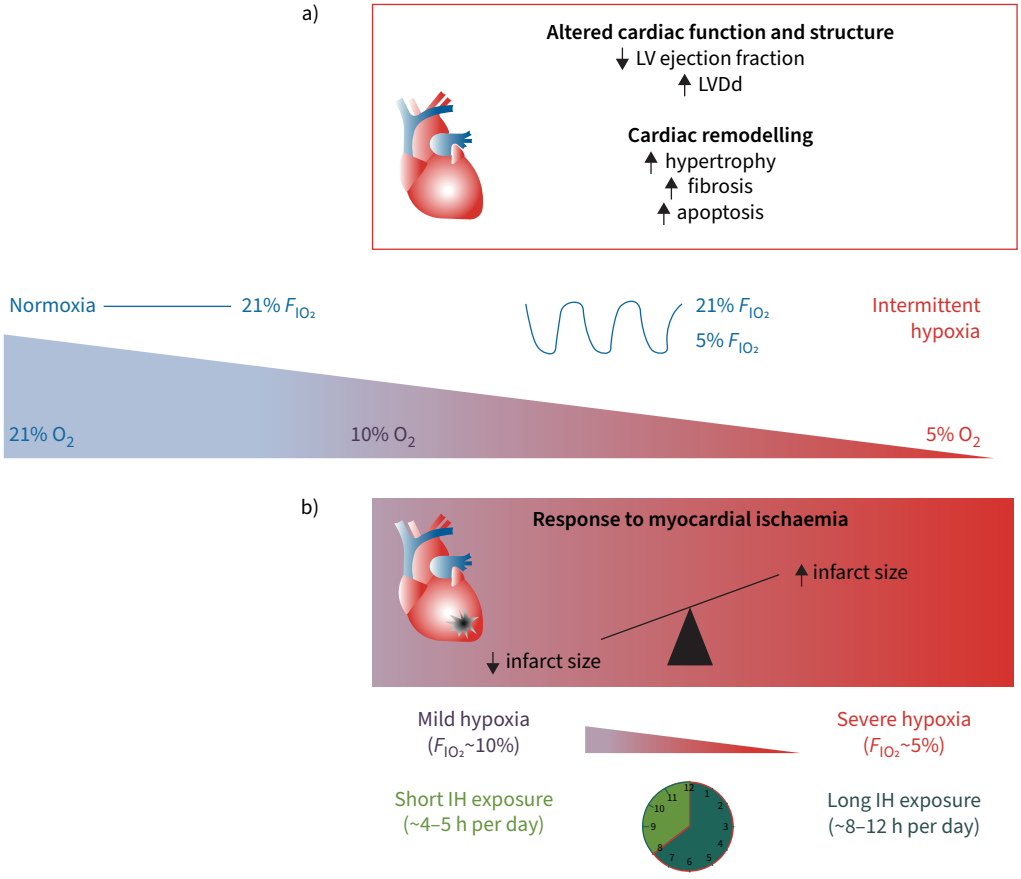

FIGURE 7 Impact of intermittent hypoxia (IH) on cardiac function, structure and response to ischaemia–reperfusion. a) Deleterious effect of IH characterised by a decrease in left ventricle (LV) ejection fraction, an increase in diastolic left ventricular diameter (LVDd), as well as hypertrophy, fibrosis and apoptosis. b) Dual effect of IH on infarct size following ischaemia–reperfusion depending on F_{IO_2} severity and IH duration per day. F_{IO_2}: inspired oxygen fraction.

male and female, with multiple comorbidities, the conclusions of such preclinical studies should be interpreted cautiously and more complex preclinical models will be necessary in the future. Moreover, in this study, we used SMD to pool studies expressing results with different units, which may reflect technical heterogeneity in measurement methods. An additional source of heterogeneity might be induced by the studies' risk of bias, which has been particularly difficult to evaluate due to the poor reporting of the methods used in the included studies, leading to a high proportion of items judged as unclear risk. Lastly, differences in laboratory environments and other unmeasured (or undescribed) parameters, such as temperature, diet, or even social interactions among animals, may also play a role in this heterogeneity.

Translational perspective

IH is considered as a major contributor to OSA-related cardiovascular consequences. This relationship is challenged by large clinical studies, suggesting the urgency to better understand the response to IH in order to improve OSA physiopathology and individualised healthcare. Fortunately, this meta-analysis aimed to investigate how different IH-controlled conditions impact cardiac remodelling, function and response to myocardial ischaemia in rodents. This systematic review aimed to highlight the importance of IH pattern, to dissect the mechanisms involved and to propose biomarkers for specific hypoxic response in order to better predict cardiovascular risk and to improve healthcare in patients with OSA.

Conclusion

Our meta-analysis demonstrated that IH systematically induces cardiac remodelling and contractile dysfunction in rodents. Importantly, most of the studies assessing these parameters applied IH protocols

that mimicked extremely severe hypoxic disease. Conversely, IH induces a dual response to myocardial ischaemia–reperfusion, which is related to hypoxia intensity and duration (figure 7). Further preclinical studies, with various IH experimental designs and rigorous follow-up of hypoxic burden, are needed 1) to determine the specific detrimental or beneficial cardiovascular effects of different IH protocols (intensity and duration), 2) to dissect the mechanisms involved, and 3) to propose biomarkers for each specific hypoxic response in order to better predict cardiovascular risk and to improve healthcare in patients with OSA.

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References

1 Benjafield AV, Ayas NT, Eastwood PR, *et al.* Estimation of the global prevalence and burden of obstructive sleep apnoea: a literature-based analysis. *Lancet Respir Med* 2019; 7: 687–698.
2 Drager LF, McEvoy RD, Barbe F, *et al.* Sleep apnea and cardiovascular disease: lessons from recent trials and need for team science. *Circulation* 2017; 136: 1840–1850.
3 Yeghiazarians Y, Jneid H, Tietjens JR, *et al.* Obstructive sleep apnea and cardiovascular disease: a scientific statement from the American Heart Association. *Circulation* 2021; 144: e56–e67.
4 Levy P, Kohler M, McNicholas WT, *et al.* Obstructive sleep apnoea syndrome. *Nat Rev Dis Primers* 2015; 1: 15015.
5 Dewan NA, Nieto FJ, Somers VK. Intermittent hypoxemia and OSA: implications for comorbidities. *Chest* 2015; 147: 266–274.
6 Marin JM, Carrizo SJ, Vicente E, *et al.* Long-term cardiovascular outcomes in men with obstructive sleep apnoea–hypopnoea with or without treatment with continuous positive airway pressure: an observational study. *Lancet* 2005; 365: 1046–1053.
7 Cuspidi C, Tadic M, Sala C, *et al.* Obstructive sleep apnoea syndrome and left ventricular hypertrophy: a meta-analysis of echocardiographic studies. *J Hypertens* 2020; 38: 1640–1649.
8 Ogilvie RP, Genuardi MV, Magnani JW, *et al.* Association between sleep disordered breathing and left ventricular function: a cross-sectional analysis of the ECHO-SOL ancillary study. *Circ Cardiovasc Imaging* 2020; 13: e009074.
9 Yu L, Li H, Liu X, *et al.* Left ventricular remodeling and dysfunction in obstructive sleep apnea: systematic review and meta-analysis. *Herz* 2020; 45: 726–738.
10 Barbe F, Sanchez-de-la-Torre A, Abad J, *et al.* Effect of obstructive sleep apnoea on severity and short-term prognosis of acute coronary syndrome. *Eur Respir J* 2015; 45: 419–427.
11 Buchner S, Satzl A, Debl K, *et al.* Impact of sleep-disordered breathing on myocardial salvage and infarct size in patients with acute myocardial infarction. *Eur Heart J* 2014; 35: 192–199.
12 Sanchez-de-la-Torre M, Sanchez-de-la-Torre A, Bertran S, *et al.* Effect of obstructive sleep apnoea and its treatment with continuous positive airway pressure on the prevalence of cardiovascular events in patients with acute coronary syndrome (ISAACC study): a randomised controlled trial. *Lancet Respir Med* 2020; 8: 359–367.
13 McEvoy RD, Antic NA, Heeley E, *et al.* CPAP for prevention of cardiovascular events in obstructive sleep apnea. *N Engl J Med* 2016; 375: 919–931.
14 da Silva Paulitsch F, Zhang L. Continuous positive airway pressure for adults with obstructive sleep apnea and cardiovascular disease: a meta-analysis of randomized trials. *Sleep Med* 2019; 54: 28–34.
15 Oldenburg O, Wellmann B, Buchholz A, *et al.* Nocturnal hypoxaemia is associated with increased mortality in stable heart failure patients. *Eur Heart J* 2016; 37: 1695–1703.
16 Nicholl DDM, Hanly PJ, Zalucky AA, *et al.* Nocturnal hypoxemia severity influences the effect of CPAP therapy on renal renin-angiotensin-aldosterone system activity in humans with obstructive sleep apnea. *Sleep* 2021; 44: zsaa228.

17 Azarbarzin A, Sands SA, Stone KL, *et al.* The hypoxic burden of sleep apnoea predicts cardiovascular disease-related mortality: the Osteoporotic Fractures in Men Study and the Sleep Heart Health Study. *Eur Heart J* 2019; 40: 1149–1157.
18 Hooijmans CR, Rovers MM, de Vries RB, *et al.* SYRCLE's risk of bias tool for animal studies. *BMC Med Res Methodol* 2014; 14: 43.
19 Murad MH, Wang Z, Chu H, *et al.* When continuous outcomes are measured using different scales: guide for meta-analysis and interpretation. *BMJ* 2019; 364: k4817.
20 Cohen J. Statistical Power Analysis for the Behavioral Sciences. New York, NY, Routledge Academic, 1988.
21 Nakagawa S, Lagisz M, O'Dea RE, *et al.* The orchard plot: cultivating a forest plot for use in ecology, evolution, and beyond. *Res Synth Methods* 2021; 12: 4–12.
22 Egger M, Davey Smith G, Schneider M, *et al.* Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; 315: 629–634.
23 Duval S, Tweedie R. Trim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics* 2000; 56: 455–463.
24 Harki O, Boete Q, Pepin JL, *et al.* Intermittent hypoxia-related alterations in vascular structure and function: a systematic review and meta-analysis of rodent data. *Eur Respir J* 2022; 59: 2100866.
25 Tamisier R, Gilmartin GS, Launois SH, *et al.* A new model of chronic intermittent hypoxia in humans: effect on ventilation, sleep, and blood pressure. *J Appl Physiol* 2009; 107: 17–24.
26 Tamisier R, Pepin JL, Remy J, *et al.* 14 nights of intermittent hypoxia elevate daytime blood pressure and sympathetic activity in healthy humans. *Eur Respir J* 2011; 37: 119–128.
27 Pengo MF, Soranna D, Giontella A, *et al.* Obstructive sleep apnoea treatment and blood pressure: which phenotypes predict a response? A systematic review and meta-analysis. *Eur Respir J* 2020; 55: 1901945.
28 Hou H, Zhao Y, Yu W, *et al.* Association of obstructive sleep apnea with hypertension: a systematic review and meta-analysis. *J Glob Health* 2018; 8: 010405.
29 Farré R, Martínez-García M A, Gozal D. Systematic reviews and meta-analyses in animal model research: as necessary, and with similar pros and cons, as in patient research. *Eur Respir J* 2022; 59: 2102438 [DOI: 10.1183/13993003.02438-2021].
30 Pujante P, Abreu C, Moreno J, *et al.* Obstructive sleep apnea severity is associated with left ventricular mass independent of other cardiovascular risk factors in morbid obesity. *J Clin Sleep Med* 2013; 9: 1165–1171.
31 Niroumand M, Kuperstein R, Sasson Z, *et al.* Impact of obstructive sleep apnea on left ventricular mass and diastolic function. *Am J Respir Crit Care Med* 2001; 163: 1632–1636.
32 Cloward TV, Walker JM, Farney RJ, *et al.* Left ventricular hypertrophy is a common echocardiographic abnormality in severe obstructive sleep apnea and reverses with nasal continuous positive airway pressure. *Chest* 2003; 124: 594–601.
33 Singh M, Hanis CL, Redline S, *et al.* Sleep apnea and galectin-3: possible sex-specific relationship. *Sleep Breath* 2019; 23: 1107–1114.
34 Shah NA, Reid M, Kizer JR, *et al.* Sleep-disordered breathing and left ventricular scar on cardiac magnetic resonance: results of the Multi-Ethnic Study of Atherosclerosis. *J Clin Sleep Med* 2020; 16: 855–862.
35 Diez J, Gonzalez A, Kovacic JC. Myocardial interstitial fibrosis in nonischemic heart disease, Part 3/4: JACC Focus Seminar. *J Am Coll Cardiol* 2020; 75: 2204–2218.
36 Dematteis M, Julien C, Guillermet C, *et al.* Intermittent hypoxia induces early functional cardiovascular remodeling in mice. *Am J Respir Crit Care Med* 2008; 177: 227–235.
37 Dematteis M, Godin-Ribuot D, Arnaud C, *et al.* Cardiovascular consequences of sleep-disordered breathing: contribution of animal models to understanding the human disease. *ILAR J* 2009; 50: 262–281.
38 Du Y, Wang X, Li L, *et al.* miRNA-mediated suppression of a cardioprotective cardiokine as a novel mechanism exacerbating post-MI remodeling by sleep breathing disorders. *Circ Res* 2020; 126: 212–228.
39 Bourdier G, Detrait M, Bouyon S, *et al.* Intermittent hypoxia triggers early cardiac remodeling and contractile dysfunction in the time-course of ischemic cardiomyopathy in rats. *J Am Heart Assoc* 2020; 9: e016369.
40 Zapater A, Sanchez-de-la-Torre M, Benitez ID, *et al.* The effect of sleep apnea on cardiovascular events in different acute coronary syndrome phenotypes. *Am J Respir Crit Care Med* 2020; 202: 1698–1706.
41 Lavie L, Lavie P. Ischemic preconditioning as a possible explanation for the age decline relative mortality in sleep apnea. *Med Hypotheses* 2006; 66: 1069–1073.
42 Lavie L, Lavie P. The double-edged sword of intermittent hypoxia–can intermittent hypoxia be both deleterious and protective in OSA? Focus on "Frequency and magnitude of intermittent hypoxia modulate endothelial wound healing in a cell culture model of sleep apnea". *J Appl Physiol* 2017; 123: 1021–1023.
43 Shah N, Redline S, Yaggi HK, *et al.* Obstructive sleep apnea and acute myocardial infarction severity: ischemic preconditioning? *Sleep Breath* 2013; 17: 819–826.
44 Verges S, Chacaroun S, Godin-Ribuot D, *et al.* Hypoxic conditioning as a new therapeutic modality. *Front Pediatr* 2015; 3: 58.
45 Linz D, Linz B, Heijman J. Sleep apnea, intermittent hypoxemia, and effects on ischemic myocardial damage: friend or foe? *Can J Cardiol* 2020; 36: 809–812.

46 Prabhakar NR, Semenza GL. Adaptive and maladaptive cardiorespiratory responses to continuous and intermittent hypoxia mediated by hypoxia-inducible factors 1 and 2. *Physiol Rev* 2012; 92: 967–1003.

47 Belaidi E, Beguin PC, Levy P, *et al.* Prevention of HIF-1 activation and iNOS gene targeting by low-dose cadmium results in loss of myocardial hypoxic preconditioning in the rat. *Am J Physiol Heart Circ Physiol* 2008; 294: H901–H908.

48 Belaidi E, Joyeux-Faure M, Ribuot C, *et al.* Major role for hypoxia inducible factor-1 and the endothelin system in promoting myocardial infarction and hypertension in an animal model of obstructive sleep apnea. *J Am Coll Cardiol* 2009; 53: 1309–1317.

49 Moulin S, Thomas A, Arnaud C, *et al.* Cooperation between hypoxia-inducible factor 1alpha and activating transcription factor 4 in sleep apnea-mediated myocardial injury. *Can J Cardiol* 2020; 36: 936–940.

50 Hegner P, Lebek S, Maier LS, *et al.* The effect of gender and sex hormones on cardiovascular disease, heart failure, diabetes, and atrial fibrillation in sleep apnea. *Front Physiol* 2021; 12: 741896.