Intermittent hypoxia-related alterations in vascular structure and function: a systematic review and meta-analysis of rodent data

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Shareable abstract (@ERSpublications)
This meta-analysis of rodent studies firmly establishes that intermittent hypoxia (IH), as a model of obstructive sleep apnoea, alters vascular pressure, remodelling and reactivity. Severity of IH and rodent characteristics contribute to this impact. https://bit.ly/3fm45fB

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Abstract

Background Obstructive sleep apnoea and the related intermittent hypoxia (IH) are widely recognised as risk factors for incident cardiovascular diseases. Numerous studies support the deleterious vascular impact of IH in rodents but an overall interpretation is challenging owing to heterogeneity in rodent species investigated and the severity and duration of IH exposure. To clarify this major issue, we conducted a systematic review and meta-analysis to quantify the impact of IH on systemic artery structure and function depending on the different IH exposure designs.

Methods We searched PubMed, Embase and Web of Science, and included 125 articles in a meta-analysis, among them 112 using wild-type rodents and 13 using apolipoprotein E knockout ($ApoE^{-/-}$) mice. We used the standardised mean difference (SMD) to compare results between studies.

Results IH significantly increased mean arterial pressure (+13.90 (95% CI 11.88–15.92) mmHg), and systolic and diastolic blood pressure. Meta-regressions showed that mean arterial pressure change was associated with strain and year of publication. IH altered vasodilation in males but not in females and increased endothelin-1-induced but not phenylephrine-induced vasoconstriction. Intima–media thickness significantly increased upon IH exposure (SMD 1.10 (95% CI 0.58–1.62); absolute values +5.23 (2.81–7.84) µm). This increase was observed in mice but not in rats and was negatively associated with age. Finally, IH increased atherosclerotic plaque size in $ApoE^{-/-}$ mice (SMD 1.08 (95% CI 0.80–1.37)).

Conclusions Our meta-analysis established that IH, independently of other confounders, has a strong effect on vascular structure and physiology. Our findings support the interest of identifying and treating sleep apnoea in routine cardiology practice.

Introduction

Obstructive sleep apnoea syndrome (OSAS) is one of the most frequent chronic diseases, affecting up to nearly 1 billion individuals worldwide [1], and is characterised by the repetitive occurrence of apnoeas and hypopnoeas during sleep [2]. OSAS is widely recognised as a risk factor for prevalent and incident cardiovascular diseases, including hypertension, atherogenesis, stroke and myocardial infarction, thus leading to increased morbidity and mortality [2–4]. Among OSAS pathophysiological mechanisms, intermittent hypoxia (IH) caused by repetitive hypoxia–reoxygenation cycles is thought to be the key intermediary mechanism leading to cardiovascular morbidity and mortality [2, 5, 6]. However, clinical studies are frequently partly flawed by confounders and the role of IH as an independent cardiovascular risk factor is still debated. It is crucial to establish from robust and consistent experimental data the role of

IH in increasing cardiovascular risk so as to better understand its contribution towards cardiovascular diseases.

Among OSA animal models developed in the last decades [5], IH exposure in rodents is by far the most commonly used worldwide. Studies on rodents exposed to IH have allowed us to dissect the contribution of different pathophysiological pathways and intermediary mechanisms such as sympathetic nervous system activation, endothelial dysfunction, inflammation or oxidative stress in triggering cardiovascular consequences. Many studies using animal models have supported the hypothesis that IH might be responsible for increased arterial blood pressure [5, 7], structural vascular remodelling [8–10], altered vascular reactivity [11, 12] and atherosclerosis progression [13–15]. However, some studies showed no effect of IH on vascular parameters (*e.g.* [16–19]) and there was heterogeneity regarding effect size. Inconsistency between studies might be explained by the disparity in rodent models and variations in patterns of IH exposure. Indeed, studies included mice or rats, predominantly males, but from different strains, of different ages and weights at baseline, and on different diets (standard *versus* high fat). There were also variations regarding the studied vascular beds, from large elastic to small muscular arteries, which might potentially account for substantial variability. Last but not least, IH patterns differed across studies. Animals were exposed to IH severities ranging from 5% to 10% inspiratory oxygen fraction (F_{IO_2}), hypoxic phases varied from 6 to 12 h per day and desaturation–reoxygenation sequences lasted from 20 s to a few minutes, with the total duration of IH exposure ranging from a few days to up to several weeks or months.

With the goal of clarifying and strengthening our knowledge, we carried out a systematic review and meta-analysis addressing the overall impact of IH on vascular parameters, namely blood pressure, vascular remodelling, arterial function and atherosclerotic lesions in systemic arteries. Subgroup analyses and meta-regressions were performed to identify the main factors accounting for heterogeneity in results, with particular interest in assessing different rodent models and IH cycle characteristics.

Methods

The protocol for the meta-analysis was recorded in PROSPERO with identifier number CRD42020169940. Owing to the very large amount of available data, this work focuses on structural and functional vascular outcomes.

Search methods and study selection

We searched PubMed, Embase and Web of Science for articles published up to 16 April 2021. The search terms were “intermittent hypoxia” AND “rodent” OR “mice” OR “rats” (see PROSPERO record for exact query). We also searched for key words and Medical Subject Headings (MeSH) related to each search term. After the initial electronic search, we screened the titles and the abstracts to retrieve relevant articles. Eligibility was considered if the articles were written in English and addressed vascular outcomes of IH in rodents. Then, the full articles were screened for inclusion and exclusion criteria. Two authors independently screened all references for inclusion and any discrepancies were resolved by discussion among the team.

We included only controlled studies with a well-established control group, *i.e.* normoxic animals, for adult rodents exposed to chronic IH. An IH cycle was defined as the repetitive occurrence of several hypoxia–reoxygenation sequences during the same day. Chronic IH was defined as a repetition of IH cycles over time, for a minimum of 1 day. All wild-type rodent models (mouse and rat, male and female, young or aged, lean or obese) with exposure to IH and compared with a normoxic group were included.

The outcomes considered as mandatory for inclusion were variables allowing characterisation of vascular structure or function. These included blood pressure (systolic (SBP)/diastolic (DBP)/mean/pulsed), arterial reactivity (vasodilatory response to 10^{-6} M acetylcholine and vasoconstriction responses to 10^{-6} M phenylephrine or 10^{-8} M endothelin-1, both *ex vivo* in cannulated vessels or vessel rings), vascular remodelling (intima–media thickness (IMT) and internal vessel diameter) and atherosclerosis plaque size in apolipoprotein E knockout ($ApoE^{-/-}$) mice.

We excluded studies without any control group (normoxic and untreated mice), studies in which hypoxia was applied continuously (*i.e.* no hypoxia–normoxia cycles) and studies in which hypoxia was combined with hypercapnic or hypobaric conditions. We also excluded studies using IH exposure in prenatal or perinatal periods and studies using transgenic animals, except for $ApoE^{-/-}$ mice that are the model of choice to study atherosclerosis plaques. Studies on pulmonary, retinal or cerebral vascular beds were also excluded.

Assessment of methodological quality

The quality assessment of studies was performed using the SYRCLE tool described by Hooijmans *et al.* [20]. This contains several types of bias: selection bias (sequence generation, baseline characteristics and allocation concealment), performance bias (randomised housing of animals and blinding of investigators), detection bias (random outcome assessment and 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. Three authors were involved and every discrepancy was discussed to achieve a shared decision.

Statistical analysis

We performed two separate meta-analyses for wild-type rodents and ApoE$^{-/-}$ mice. For each outcome, data were abstracted and analysed using standardised mean difference (SMD)=(M_c−M_e)/SD, where M_c is the mean of the outcome measure in the control group, M_c is the mean of the outcome measure in the experimental group and SD is the pooled standard deviation of the two groups [21]. SMD >0.8 was considered as large, 0.5–0.8 as moderate and 0.2–0.5 as small [21]. In case of a missing standard deviation we calculated or estimated it from confidence intervals, standard errors, t-values, p-values or F-values [22]. The remaining standard deviations were imputed using the mean outcome-specific standard deviation from other included studies. All results are represented using orchard plots, an innovative data visualisation tool well adapted for displaying the results of a large number of outcomes (supplementary figure S1) [23].

To facilitate interpretation of some results, we also performed meta-analyses using natural mean differences for arterial pressure outcomes. We have back-transformed SMD to natural mean differences using the median standard deviation from the control groups of included studies using the target unit for IMT outcome [24]. SMD are expressed with 95% confidence intervals. For study descriptions, we used median and interquartile range (IQR).

Given the high anticipated heterogeneity in included studies, we performed random effects meta-analysis by the restricted maximum-likelihood estimator method [25]. Moreover, to account for correlation among multi-arm studies we constructed a hierarchical/mixed effect model with a random intercept for study. We explored sources of heterogeneity through pre-specified subgroup analyses and meta-regressions according to population (species, strain, gender, age, diet and body weight), year of publication and details of IH protocols (F_{IO_2} during hypoxic phases, duration of hypoxic and normoxic phases, frequency, duration per day, and total duration of exposure).

Given the large number of studied outcomes, we performed meta-regressions only on pre-specified primary outcomes: mean arterial pressure (MAP), IMT, response of vessel rings to acetylcholine and atherosclerosis lesion size in ApoE$^{-/-}$ mice. We first performed univariate meta-regressions on study and animal characteristics (age and body weight were adjusted on species). We then added the significant predictors ($p<0.2$) in meta-regression models evaluating IH protocol parameters. Given the exploratory nature of these analyses we considered all p-values <0.05 as significant. Lastly, to assess the robustness of the findings, we performed sensitivity analyses by excluding potential outliers for significant meta-regressions.

Funnel plot asymmetry was also explored for primary outcomes using Egger's regression test, as recommended by the Cochrane Handbook for Systemic Reviews of Interventions [26], with $p<0.1$ suggesting publication bias. We also performed a trim-and-fill analysis to assess the impact of small study effects on the meta-analyses results [27].

All statistical analyses were performed using R version 3.6.2 (www.r-project.org).

Results

Our literature review yielded 5127 references, among which we ultimately selected 125 studies for inclusion in the meta-analysis: 112 in wild-type rodents and 13 using ApoE$^{-/-}$ mice (figure 1). Supplementary table S1 and supplementary figure S2 present vascular outcomes available across the studies, settings of hypoxic exposure and experimental designs.

Among the 112 studies on wild-type rodents, 24 were performed in mice (23 in C57BL/6 mice and one in 129S1 mice) and 88 in rats (60 in Sprague-Dawley, 24 in Wistar and four in other strains of rats). At study inclusion, median (IQR) body weight and age were 25.75 (22.1–27.1) g and 8.0 (7–11) weeks for mice and 275 (200–325) g and 9 (8–12.7) weeks for rats, respectively. Males were used in 103 studies, females in four studies, and both males and females in one study, and four studies did not report the sex of the animals. Animals received standard diet in 93 studies, high-fat diet in one study, and both standard and high-fat diet in four studies; diet was not specified in 13 studies.

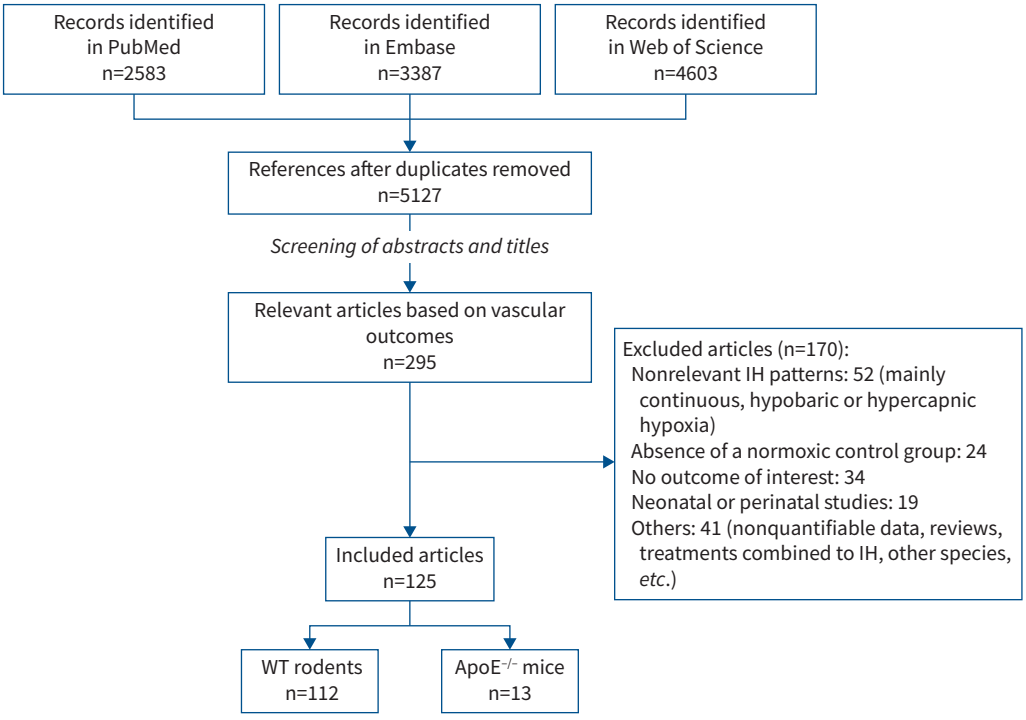

FIGURE 1 Flow diagram of the study. IH: intermittent hypoxia; WT: wild-type; ApoE$^{-/-}$: apolipoprotein E knockout.

For the studies in ApoE$^{-/-}$ mice, median (IQR) weight was 27 (25.9–29.5) g and age was 12 (8–14) weeks. Nine studies used males, three used both males and females, and one did not report the sex. The diet was standard in five studies, high fat in six studies, and both standard and high fat in two studies.

Concerning IH protocols (supplementary figure S2), median (IQR) values of F_{IO_2} during hypoxic periods were 5.25% (5–9%), desaturation during 60 (30–102.5) s followed by 90 (30–217.5) s of reoxygenation and return to a F_{IO_2} of 21% (normoxia). Cycles were repeated on average for 8 h per day for a median (IQR) duration of 21 (10–35) days. 43% of studies used a F_{IO_2} of 5%, 11% used a F_{IO_2} of 6% and 19% used a F_{IO_2} of 10% during hypoxic phases. 14% of studies had a duration of 7 days, 19% a duration of 14 days, 11% a duration of 21 days, 8% a duration of 28 days and 22% a duration of 35 days; in total, 14% of studies exposed for ⩾42 days.

The number of included studies for each outcome is shown in supplementary table S1. Outcomes were excluded from statistical analysis when less than three studies reported them. In wild-type animals, this was the case for pulsed arterial pressure, atherosclerotic plaques, endothelial permeability, vasoconstriction in cannulated vessels and compliance/pulse wave velocity. In ApoE$^{-/-}$ mice, this was the case for all outcomes except for lesion size.

Impact of IH on arterial blood pressure

IH significantly increased SBP, DBP and MAP in systemic vessels of wild-type rodents (figure 2a–c). MAP SMD was 1.43 (95% CI 1.13–1.73; I^2=75.85%) corresponding to a mean increase of 13.90 (95% CI 11.88–15.92) mmHg after IH. Similarly, SBP increased by 13.13 (95% CI 10.80–15.47) mmHg and DBP by 12.24 (95% CI 9.01–15.47) mmHg. Forest plots for SBP, DBP and MAP expressed in mmHg are shown in supplementary figure S3a–c.

Subgroup analyses showed a significant heterogeneity according to strain (test for subgroup difference p<0.01): MAP increased in mice (C57BL/6) as well as in rats (Wistar and Sprague-Dawley) but not in Fischer 344, Wistar Kyoto or lean Zucker rats, although the number of studies was very limited for these strains (table 1 and supplementary figure S4). Meta-regression analyses for IH parameters after adjustment

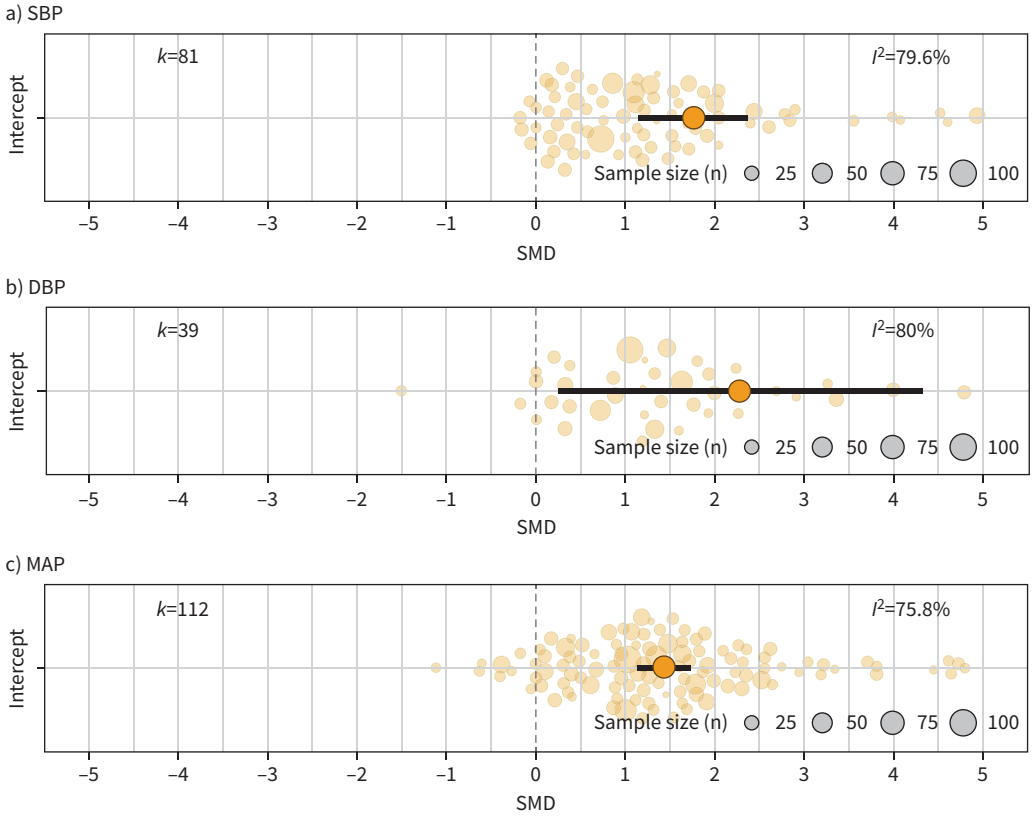

FIGURE 2 Intermittent hypoxia increases blood pressure in systemic vessels of wild-type animals. Orchard plots showing standardised mean difference (SMD) for a) systolic blood pressure (SBP), b) diastolic blood pressure (DBP) and c) mean arterial pressure (MAP). *k* represents the number of effect sizes per estimate.

for significant confounders in univariate meta-regression (strain and year of publication) showed that a lower F_{IO_2} during hypoxic phases and total duration of exposure tended to be associated with a higher MAP (p=0.11 and p=0.07, respectively). Moreover, total duration of exposure was significantly associated with MAP in multivariate meta-regressions after adjusting for all IH parameters (p=0.046).

Impact of IH on arterial reactivity

Vasodilation tests, assessed on cannulated arteries as well as on arterial rings, showed that reactivity to acetylcholine (10^{-6} M) significantly decreased after IH in both cannulated arteries (SMD −1.26 (95% CI −2.10–−0.41); I^2=62.10%) (figure 3a) and arterial rings (SMD −2.53 (95% CI −4.06–−1.00); I^2=93.29%) (figure 3b). IH also increased vasoconstriction due to endothelin-1 (10^{-8} M) (SMD 1.11 (95% CI 0.22–2.01); I^2=0.00%) (figure 3c). However, there was no impact of IH on the vasoconstriction induced by phenylephrine (10^{-6} M) (SMD 0.04 (95% CI −0.60–0.68); I^2=41.82%) (figure 3d).

Subgroup analyses showed that the IH-induced decrease in acetylcholine-dependent vasodilation was observed in male but not in female rodents (SMD 0.12 (95% CI −1.82–2.06) in females *versus* −1.78 (95% CI −3.12–−0.44) in males; p<0.01) (supplementary figure S5). Meta-regression analyses for IH parameters after adjustment for the significant confounder in univariate meta-regression (sex) showed that F_{IO_2} was significantly associated with vasodilation impairment, especially at moderate hypoxia levels (*i.e.* in the range of 10% F_{IO_2}) (figure 3e and table 1). However, after adjusting for the other IH parameters, this association did not persist (p=0.15).

Impact of IH on vascular remodelling

In wild-type rodents, IMT significantly increased after IH (SMD 1.10 (95% CI 0.58–1.62); I^2=76.57%) (figure 4a) with an increase of IMT of 5.23 (95% CI 2.81–7.84) µm. In contrast, inner vessel diameter did not significantly change after IH (figure 4b). Subgroup analyses for IMT showed a much stronger effect of

TABLE 1 Meta-regression analyses for the main outcomes: mean arterial pressure (MAP), dilation of artery rings, intima–media thickness (IMT) and atherosclerosis lesions in apolipoprotein E knockout ($ApoE^{-/-}$) mice

Moderator	MAP			Artery ring dilation			IMT			$ApoE^{-/-}$ lesions		
	n	Slope	p-value	n	Slope	p-value	n	Slope	p-value	n	Slope	p-value
Univariate meta-regressions												
Strain	112		0.00#,*	16		0.47	28		0.05#,*	25		0.86
Diet	102		0.62	16		0.37	NA			25		0.38
Species	112		0.10	16		0.47	28		0.03#,*	NA		
Gender	110		0.85	14		0.00#	23		0.96	24		0.39
Body weight	82	−0.001	0.07	12	0.041	0.06	17	−0.004	0.15#	13	−0.145	0.06#
Year of publication	112	0.029	0.05#,*	16	−0.142	0.59	28	0.086	0.11#	25	−0.038	0.38
Age	47	−0.015	0.15	12	0.0004	0.99	21	−0.026	0.014	20	0.012	0.72
Univariate adjusted meta-regression on IH parameters												
F_{IO_2}	112	−0.103	0.11	16	−0.734	0.002*	28	0.118	0.61	25	0.260	0.32
Duration of exposure	112	0.015	0.07	16	−0.023	0.60	28	0.027	0.07	25	0.0011	0.68
Duration of IH per day	112	−0.073	0.55	16	0.050	0.90	28	−0.0003	0.99	25	0.044	0.63
Duration of reoxygenation phase	112	0.0014	0.30	16	−0.005	0.61	28	0.0011	0.86	25	−0.009	0.35
Duration of hypoxic phase	112	−0.0002	0.89	16	−3.744	0.07	28	0.0047	0.59	25	−0.005	0.38

IH: intermittent hypoxia; F_{IO_2}: inspiratory oxygen fraction. #: p<0.2 indicates moderators included in the multivariate model to adjust meta-regressions on IH parameters. *: p<0.05.

IH in mice (SMD 1.34 (95% CI 0.55–2.14)) than in rats (SMD 0.40 (95% CI −0.13–0.93)) (p=0.03 for subgroup difference) (supplementary figure S6). Univariate analysis also showed that age was negatively associated with IMT thickening (p=0.014) (figure 4c and table 1). Meta-regression analysis for IH parameters after adjustment for significant predictors in univariate meta-regression (strain, species, year of publication and age) were not significant for IMT at a p-value of 0.05, but total duration of exposure tended to be associated with IMT thickening (p=0.07) (figure 4d and table 1). After adjustment for other IH parameters in multivariate meta-regression, duration of exposure was significantly associated with IMT (p=0.046).

Impact of IH on atherosclerosis lesions in $ApoE^{-/-}$ mice

Since vascular remodelling is an early step in the process of atherogenesis and because wild-type C57BL/6 mice are resistant to atherosclerosis, we included $ApoE^{-/-}$ mice in the meta-analysis, as a recognised model of susceptibility to atherosclerosis. The analysis showed that IH strongly increased atheromatous lesion size in $ApoE^{-/-}$ mice (SMD 1.08 (95% CI 0.80–1.37); I^2=27.5%) (figure 5). Meta-regression analysis for IH parameters showed that IH parameters were not significantly associated with lesion size in $ApoE^{-/-}$ mice, except for a strong trend towards significance regarding the animal's body weight (p=0.06) (table 1).

Risk of bias of studies

The risk of bias of studies was assessed using the SYRCLE tool [20]. The results are presented in supplementary figure S7 and supplementary table S2. Items for which the risk of bias was low were selective outcome reporting (selection bias, 35% of studies are at low risk), sequence generation (39% low risk), baseline characteristics (48% low risk) and incomplete outcome data (attrition bias, 56% low risk). However, incomplete outcome data was also the criterion with the highest percentage of high-risk studies (23%). Finally, several outcomes, mainly categorised as performance and detection bias, were almost never mentioned and therefore scored as "unclear risk": allocation concealment, randomised housing, blinding of investigators, random outcome assessment and blinding of outcome assessor.

Small study effect

Funnel plots are presented in supplementary figure S8 for MAP, ring dilation, IMT and plaque size. For these four items, the asymmetric distribution of studies and a significant Egger regression test indicating a clear small study effect were observed. However, the SMD remained significant for all outcomes after correcting for missing studies (trim-and-fill analysis), meaning a consistent effect of IH on the outcomes (supplementary table S3).

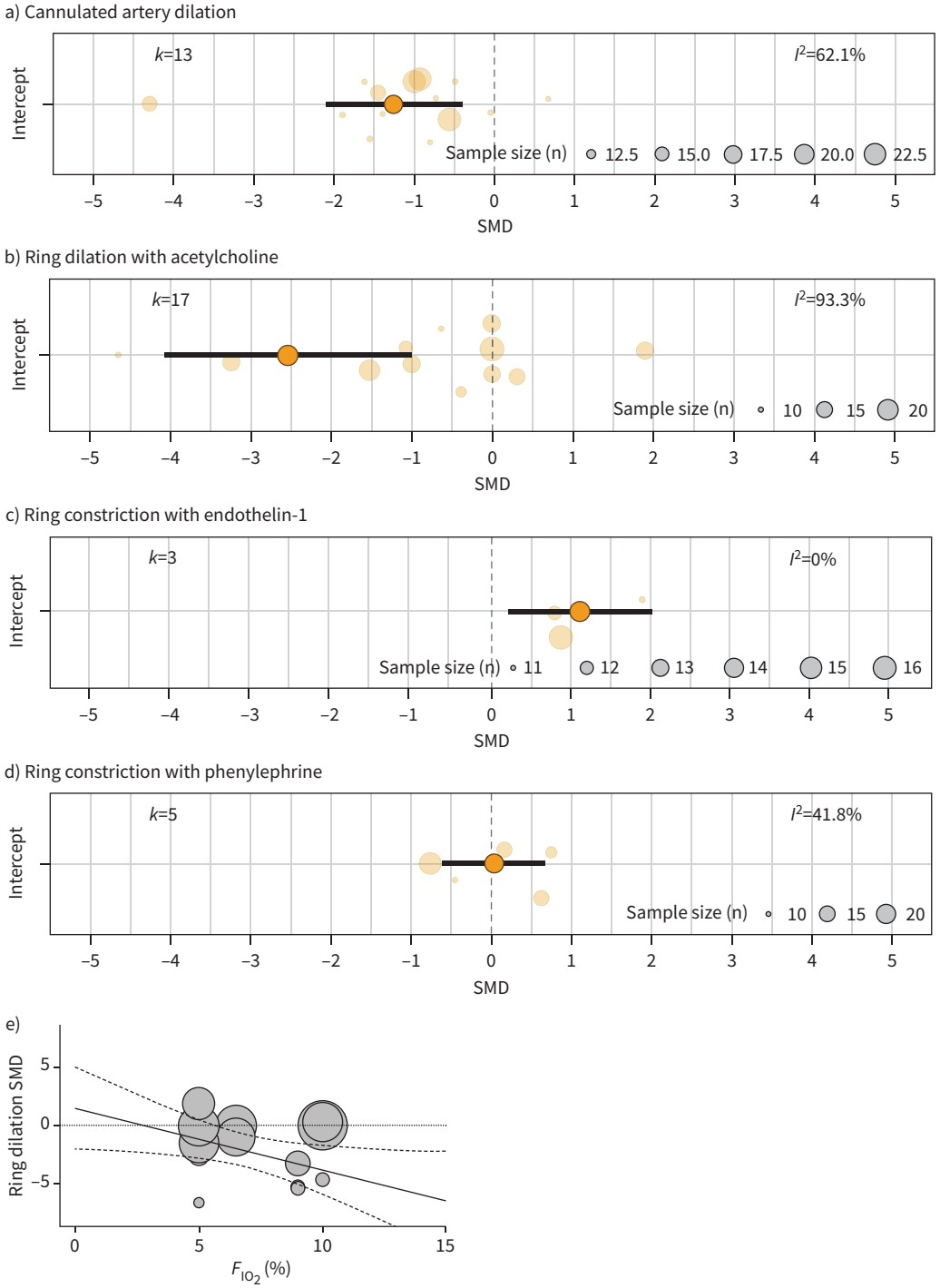

FIGURE 3 Intermittent hypoxia reduces vasodilation in response to acetylcholine and increases vasoconstriction in response to endothelin-1 but not to phenylephrine. Orchard plots showing standardised mean difference (SMD) for a) cannulated artery dilation, b) artery ring dilation with acetylcholine (10^{-6} M), c) artery ring constriction with endothelin-1 (10^{-8} M) and d) artery ring constriction with phenylephrine (10^{-6} M). *k* represents the number of effect sizes per estimate. e) Association between ring vasodilation and inspiratory oxygen fraction (F_{IO_2}) ($p<0.01$, slope −0.73).

Discussion

One of the main features of OSAS is IH, which represents the major trigger for cardiovascular complications [2]. A large corpus of studies in animals report diverse effect sizes for the impact of IH on

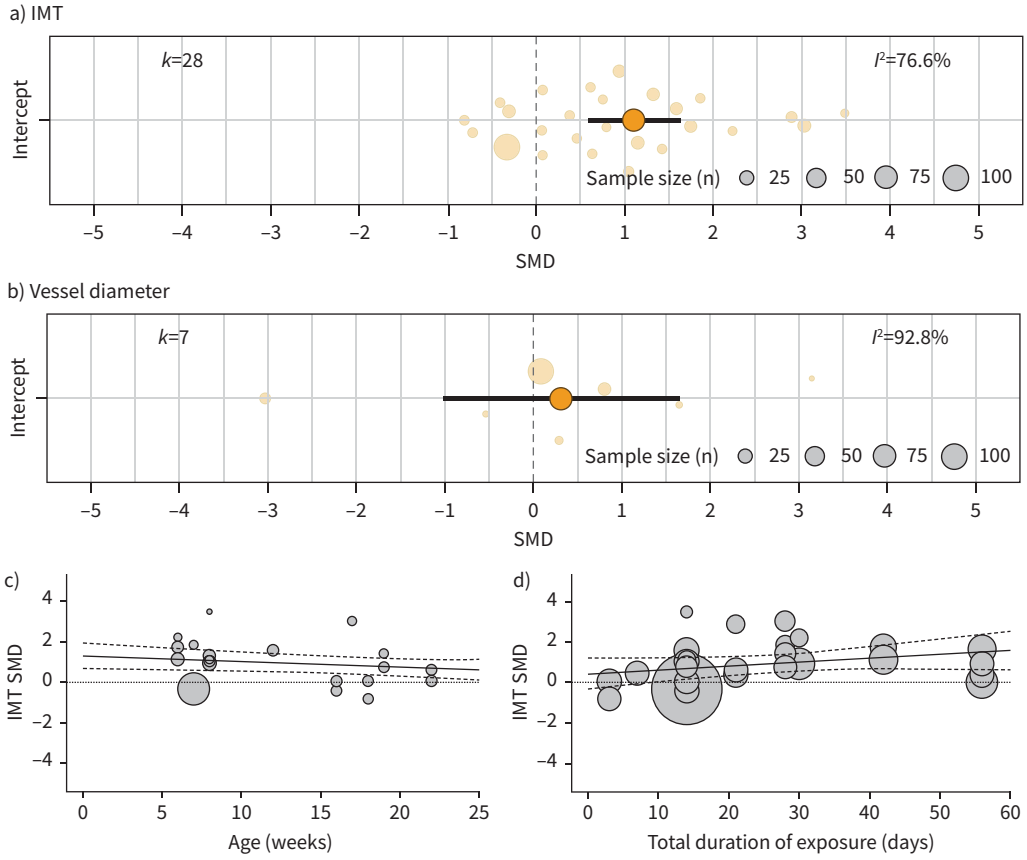

FIGURE 4 Intermittent hypoxia provokes arterial wall structural remodelling. Orchard plots showing standardised mean difference (SMD) for a) intima–media thickness (IMT) of systemic arteries and b) vessel luminal diameter. *k* represents the number of effect sizes per estimate. c) Univariate meta-regressions showing the negative correlation between IMT and rodent age (p=0.014, slope −0.03). d) Univariate adjusted meta-regression showing a strong trend towards positive correlation between IMT and total duration of exposure in days (p=0.07, slope 0.03).

vascular parameters such as arterial pressure, altered vascular reactivity or remodelling. However, there is heterogeneity or even inconsistencies among the published results and, to date, no meta-analysis has been done to assess the impact of IH on these specific parameters. Our meta-analysis firmly establishes that IH, in the absence of the confounders flawing human studies, triggers blood pressure elevation, alterations in vasodilation and atherosclerosis. Some of these responses were proportional to the hypoxic burden and duration of exposure. Another lesson was to delineate the different responses depending on the species, strains, sex and age of exposed animals.

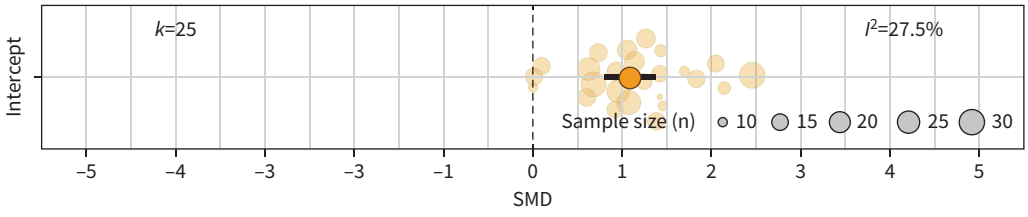

FIGURE 5 Intermittent hypoxia increases atherosclerotic plaque size in apolipoprotein E knockout ($ApoE^{-/-}$) mice. Orchard plot showing standardised mean difference (SMD) for atherosclerosis lesion size. *k* represents the number of effect sizes per estimate.

Impact of IH on vascular parameters

Our meta-analysis confirmed that IH has a clear and significant impact on the primary outcomes: arterial pressure, vessel reactivity, IMT and atherosclerotic lesions (SMD always >0.7). This is consistent with the known vascular effects of OSAS [2, 3], supporting the relevance of these IH models in rodents to human pathology. In particular, while clinical studies are often difficult to interpret due to comorbidities, rodent studies suggest that IH *per se* may be the main cause of the vascular consequences of OSA.

Our meta-analysis showed that IH is associated with a significant increase in MAP, which is consistent with the elevation in sympathetic activity and blood pressure occurring in healthy volunteers submitted to 14 nights of IH [28]. Interestingly, despite the lack of significance, meta-regression analyses suggest that F_{IO_2} (*i.e.* the hypoxic burden) tended to be associated with MAP (p=0.11), suggesting that the severity of hypoxia could be a key element for increased risk of hypertension. This could be of high clinical significance since epidemiological studies [29, 30] already suggest a dose–response relationship between OSA severity, as defined by the apnoea–hypopnoea index (AHI), and hypertension. Also, responses to continuous positive airway pressure, the primary therapy for OSA, are related to the severity of hypoxia at the time of OSA diagnosis (see meta-analyses [31, 32]). This suggests that beyond confounders (*e.g.* obesity or metabolic syndrome) IH may be the main parameter accounting for increased blood pressure in OSA. It also suggests that parameters such as minimal oxygen saturation or time spent at <90% oxygen saturation should be used to describe more precisely OSA severity and hypoxic burden, rather than AHI which does not necessarily reflect the patient's hypoxic burden.

In vascular reactivity studies, we observed that IH significantly altered endothelium-dependent vasodilation in response to acetylcholine. This is in line with studies in humans suggesting that OSA alters endothelial function [33] and is associated with arterial stiffness [34, 35]. Our group recently reported in an individual participant meta-analysis that among adults without overt cardiovascular disease, severe OSA (AHI $\geqslant$30 events·h^{-1}) was independently associated with an increased risk of endothelial dysfunction that may predispose to late cardiovascular events [36]. Moreover, vasoconstriction in response to endothelin-1 was enhanced, while vasoconstriction in response to phenylephrine was not altered by IH. Other vasoconstrictors, such as angiotensin II, have only been sparsely studied and the lack of data did not allow a meta-analysis. Interestingly, endothelin-1-induced vasoconstriction was largely studied with IH protocols that included 5% carbon dioxide in the air breathed by animals. IH combined with hypercapnia also increased the contractile response to endothelin-1 [37, 38]. Our meta-analysis thus suggests that IH, rather than hypercapnia, may be responsible for the endothelin-1 response.

In this meta-analysis, we did not have sufficient statistical power to allow comparison of the reactivity of different vascular beds after IH. However, some studies report some differences in reactivity among vascular beds, in particular in small muscular *versus* large elastic arteries [38]. More studies are needed to allow a meta-analysis on the effects on various vascular beds.

IH-induced vascular remodelling in rodents, as characterised by an augmentation in the IMT, is consistent with what is observed in humans [39, 40]. Our results are also consistent with a recent meta-analysis limited to aorta IMT in mice [41]. Although not reaching significance in univariate analysis (p=0.07), IMT was associated with the total duration of exposure in multivariate analysis. This suggests a progressive remodelling of arteries over time. Interestingly, internal vessel diameter was not modified in rodents, while in humans it is postulated that OSA could induce an increase in diameter, at least in some patients and vessels [42, 43]. IH models in rodents might rapidly attain the late characteristics of the disease such as thickening of the media following changes in the inner diameter of vessels. Other remodelling parameters, *e.g.* compliance or elasticity, could not be included in our meta-analysis due to insufficient studies. However, IH in rodents is known to induce disorganisation of the elastin fibre network [9, 44, 45], reduced vessel distension and increased stiffness [46, 47]. Taken together, IH induces structural remodelling along with alterations in vasoreactivity (blunted vasodilation and increased vasoconstriction) that could act synergistically to increase blood pressure.

Since increased IMT suggests ongoing atherogenesis in wild-type rodents, we included ApoE$^{-/-}$ mice in the meta-analysis because they are susceptible to atherosclerosis and a model of choice to study the impact of IH on atherosclerotic lesions. As expected, we found that IH strongly increased atherosclerotic lesions, consistent with the remodelling observed in wild-type animals [9] and with the known pro-atherogenic consequences of OSAS in humans [48, 49]. Interestingly, diet (standard *versus* high fat) did not significantly modulate plaque size after IH, suggesting that IH is a robust inducer of plaques, independent of a high-fat diet.

We performed meta-regression analyses to determine whether the variability of IH protocols could modulate the impact of IH. Apart from the associations mentioned earlier, other meta-regressions found no

significant effect of IH parameters on the selected vascular outcomes. This would suggest that IH has a robust impact on these outcomes, whatever the duration or severity of IH (in the range of our inclusion criteria). Interestingly, F_{IO_2} was always ⩽10% in the included studies, corresponding to the very severe hypoxia that occurs in the most severe OSAS patients. A less severe hypoxic burden has been little investigated in animal experiment designs. This needs investigation in future animal studies because the impact of OSA treatments in reducing cardiovascular consequences is mostly debated for the mild-to-moderate spectrum of the disease.

Contribution of animal characteristics to IH impact

In univariate analyses, we investigated the contribution of strain, sex, age, diet, body weight and year of publication on IH effects. The species and/or the strain significantly impacted MAP and IMT, suggesting that the choice of species/strain of mice or rats is important when designing a study. MAP is consistently elevated in the most frequently used models such as C57BL/6 mice or Sprague-Dawley and Wistar rats. However, MAP was not found to be elevated in Fischer, Wistar Kyoto or lean Zucker rats, although the very small number of studies using these strains probably accounts for this absence of statistical effect. Vascular remodelling as assessed by IMT is much more pronounced in mice than in rats; rats may thus not be a good model to study remodelling. Our meta-analysis may help researchers to choose the most appropriate models according to the objectives of their study.

Analysis by sex showed that the alteration of vessel dilation induced by IH is found in males, but not in females, despite the small number of studies using females (n=5), suggesting a robust difference in the impact of IH on vasodilation between males and females. This may reflect a sex-related sensitivity to the IH stimulus regarding this particular outcome, consistent with the known stimulation of endothelial-dependent vasodilation by oestrogens [50]. It may underlie the fact that, although most OSAS patients are men, specific studies of the vascular consequences of OSAS in women are necessary, although they are under-represented in the current literature [34, 51].

Animal age was inversely associated with IH-induced intima–media thickening, suggesting that young animals may be more sensitive to IH in terms of vascular remodelling. This is consistent with data in humans [32]. Age had no significant association with other parameters. However, the vast majority of studies were performed in young animals (8–9 weeks old) and the very few using animals older than 50 weeks had to be considered as outliers. There is a lack of information about the effects of IH in older animals, indicating a need for further studies, particularly as OSA is predominant in humans aged over 50 years and not in young adults.

Beside predominantly using young and male animals, animal models currently used present some limitations regarding their relevance for the clinical picture of OSA. First, only the IH component of sleep apnoea is modelled, while sleep fragmentation and increased respiratory efforts are not considered in the IH models. IH is generally applied during daytime, corresponding to rodent's sleep, but in some cases IH has been applied in awake animals. Moreover, in most models carbon dioxide is not controlled as OSA-related intermittent hypercapnia is difficult to mimic. Finally, IH applied to animals is generally severe: 43% of studies use a F_{IO_2} of 5% corresponding to severe OSA with arterial oxygen saturation (S_{aO_2}) ranges achieving 60–80% [5] and only 19% of studies use a F_{IO_2} of 10% corresponding to S_{aO_2} variations encountered in moderate-to-severe OSA patients [5]. There is a need for implementing international consensus statements for fixing shared experimental protocols of IH exposures in terms of severity, circadian alignment and carbon dioxide monitoring.

Risk of bias and limitations of the analysis

Funnel plots and Egger regression tests evidenced a small study effect for all the outcomes studied. Such an effect could have multiple reasons: selective reporting of results or publication bias, nonexhaustive research strategy, poor methodological quality of small studies leading to overestimation of results, true heterogeneity in the results, or chance [52]. One of the potential limitations of our research strategy is the exclusion of non-English language studies because of the difficulty to assess the eligibility criteria of such studies, notably regarding IH protocols. However, interestingly the SMD remained stable for the four main outcomes after the trim-and-fill analyses correcting for missing values, suggesting a limited impact of the small study effect on the results. A publication bias is not unusual in animal studies, and is probably mainly due to selective reporting such as nonpublication of negative results and selection of publishable outcomes. This could be associated with the frequent reluctance of journals to publish negative results. To avoid this reporting bias, we suggest that journals accept to publish animal study protocols or negative results and that authors pre-register their protocols, as is done for clinical studies. For many of the listed items the risk of bias assessed with the SYRCLE risk-of-bias tool was quite high. This is in line with the

poor SYRCLE scores in many other animal study meta-analyses. We argue for improvement of research and publication practices with regard to laboratory animal studies, and the widespread adoption and implementation of the SYRCLE guidelines.

Another limitation of this meta-analysis was the heterogeneity of the outcomes and units of measurement. The use of SMD was intended to deal with this, but our analyses still showed strong heterogeneity for most of the outcomes studied. Statistical analyses only partly succeeded in identifying factors that could explain this heterogeneity, although some characteristics such as species, sex or certain IH properties were suggested. There may be other underlying factors that could potentially explain the heterogeneity of results that were not investigated in our study, such as laboratory-, experimentation- or investigator-dependent effects.

Conclusions

To the best of our knowledge, this is the first meta-analysis of animal studies on the vascular impact of IH. The meta-analysis based on a large corpus of articles evidenced the clear impact of IH on arterial pressure, reactivity and vascular remodelling, summarised as a graphical abstract in supplementary figure S9. We identified some features of IH, in particular F_{IO_2} during hypoxia, which were sometimes associated with an amplified impact of IH. However, in most cases the impact of IH was independent of the precise pattern of IH exposure, suggesting that whatever its modality, aimed at mimicking OSA in humans, IH had a robust effect on rodent vessel structure and function.

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