EUROPEAN RESPIRATORY *journal*

FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Early View

Review

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Please cite this article as: Harki O, Boete Q, Pépin J-L, *et al*. Intermittent hypoxia-related alterations in vascular structure and function: a systematic review and meta-analysis of rodent data. *Eur Respir J* 2021; in press (https://doi.org/10.1183/13993003.00866-2021).

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

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Abbreviated title: Intermittent hypoxia alters vascular structure and function

Key Words: Systematic review, meta-analysis, intermittent hypoxia, arterial pressure, vascular reactivity, vascular remodeling.

Take-home message : Our meta-analysis of rodent studies firmly establishes that intermittent hypoxia, as a model of obstructive sleep apnea, alters vascular pressure, remodeling and reactivity. Severity of IH and rodent characteristics contribute to this impact.

Abstract

Obstructive Sleep Apnea and the related intermittent hypoxia (IH) are widely recognized 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.

We searched PubMed, Embase and Web of Sciences and included 125 articles in a meta-analysis, among them 112 using wild-type rodents and 13 using Apolipoprotein E knock-out mice. We used the standardized mean difference (SMD) to compare results between studies.

IH significantly increased mean arterial pressure (+ 13.90 mmHg (95% CI [11.88; 15.92]), 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 [0.58; 1.62], absolute values: +5.23 (2.81-7.84)). 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 [0.80; 1.37]).

To conclude, 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 apnea in routine cardiology practice.

Introduction

Obstructive Sleep Apnea Syndrome (OSAS) is one of the most frequent chronic diseases, affecting up to nearly one billion individuals worldwide,[1] and is characterized by the repetitive occurrence of apneas and hypopneas during sleep[2]. OSAS is widely recognized as a risk factor for prevalent and incident cardiovascular (CV) 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 CV 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 toward 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 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 CV 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 (for example[16–19]) and there was heterogeneity regarding effect size. Inconsistency between studies might be explained by 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 diet). 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% FiO_2, hypoxic phases varied from 6 to 12 hours per day, and desaturation-reoxygenation sequences lasted from 20 seconds 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 remodeling, 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 the PROSPERO registry under the number CRD42020169940 (https://www.crd.york.ac.uk/prospero/display_record.php?ID=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, Web of Science and EMBASE for articles published up to April 16th, 2021. The search terms were “Intermittent hypoxia” AND “Rodent” OR “mice” OR “rat” (see PROSPERO record for exact query). We also searched for keywords and 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 they were written in English and addressed vascular outcomes of intermittent hypoxia in rodents. Then, the full manuscripts were screened for inclusion and exclusion criteria. Two authors independently screened all references for inclusion, and 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 intermittent hypoxia. An intermittent hypoxia 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 one day. All wild-type rodent models (mouse and rat, male and female, young or aged, lean or obese) with exposure to intermittent hypoxia and compared to a normoxic group were included.

The outcomes considered as mandatory for inclusion were variables allowing characterization of vascular structure or function. This 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 remodeling (Intima-Media Thickness (IMT), internal vessel diameter), and atherosclerosis plaque size in apolipoprotein E knock-out (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), or 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 (randomized 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. 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 analyzed using standardized mean difference, 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 group, and SD is the pooled standard deviation of the two groups.[21] A SMD>0.8 was considered as large, 0.5-0.8 as moderate, and 0.2-0.5 small[22]. In case of a missing SD we calculated or estimated it from confidence intervals, standard errors, t values, P values or F values.[23] The remaining SD were imputed using the mean outcome-specific SD from other included studies. All results are represented using orchard plots, an innovative data visualization tool well adapted for displaying the results of a large number of outcomes[24] (supplementary figure 1).

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 SD from the control groups of included studies using the target unit for IMT outcome[2525]. SMD are expressed with 95% confidence intervals. For study descriptions, we used medians and interquartile ranges (IQR).

Given the high anticipated heterogeneity in included studies we performed random effect meta-analysis by the restricted maximum-likelihood estimator method.[26] 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 intermittent hypoxia protocols (oxygen fraction (FiO_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 blood pressure (MAP), intima-media thickness, 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). Then, we added the significant predictors ($p<0.2$) in meta-regression models evaluating intermittent hypoxia (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[27], 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[28].

All statistical analyses were performed using R statistical software (version 3.6.2).

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 1 and supplementary figure 2 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, 1 in 129S1 mice) and 88 in rats (60 in Sprague-Dawley, 24 in Wistar and 4 in other strains of rats). At study inclusion, median body weight and age were 25.75g (IQR 22.1-27.1) and 8.0 (7-11) weeks for mice and 275g (200-325) and 9 (8-12.7) weeks for rats respectively. Males were used in 103 studies, females in 4 studies, both males and females in one study, and 4 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 4 studies; diet was not specified in 13 studies.

For the studies in ApoE-/- mice, median weight was 27g (25.9-29.5) and median age was 12 (8- 14) weeks. Nine studies used males, 3 used both males and females and 1 did not report the sex. The diet was standard in 5 studies, high fat in 6 studies and both standard and high fat in 2 studies.

Concerning IH protocols (Supplementary figure 2), median and IQ values of FiO_2 during hypoxic periods were of 5.25% (IQR 5-9), desaturation during 60 (30-102.5) seconds followed by 90 (30-217.5) seconds of reoxygenation and return to a FiO_2 of 21% (normoxia). Cycles were repeated on average for 8h/day for a median duration of 21 (10-35) days. Forty-three percent of studies used a FiO_2 of 5%, 11% used a FiO_2 of 6%, and 19% used a FiO_2 of 10% during hypoxic phases. Fourteen percent 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 1. Outcomes were excluded from statistical analysis when <3 studies reported them. In wild-type animals, this was the case for pulsed arterial pressure, atherosclerotic plaques, endothelial permeability, vasoconstriction in cannulated vessels, 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

Intermittent hypoxia significantly increased systolic, diastolic and mean arterial pressure in systemic vessels of wild-type rodents (Figure 2A-C). Mean arterial pressure (MAP) SMD was 1.43, CI [1.13-1.73], I^2 = 75.85% corresponding to a mean increase of 13.90 mmHg (CI [11.88; 15.92]) after IH. Similarly, SBP increased by 13.13 [10.80; 15.47] mmHg, and DBP by 12.24 [9.01; 15.47] mmHg. Forest plots for SBP, DBP and MAP expressed in mmHg are shown in Supplementary Figure 3A, B, C.

Subgroup analyses showed a significant heterogeneity according to strain (test for subgroup difference <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 4). Meta-regression analyses for IH parameters after adjustment for significant confounders in univariate meta-regression (strain and year of publication) showed that a lower FiO_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 metaregressions 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 Ach (10^{-6}M) significantly decreased after IH in both cannulated arteries SMD=-1.26 [-2.10;-0.41], I^2 =62.1% (Figure 3A), and arterial rings SMD=-2.53 [-4.06;-1.00], I^2 = 93.29% (Figure 3B). IH also increased vasoconstriction due to endothelin-1 (10^{-8}M) (SMD=1.11 [0.22; 2.01], I^2 =41.82%) (Figure 3C). On the other hand, there was no impact of IH on the vasoconstriction induced by phenylephrine (10^{-6}M) (SMD=0.04 [-0.6; 0.68], I^2 =41.82%) (Figure 3D).

Subgroup analyses showed that the IH-induced decrease in Ach-dependent vasodilation was observed in male but not in female rodents (SMD 0.12 [-1.82; 2.06] in females vs -1.78 [-3.12; -0.44] in males, p<0.01) (supplementary figure 5). Meta-regression analyses for IH parameters after adjustment for the significant confounder in univariate meta-regression (sex) showed that FiO_2 was significantly associated with vasodilation impairment, especially at moderate hypoxia levels (i.e. in the range of 10% FiO_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 remodeling

In wild-type rodents, intima-media thickness significantly increased after IH (SMD 1.10 [0.58; 1.62], I^2 = 76.5%) (Figure 4A) with an increase of IMT of 5.23 (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 IH in mice (SMD 1.34 [0.55; 2.14]) than in rats (SMD 0.40 [-0.13; 0.93]) (p=0.03 for subgroup difference) (supplementary figure 6). 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 metaregression, duration of exposure was significantly associated with IMT (p=0.046).

Impact of IH on atherosclerosis lesions in ApoE-/- mice

Since vascular remodeling 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 recognized model of susceptibility to atherosclerosis. The analysis showed that IH strongly increased atheromatous lesion size in ApoE-/- mice (SMD 1.08 [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 7 and supplementary table 2. Items for which the risk of bias was low were selective outcome reporting (selection bias, 35% low risk), sequence generation (39% of studies are at 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 categorized as performance and detection bias, were almost never mentioned and therefore scored as "unclear risk": allocation concealment,

randomized housing, blinding of investigators, random outcome assessment, blinding of outcome assessor.

Small study effect

Funnel plots are presented in supplementary figure 8 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 3).

Discussion

One of the main features of obstructive sleep apnea syndrome is intermittent hypoxia which represents the major trigger for cardiovascular complications[29]. A large corpus of studies in animals report diverse effect sizes for the impact of intermittent hypoxia on vascular parameters such as arterial pressure, altered vascular reactivity or remodeling. 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 intermittent hypoxia, 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.

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, intima-media thickness and atherosclerotic lesions (standard mean differences always > 0.7). This is consistent with the known vascular effects of OSAS[3,29], supporting the relevance of these IH models in rodents to the 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 intermittent hypoxia[30]. Interestingly, despite the lack of

significance meta-regression analyses suggest that FiO_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[31,32] already suggest a dose response relationship between OSA severity, as defined by the Apnea-Hypopnea Index, and hypertension. Also, responses to continuous positive airway pressure, the primary therapy for OSA, is related to the severity of hypoxia at the time of OSA diagnosis (see meta-analyses [33,34]). This suggests that beyond confounders (such as 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 the 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[35], and is associated with arterial stiffness[36,37]. Our group recently reported in an individual participant meta-analysis that among adults without overt CV disease, severe OSA (AHI ≥30) was independently associated with an increased risk of endothelial dysfunction that may predispose to late CV events[38]. 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% CO_2 in the air breathed by animals. IH combined with hypercapnia also increased the contractile response to ET-1[39,40]. Our meta-analysis thus suggests that IH, rather than hypercapnia, may thus be responsible for the ET-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 the reactivity among vascular beds, in particular in small muscular vs large elastic arteries[40]. More studies are needed to allow a meta-analysis on the effects on various vascular beds.

IH-induced vascular remodeling in rodents, as characterized by an augmentation in the intima-media thickness, is consistent with what is observed in humans[41,42]. Our results are also consistent with a recent meta-analysis limited to aorta IMT in mice[43]. Although not reaching significance in univariante analysis (p=0.07), the IMT was associated with the total duration of exposure in multivariate analysis. This suggests a progressive remodeling of arteries over time. Interestingly, internal vessel diameter was not modified in rodents while, in humans, it is postulated that obstructive sleep apnea could

induce an increase in diameter, at least in some patients and vessels[44,45]. IH models in rodents might rapidly attain the late characteristics of the disease such as thickening the media following changes in the inner diameter of vessels. Other remodeling parameters, such as compliance or elasticity, could not be included in our meta-analysis due to insufficient studies. However, IH in rodents is known to induce disorganization of the elastin fiber network[9,46,47], reduced vessel distension and increased stiffness[48,49]. Taken together, IH induces structural remodeling 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 remodeling observed in wild-type animals[9], and with the known pro-atherogenic consequences of OSAS in humans[50,51]. Interestingly, diet (standard vs 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 above, 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, FiO_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 or 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 remodeling as assessed by IMT is much more pronounced in

mice than in rats; rats may thus not be a good model to study remodeling. 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 sensibility to the IH stimulus regarding this particular outcome, consistent with the known stimulation of endothelial-dependent vasodilation by oestrogens[52]. 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[36,53].

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 remodeling. This is consistent with data in humans[34]. 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 obstructive sleep apnea is predominant in humans aged over 50 and not in young adults.

Beside predominantly using young and male animals, animal models currently used present some limitations regarding their relevance for clinical picture of OSA. First, only the IH component of sleep apnea 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 CO_2 is not controlled as OSA-related intermittent hypercapnia is difficult to mimic. Finally, intermittent hypoxia applied to animals is generally severe : 43% of studies use a FiO2 of 5% corresponding to severe OSA with SaO2 ranges achieving 60-80%[5], and only 19% of studies use a FiO2 of 10% corresponding to SaO2 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 CO_2 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, non-exhaustive research strategy, poor methodological quality of small studies leading to overestimation

of results, true heterogeneity in the results or chance[54]. 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 non-publication 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 authors to 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.

Conclusion

To our knowledge, this is the first meta-analysis of animal studies on the vascular impact of intermittent hypoxia. The meta-analysis based on a large corpus of articles evidenced the clear impact of IH on arterial pressure, reactivity and vascular remodeling. We identified some features of IH, in particular FiO_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 obstructive sleep apnea in humans, IH had a robust effect on rodent vessel structure and function.

Funding

This work was supported by the “Fondation du Souffle / Fondation pour la Recherch en Santé Respiratoire” (PhD fellowship for O.H.), Fondation de l’Avenir, Fondation Agir Pour les Maladies Chroniques, and ANR Arterylastic grant ANR-18-CE-18-0001 (PhD fellowship for Q.B.).

Acknowledgments

The authors thank Axel Couturier for helpful discussions and Alison Foote for critical reading and English editing.

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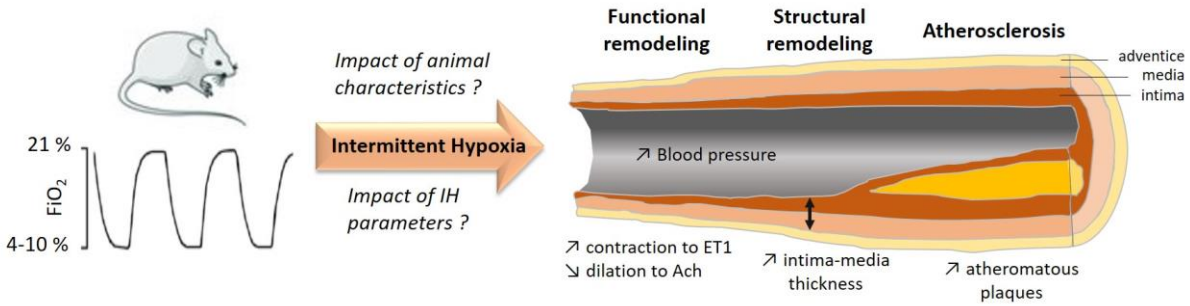
Table 1. Meta-regression analyses for the main outcomes: MAP, dilation of artery rings, IMT, atherosclerosis lesions in ApoE-/- mice ("ApoE lesion"). Bold figures indicate significance at p<0.05, ¥ p<0.2 indicates moderators included in the multivariate model to adjust meta-regressions on IH parameters.

	MAP			Arteries rings dilation			IMT			ApoE lesion		
Moderator	n	slope	p-val	n	slope	p-val	n	slope	p-val	n	slope	p-val
Univariate metaregressions												
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 metaregression on IH parameters												
FiO_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

Figure legends



Graphical abstract : Our meta-analysis included rodents exposed to experimental intermittent hypoxia. We demonstrate that IH significantly increased blood pressure, altered vasodilation and increased vasoconstriction, increased intima-media thickness and atherosclerosis plaques. Altogether, IH is responsible for structural and functional vascular alterations in obstructive sleep apnea.

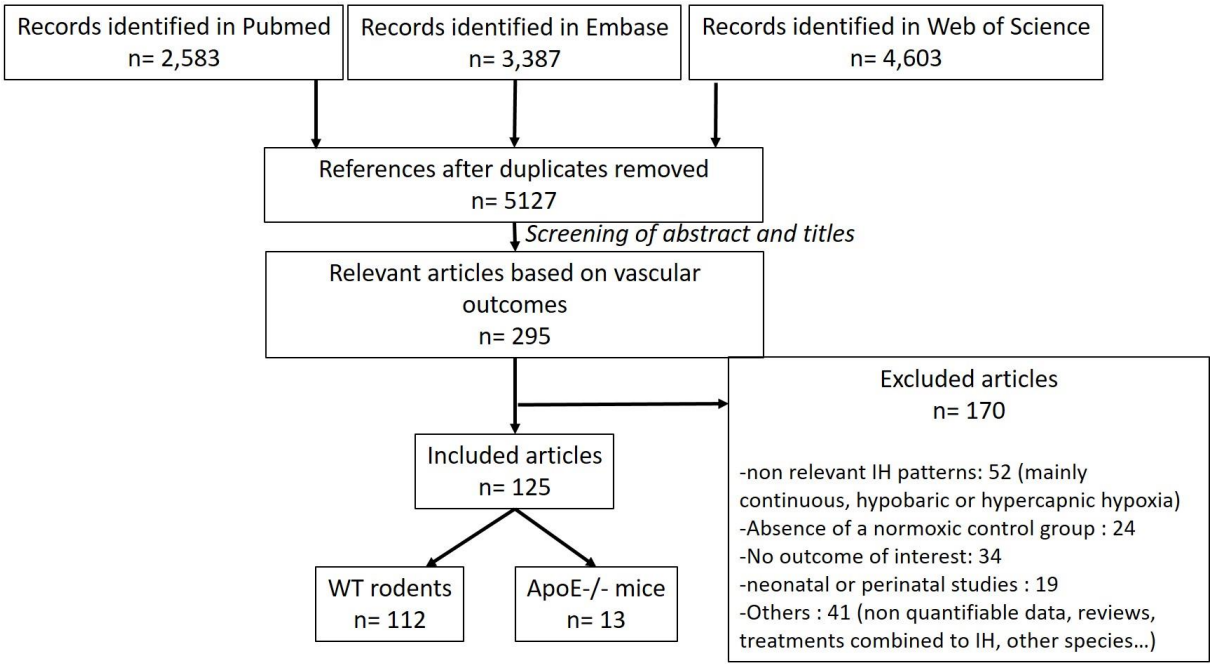


Figure 1: Flow diagram of the study

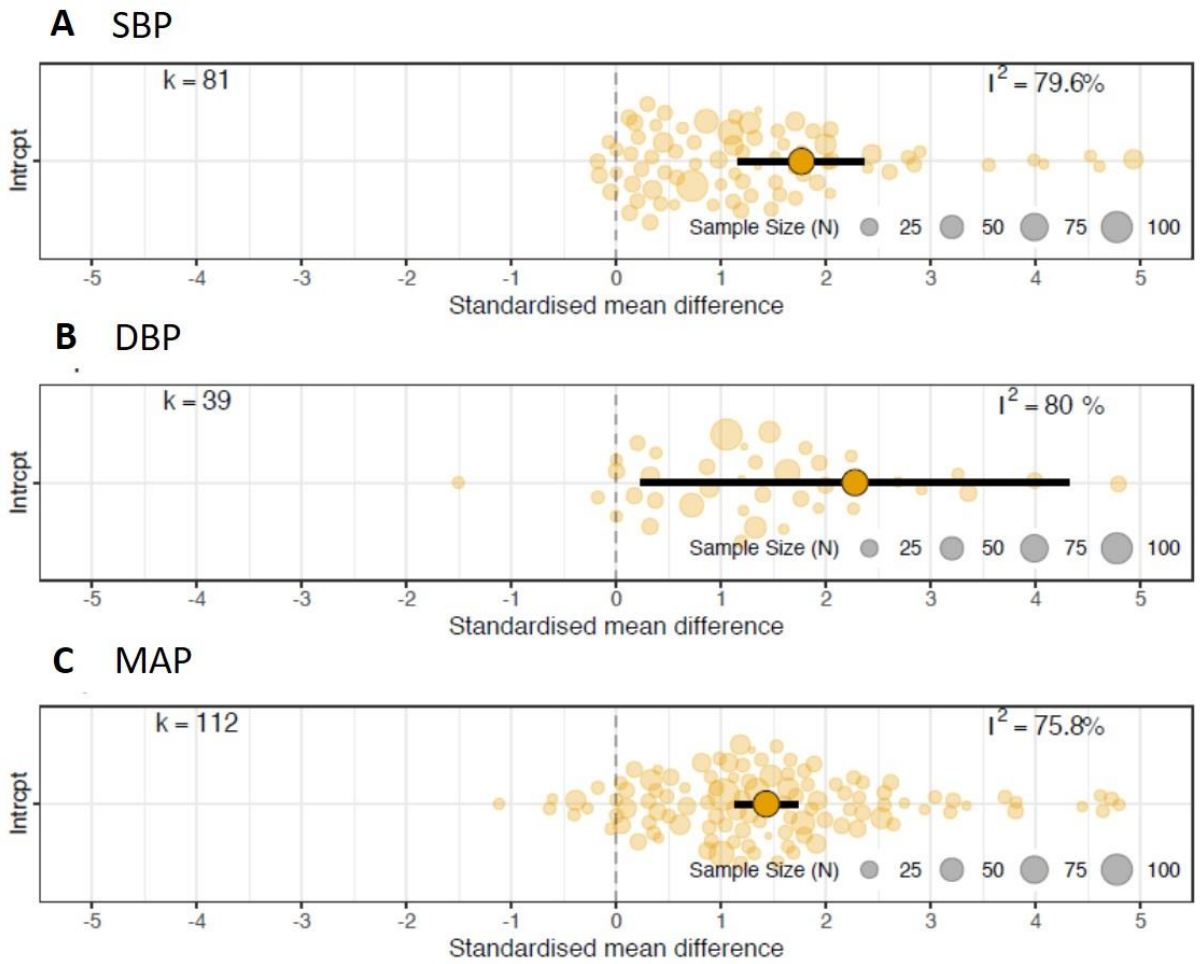


Figure 2: IH increases blood pressure in systemic vessels of wild-type animals. Orchard plots showing SMD for: A) systolic blood pressure (SBP), B) diastolic blood pressure (DBP), C) mean arterial pressure (MAP).

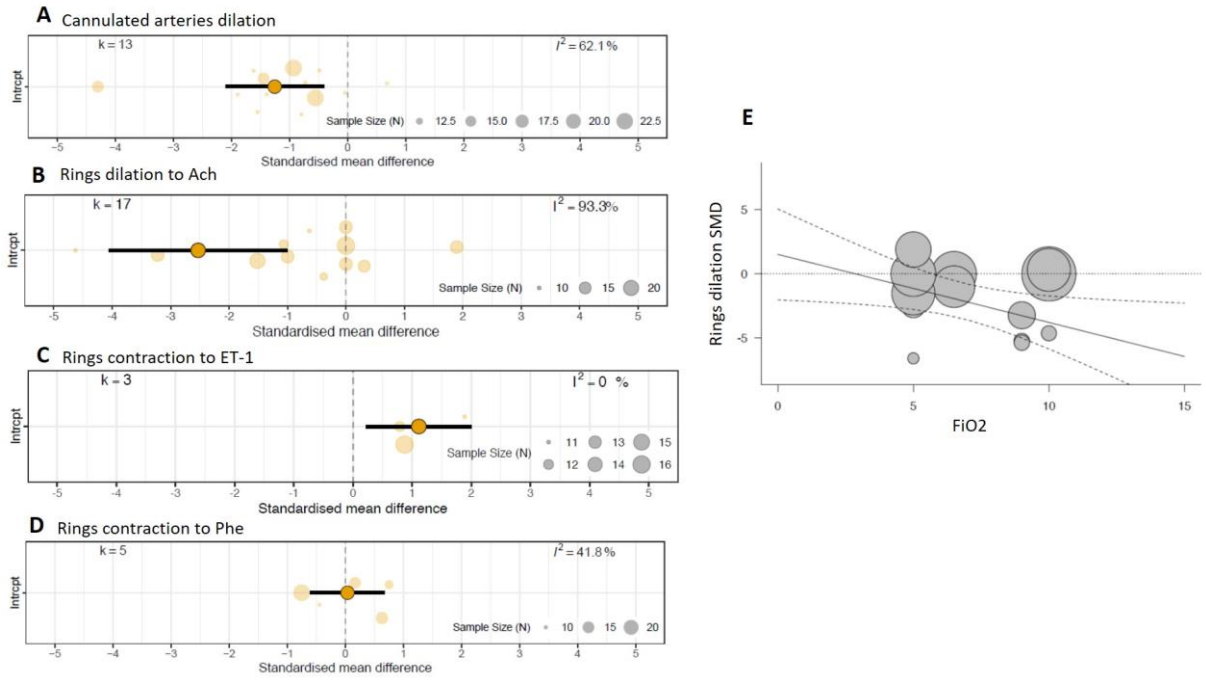


Figure 3: IH reduces vasodilation in response to Acetylcholine and increases vasoconstriction in response to ET-1 but not to Phenylephrine. Orchard plots showing SMD for A) cannulated artery dilation, B) artery ring dilation with Acetylcholine (10^{-6}M), C) artery ring constriction with Endothelin-1 (10^{-8}M), D) artery ring constriction with Phenylephrine (10^{-6}M). E) Association between ring vasodilation and FiO_2 ($p<0.01$, slope= -0.73).

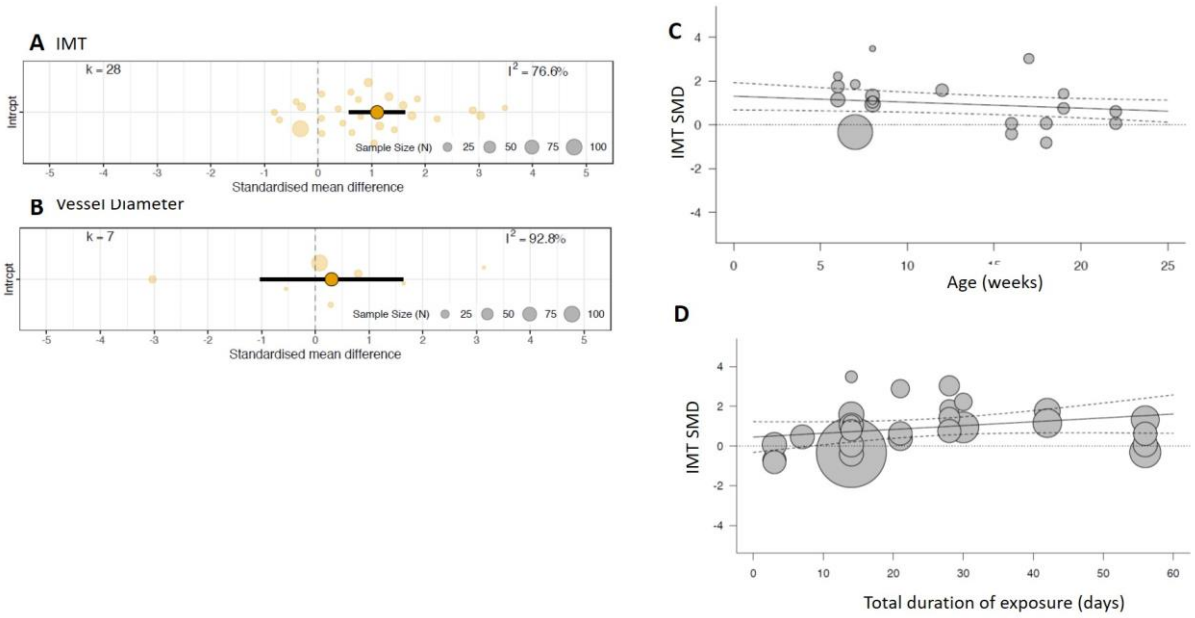


Figure 4: IH provokes arterial wall structural remodeling. Orchard plots showing SMD for A) intima-media thickness (IMT) of systemic arteries, B) vessel luminal diameter. C) Univariate meta-regressions showing the negative correlation between IMT and rodent age (p=0.014, slope = -0.03). D) Univariate 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).

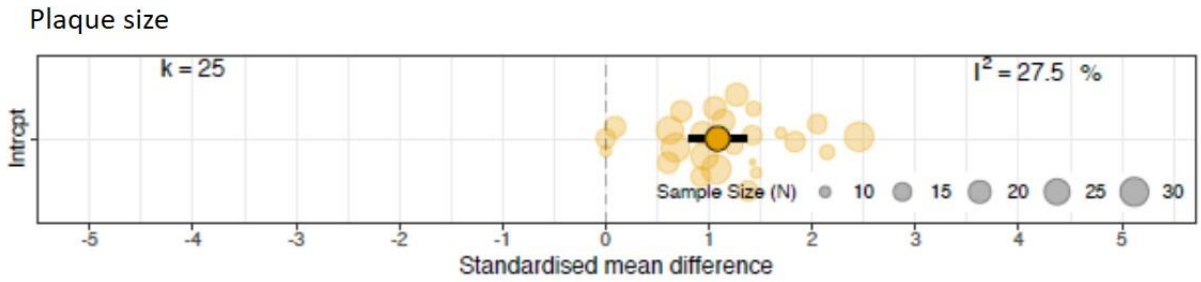
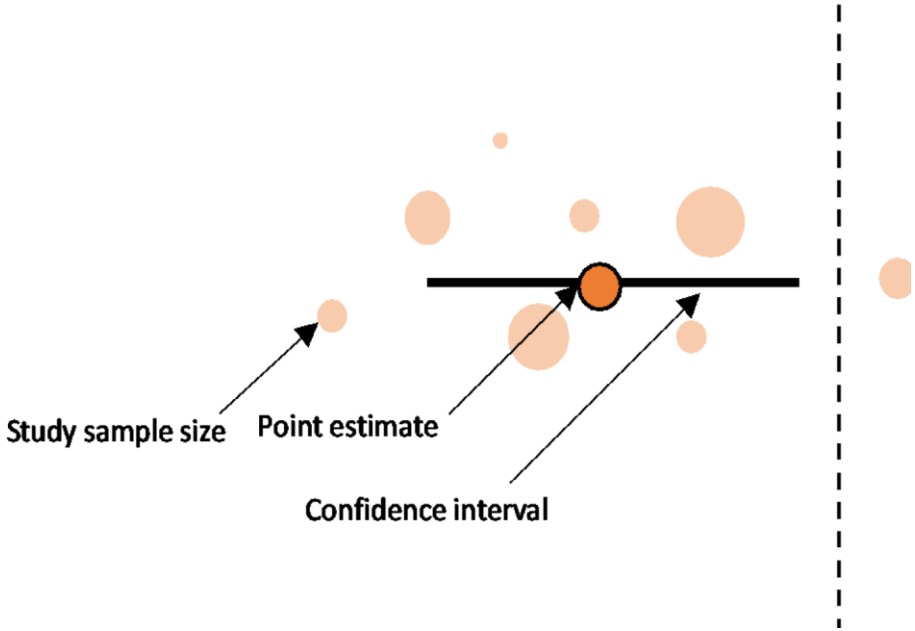


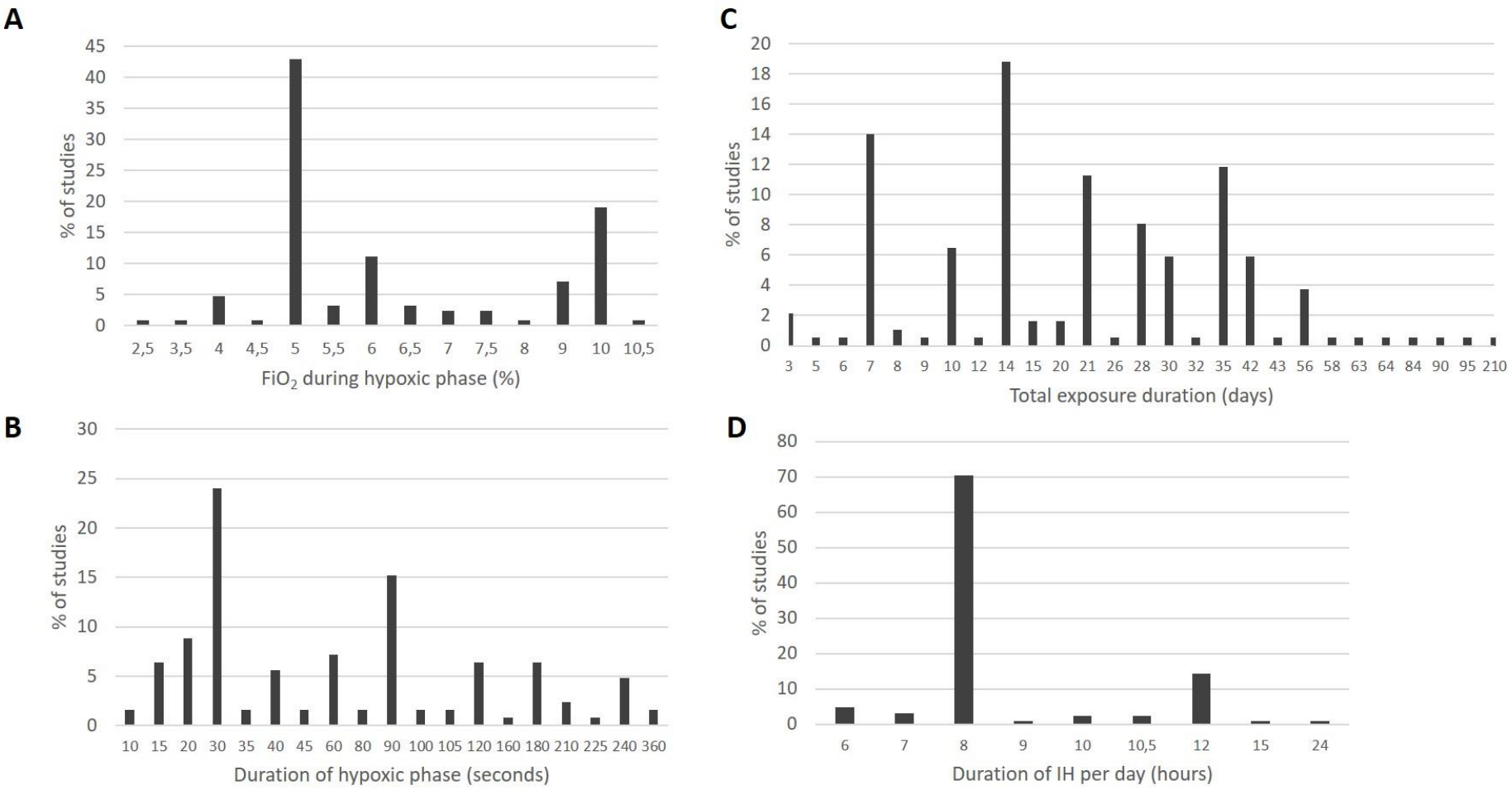
Figure 5: IH increases atherosclerotic plaque size in ApoE-/- mice. Orchard plot showing SMD for atherosclerosis lesion size.

Supplemental data

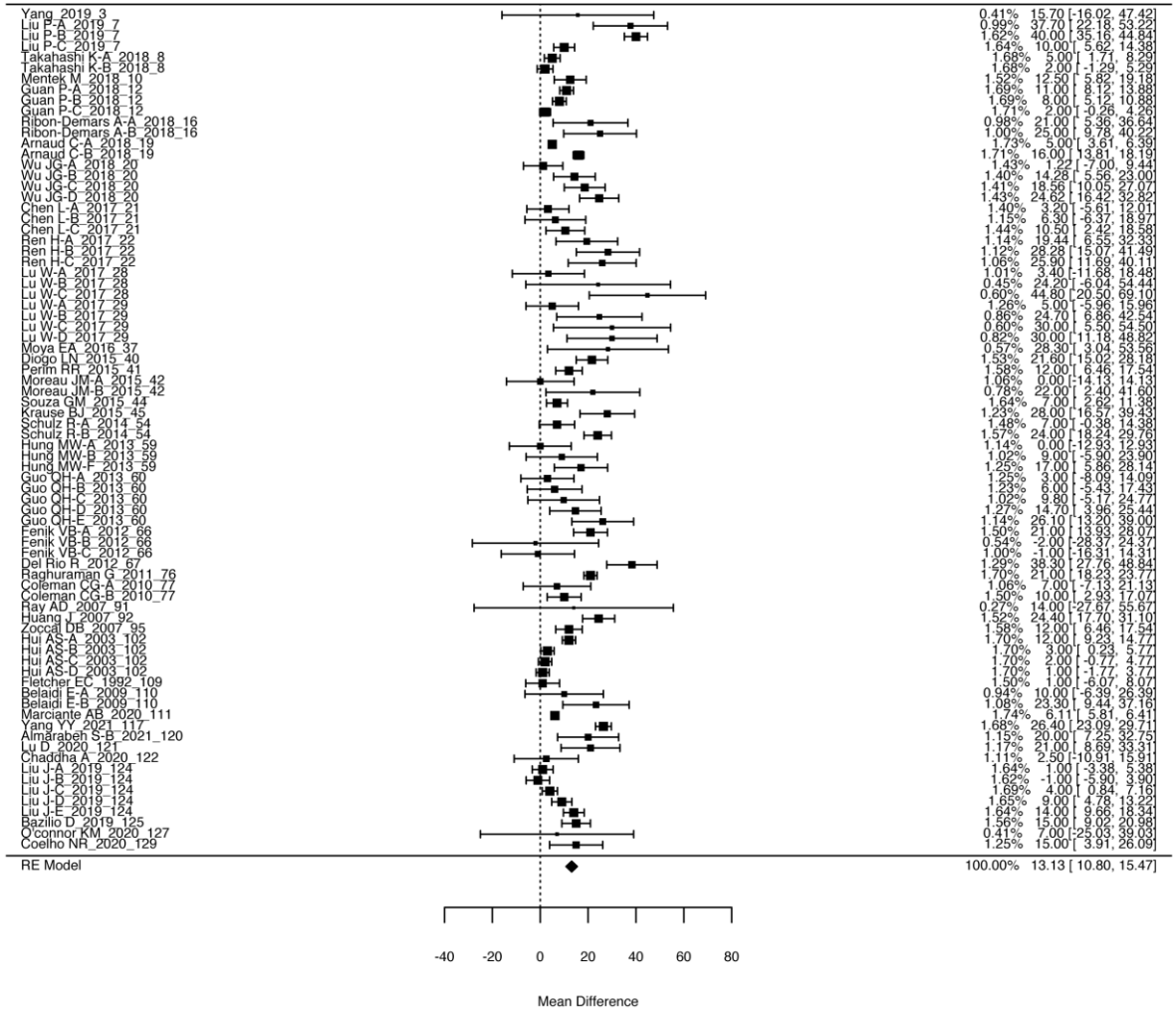
Supplementary Figure 1

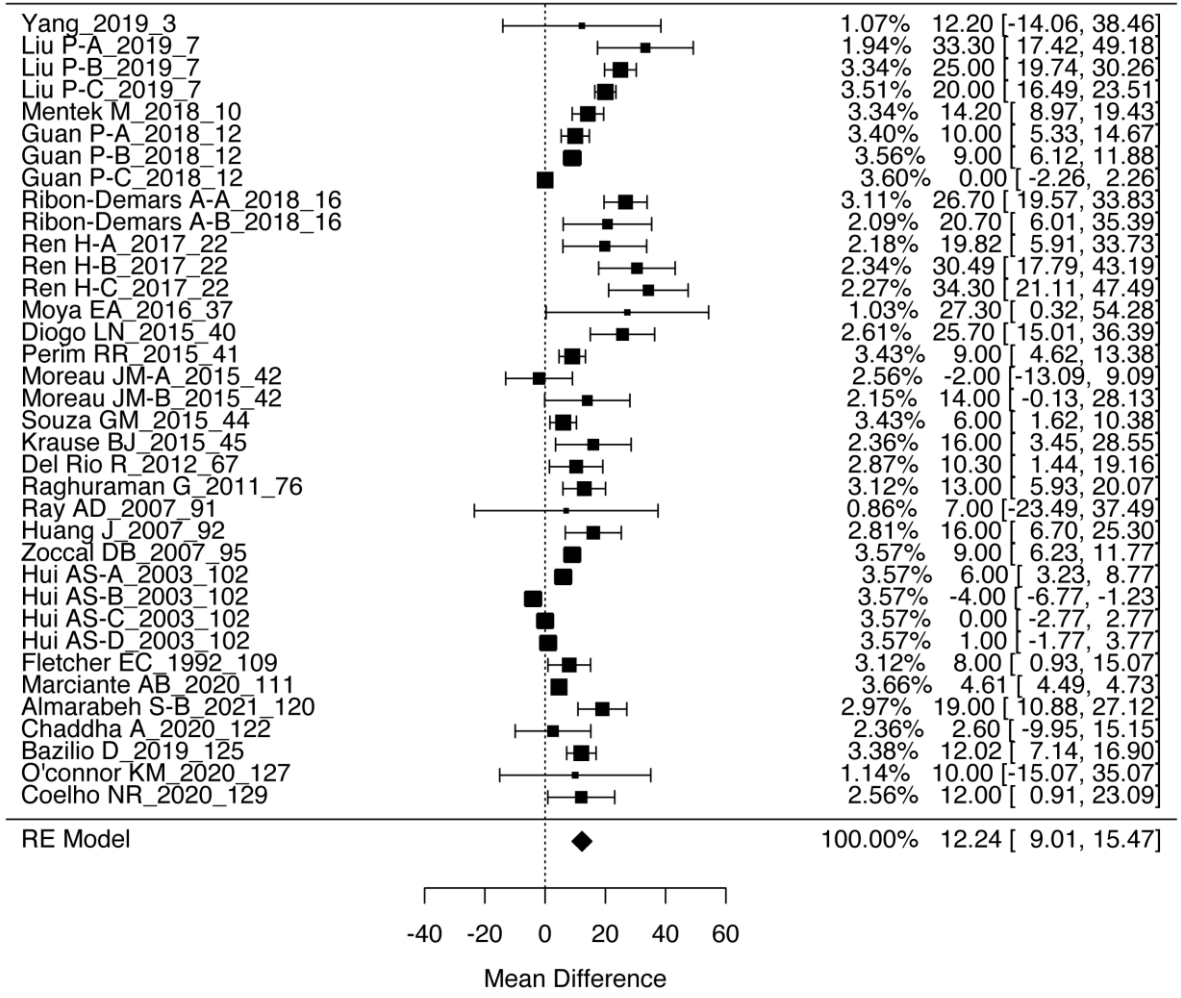


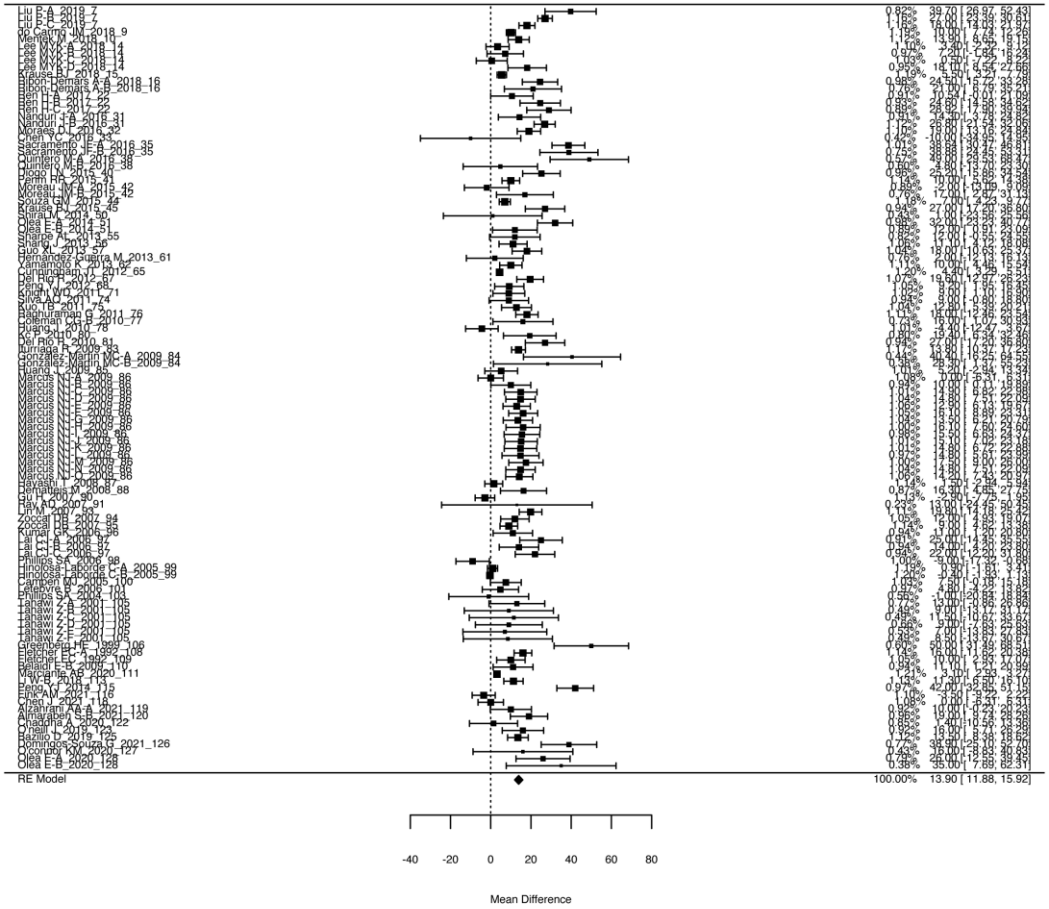
Supplementary Figure 1: Orchard plot example showing the meaning of the different parts of the plot.



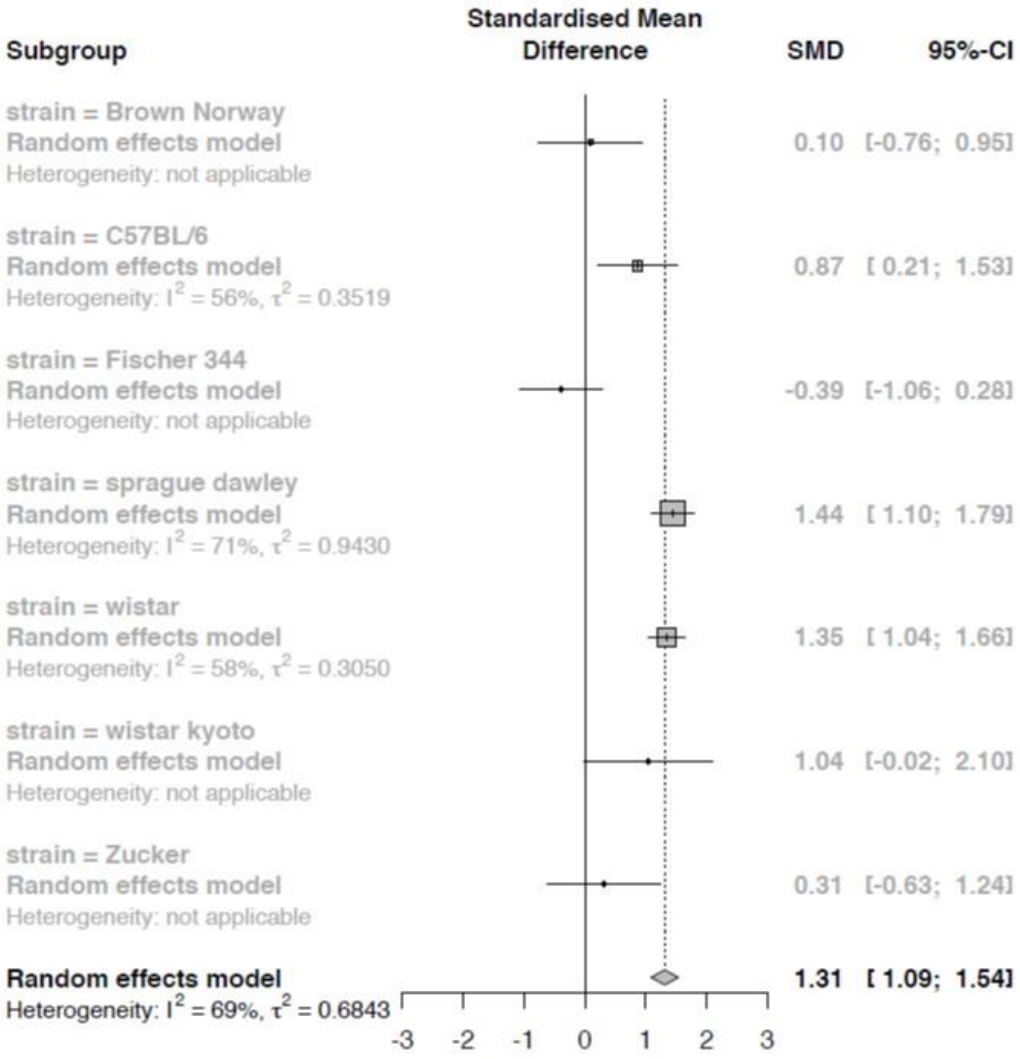
Supplementary Figure 2: Description of the number of studies included depending on four IH parameters: A) FiO_2 during hypoxic phase (in %), B) Duration of each hypoxic phase (in seconds), C) Total duration of exposure (in days), D) Duration of IH exposure per day (in hours).


Yang_2019_3 0.41% 15.70 [-16.02, 47.42]
Liu P-A_2019_7 0.99% 37.70 [22.18, 53.22]
Liu P-B_2019_7 1.62% 40.00 [35.16, 44.84]
Liu P-C_2019_7 1.64% 10.00 [5.62, 14.38]
Takahashi K-A_2018_8 1.68% 5.00 [1.71, 8.29]
Takahashi K-B_2018_8 1.68% 2.00 [-1.29, 5.29]
Mentek M_2018_10 1.52% 12.50 [5.82, 19.18]
Guan P-A_2018_12 1.69% 11.00 [8.12, 13.88]
Guan P-B_2018_12 1.69% 8.00 [5.12, 10.88]
Guan P-C_2018_12 1.71% 2.00 [-0.28, 4.28]
Ribon-Demars A-A_2018_16 0.98% 21.00 [5.36, 36.64]
Ribon-Demars A-B_2018_16 1.00% 25.00 [9.78, 40.22]
Arnaud C-A_2018_19 1.73% 5.00 [3.61, 6.39]
Arnaud C-B_2018_19 1.71% 16.00 [13.81, 18.19]
Wu JG-A_2018_20 1.43% 1.22 [-7.00, 9.44]
Wu JG-B_2018_20 1.40% 14.28 [5.56, 23.00]
Wu JG-C_2018_20 1.41% 18.56 [10.05, 27.07]
Wu JG-D_2018_20 1.43% 24.62 [16.42, 32.82]
Chen L-A_2017_21 1.40% 3.20 [-5.61, 12.01]
Chen L-B_2017_21 1.15% 6.30 [-6.37, 18.97]
Chen L-C_2017_21 1.44% 10.50 [2.42, 18.58]
Ren H-A_2017_22 1.14% 19.44 [6.55, 32.33]
Ren H-B_2017_22 1.12% 28.28 [15.07, 41.49]
Ren H-C_2017_22 1.06% 25.90 [11.69, 40.11]
Lu W-A_2017_28 1.01% 3.40 [-11.68, 18.48]
Lu W-B_2017_28 0.45% 24.20 [-6.04, 54.44]
Lu W-C_2017_28 0.60% 44.80 [20.50, 69.10]
Lu W-A_2017_29 1.26% 5.00 [-5.96, 15.96]
Lu W-B_2017_29 0.86% 24.70 [6.86, 42.54]
Lu W-C_2017_29 0.60% 30.00 [5.50, 54.50]
Lu W-D_2017_29 0.82% 30.00 [11.18, 48.82]
Moya EA_2016_37 0.57% 28.30 [3.04, 53.56]
Diogo LN_2015_40 1.53% 21.60 [15.02, 28.18]
Perlm RR_2015_41 1.58% 12.00 [6.46, 17.54]
Moreau JM-A_2015_42 1.06% 0.00 [-14.13, 14.13]
Moreau JM-B_2015_42 0.78% 22.00 [2.40, 41.60]
Souza GM_2015_44 1.64% 7.00 [2.62, 11.38]
Krause BJ_2015_45 1.23% 28.00 [16.57, 39.43]
Schulz R-A_2014_54 1.48% 7.00 [-0.38, 14.38]
Schulz R-B_2014_54 1.57% 24.00 [18.24, 29.76]
Hung MW-A_2013_59 1.14% 0.00 [-12.93, 12.93]
Hung MW-B_2013_59 1.02% 9.00 [-5.90, 23.90]
Hung MW-F_2013_59 1.25% 17.00 [5.86, 28.14]
Guo QH-A_2013_60 1.25% 3.00 [-8.09, 14.09]
Guo QH-B_2013_60 1.23% 6.00 [-5.43, 17.43]
Guo QH-C_2013_60 1.02% 9.80 [-5.17, 24.77]
Guo QH-D_2013_60 1.27% 14.70 [3.96, 25.44]
Guo QH-E_2013_60 1.14% 26.10 [13.20, 39.00]
Fenik VB-A_2012_66 1.50% 21.00 [13.93, 28.07]
Fenik VB-B_2012_66 0.54% -2.00 [-28.37, 24.37]
Fenik VB-C_2012_66 1.00% -1.00 [-16.31, 14.31]
Del Rio R_2012_67 1.29% 38.30 [27.76, 48.84]
Raghuraman G_2011_76 1.70% 21.00 [18.23, 23.77]
Coleman CG-A_2010_77 1.06% 7.00 [-7.13, 21.13]
Coleman CG-B_2010_77 1.50% 10.00 [2.93, 17.07]
Ray AD_2007_91 0.27% 14.00 [-27.67, 55.67]
Huang J_2007_92 1.52% 24.40 [17.70, 31.10]
Zoccali DB_2007_95 1.58% 12.00 [6.46, 17.54]
Hui AS-A_2003_102 1.70% 12.00 [9.23, 14.77]
Hui AS-B_2003_102 1.70% 3.00 [0.23, 5.77]
Hui AS-C_2003_102 1.70% 2.00 [-0.77, 4.77]
Hui AS-D_2003_102 1.70% 1.00 [-1.77, 3.77]
Fletcher EC_1992_109 1.50% 1.00 [-6.07, 8.07]
Belaidi E-A_2009_110 0.94% 10.00 [-6.39, 26.39]
Belaidi E-B_2009_110 1.08% 23.30 [9.44, 37.16]
Marciante AB_2020_111 1.74% 6.11 [5.81, 6.41]
Yang YY_2021_117 1.68% 26.40 [23.09, 29.71]
Almarabeh S-B_2021_120 1.15% 20.00 [7.25, 32.75]
Lu D_2020_121 1.17% 21.00 [8.69, 33.31]
Chaddha A_2020_122 1.11% 2.50 [-10.91, 15.91]
Liu J-A_2019_124 1.64% -1.00 [-3.38, 5.38]
Liu J-B_2019_124 1.62% -1.00 [-5.90, 3.90]
Liu J-C_2019_124 1.69% 4.00 [0.84, 7.16]
Liu J-D_2019_124 1.65% 9.00 [4.78, 13.22]
Liu J-E_2019_124 1.64% 14.00 [9.66, 18.34]
Bazilio D_2019_125 1.56% 15.00 [9.02, 20.98]
O'connor KM_2020_127 0.41% 7.00 [-25.03, 39.03]
Coelho NR_2020_129 1.25% 15.00 [3.91, 26.09]
RE Model 100.00% 13.13 [10.80, 15.47]
-40
-20
0
20
40
60
80
Mean Difference

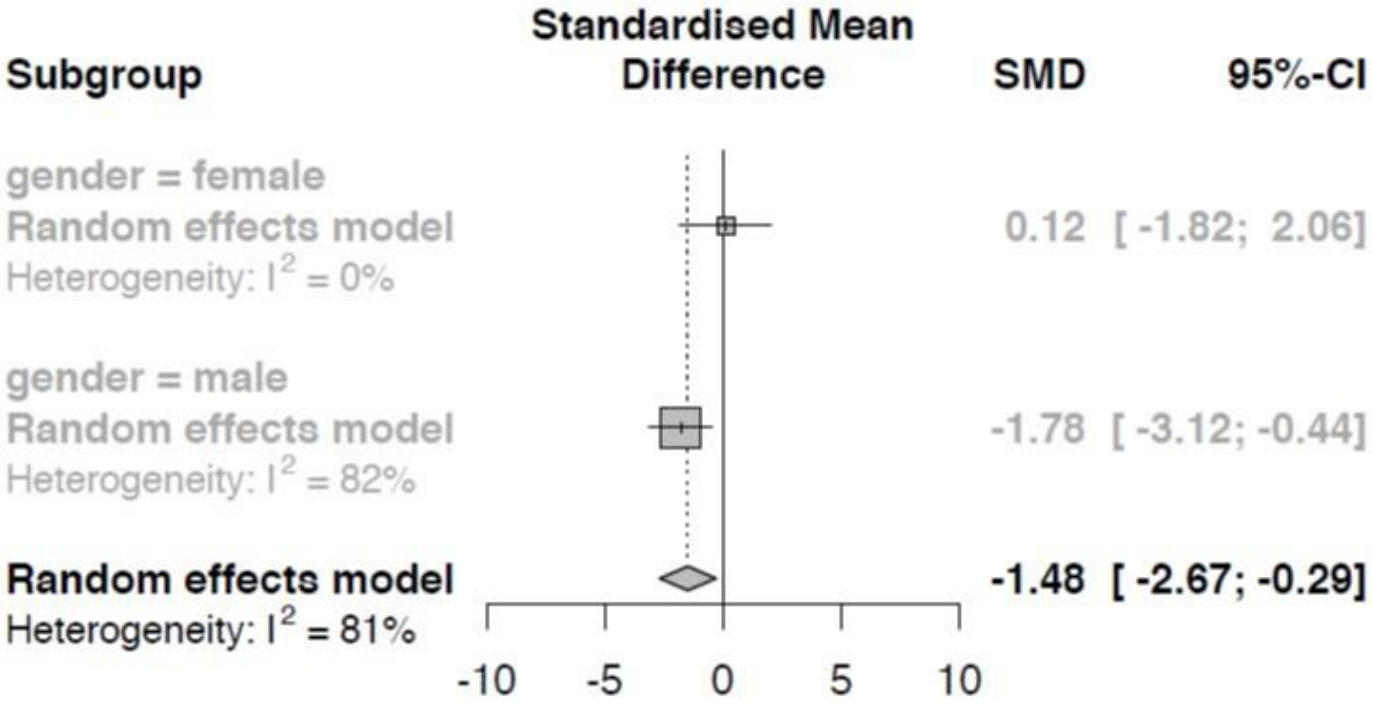

Yang_2019_3 1.07% 12.20 [-14.06, 38.46]
Liu P-A_2019_7 1.94% 33.30 [17.42, 49.18]
Liu P-B_2019_7 3.34% 25.00 [19.74, 30.26]
Liu P-C_2019_7 3.51% 20.00 [16.49, 23.51]
Mentek M_2018_10 3.34% 14.20 [8.97, 19.43]
Guan P-A_2018_12 3.40% 10.00 [5.33, 14.67]
Guan P-B_2018_12 3.56% 9.00 [6.12, 11.88]
Guan P-C_2018_12 3.60% 0.00 [-2.26, 2.26]
Ribon-Demars A-A_2018_16 3.11% 26.70 [19.57, 33.83]
Ribon-Demars A-B_2018_16 2.09% 20.70 [6.01, 35.39]
Ren H-A_2017_22 2.18% 19.82 [5.91, 33.73]
Ren H-B_2017_22 2.34% 30.49 [17.79, 43.19]
Ren H-C_2017_22 2.27% 34.30 [21.11, 47.49]
Moya EA_2016_37 1.03% 27.30 [0.32, 54.28]
Diogo LN_2015_40 2.61% 25.70 [15.01, 36.39]
Perim RR_2015_41 3.43% 9.00 [4.62, 13.38]
Moreau JM-A_2015_42 2.56% -2.00 [-13.09, 9.09]
Moreau JM-B_2015_42 2.15% 14.00 [-0.13, 28.13]
Souza GM_2015_44 3.43% 6.00 [1.62, 10.38]
Krause BJ_2015_45 2.36% 16.00 [3.45, 28.55]
Del Rio R_2012_67 2.87% 10.30 [1.44, 19.16]
Raghuraman G_2011_76 3.12% 13.00 [5.93, 20.07]
Ray AD_2007_91 0.86% 7.00 [-23.49, 37.49]
Huang J_2007_92 2.81% 16.00 [6.70, 25.30]
Zoccal DB_2007_95 3.57% 9.00 [6.23, 11.77]
Hui AS-A_2003_102 3.57% 6.00 [3.23, 8.77]
Hui AS-B_2003_102 3.57% -4.00 [-6.77, -1.23]
Hui AS-C_2003_102 3.57% 0.00 [-2.77, 2.77]
Hui AS-D_2003_102 3.57% 1.00 [-1.77, 3.77]
Fletcher EC_1992_109 3.12% 8.00 [0.93, 15.07]
Marciante AB_2020_111 3.66% 4.61 [4.49, 4.73]
Almarabeh S-B_2021_120 2.97% 19.00 [10.88, 27.12]
Chaddha A_2020_122 2.36% 2.60 [-9.95, 15.15]
Bazilio D_2019_125 3.38% 12.02 [7.14, 16.90]
O'connor KM_2020_127 1.14% 10.00 [-15.07, 35.07]
Coelho NR_2020_129 2.56% 12.00 [0.91, 23.09]
RE Model 100.00% 12.24 [9.01, 15.47]
-40 -20 0 20 40 60
Mean Difference



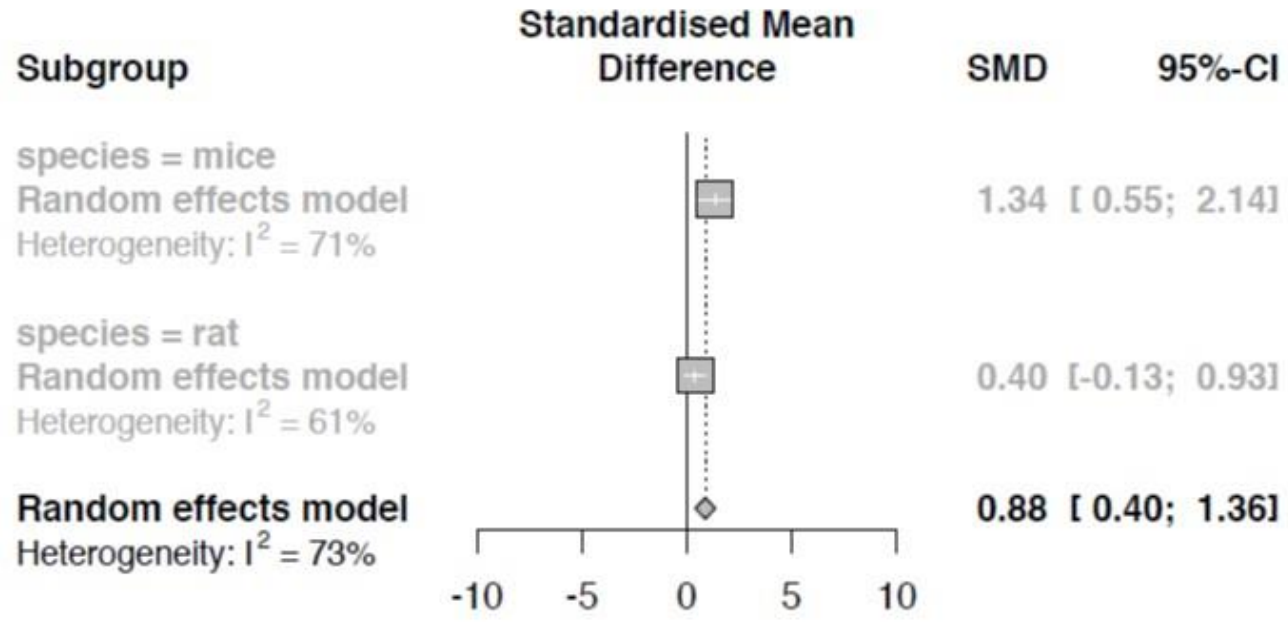
Supplementary Figure 3: Forest plots for Systolic Blood Pressure (A), Diastolic Blood Pressure (B) and Mean Arterial Pressure (C). Mean differences are expressed in mmHg with 95% CI.



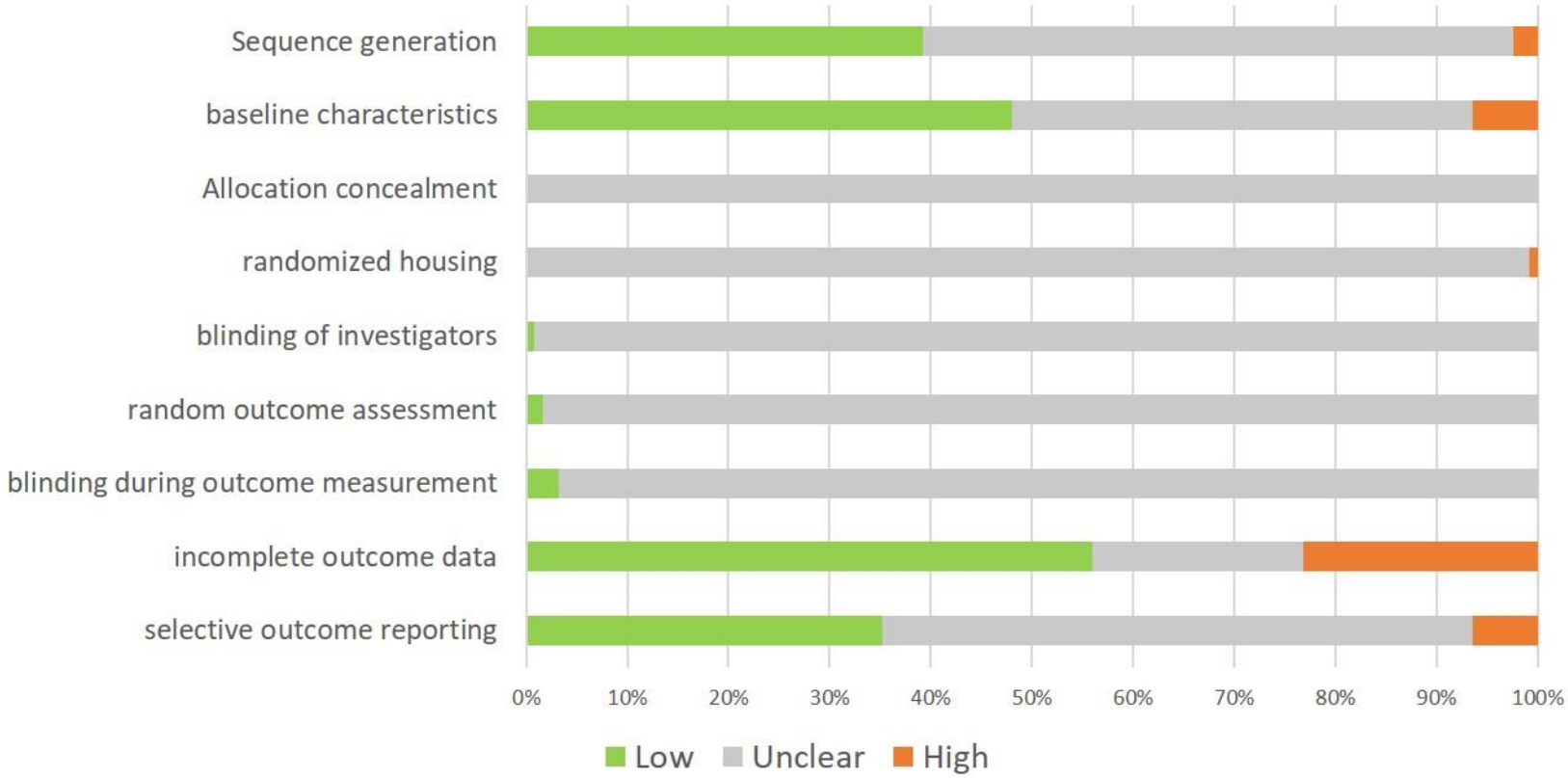
Supplementary Figure 4: Subgroup analysis for MAP according to the strain of wild-type animals. $p<0.01$ for subgroup differences.



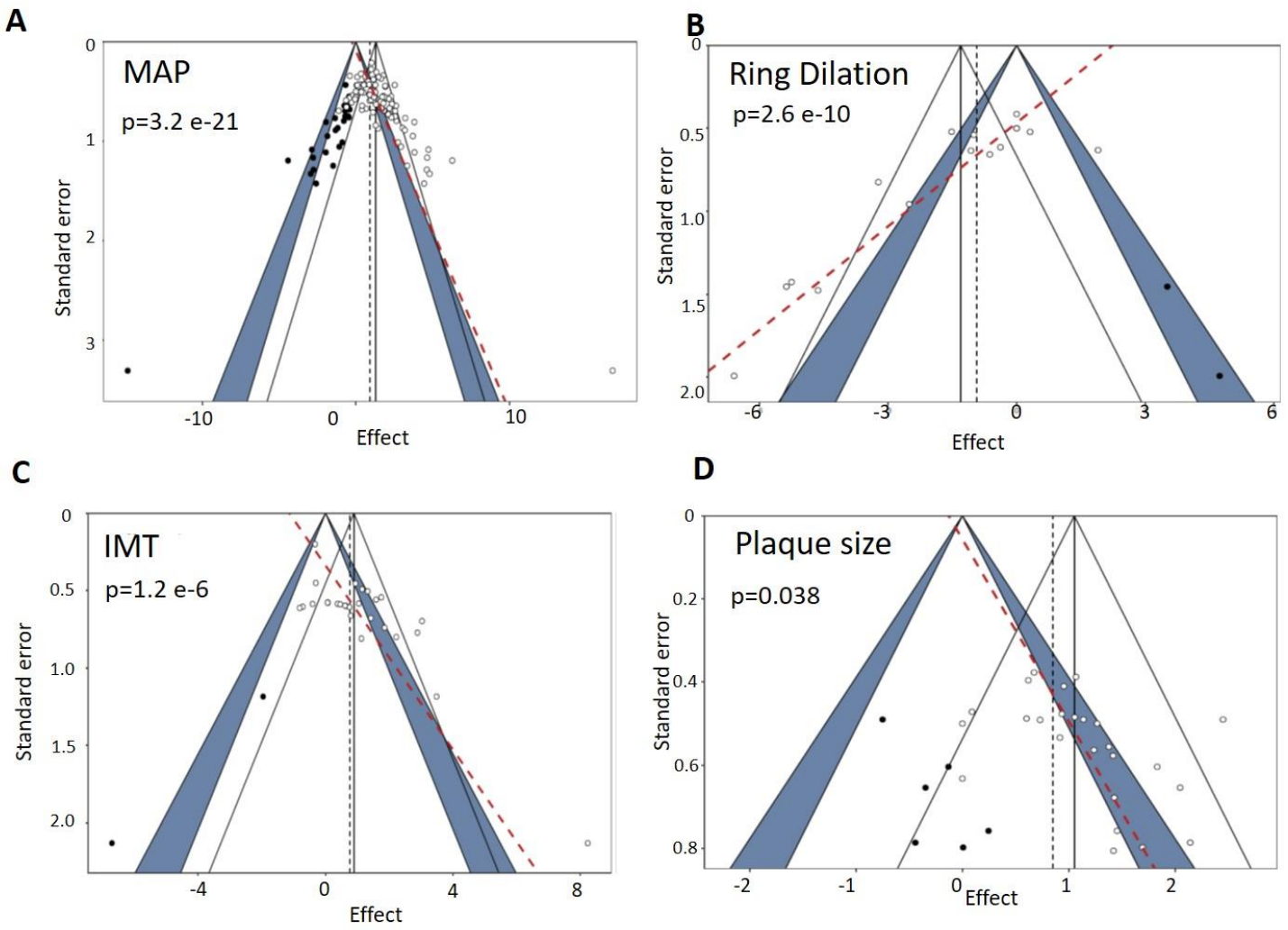
Supplementary Figure 5: Subgroup analyses for vessel ring dilation according to the gender of rodents. $p<0.01$ for female vs male.



Supplementary Figure 6: Subgroup analysis for IMT according to the species of rodent. p=0.05 for mice vs rats.



Supplementary Figure 7: **Risk of bias of studies assessed with the SYRCLE tool.** For each item of the SYRCLE tool, the percentage of studies scored low/unclear/high risk of bias is shown.



Supplementary Figure 8: Funnel plots showing publication bias for the main outcomes: MAP (A), artery ring dilation (B), IMT (C), atherosclerosis lesions in ApoE-/- mice (D).

Supplementary Table 1: Description of studies included in the meta-analysis. Abbreviations: nm non mentioned; m male; f female; HF High Fat diet.

Due to its large size, this table could not be embedded in the text. See excel file

Supplementary table 2: Risk of bias of studies, according to the 9 outcomes of the SYRCLE quality tool. L=Low, U= Unclear, H= High risk of bias.

Due to its large size, this table could not be embedded in the text. See excel file

Supplementary Table 3: Trim and Fill analysis for correction of small study effect. The table indicates the corrected SMD, confidence interval and I^2 after Trim and Fill analysis. All SMD remain significant after correction.

	Number of missing studies	Total number of studies	SMD	CI inf	CI sup	I^2
MAP	24	136	0.98	0.73	1.24	86%
RVD	0	16	-1.62	-2.71	-0.54	91%
IMT	0	28	0.94	0.51	1.37	77%
LESION	6	31	0.87	0.6	1.14	50%