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

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Key Words:	systematic review, meta-analysis, intermittent hypoxia, arterial pressure, vascular reactivity, vascular remodeling
Abstract:	<p>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.</p> <p>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.</p> <p>We searched PubMed and included 104 articles in a meta-analysis, among them 92 using wild-type rodents and 12 using Apolipoprotein E knock-out mice. We used the standardized mean difference (SMD) to compare results between studies.</p> <p>IH significantly increased mean arterial pressure (+ 13.77 mmHg (95% CI [11.73; 15.82])), systolic and diastolic blood pressure. Meta-regressions showed that hypoxia severity (FiO<sub>2</sub>) was associated with mean arterial pressure. 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 1.05, CI [0.52; 1.58], absolute values: + 3.23 μm (1.61-5.00)). This increase was observed in mice but not in rats, and</p>

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	<p>was negatively associated with age. Finally IH increased atherosclerotic plaque size in ApoE<sup>-/-</sup> mice (SMD 1.1, CI [0.82; 1.41]). 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.</p>

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**Professor Martin Kolb**  
**Editor-in Chief**  
***European Respiratory Journal***

Grenoble, March 22, 2021

Dear Professor Kolb,

We are pleased to submit our manuscript entitled “Intermittent hypoxia-related alterations in vascular structure and function: a systematic review and meta-analysis of rodent data”, by O. Harki et al., for your consideration for publication as a review article in the *European Respiratory Journal*.

Intermittent hypoxia is a key feature of Obstructive Sleep Apnea (OSA), a very common respiratory disease associated with major cardiovascular morbidity and mortality. Among OSA complications are hypertension, atherosclerosis, and myocardial infarction. However, clinical studies are often partly flawed by confounders and the role of IH as an independent cardiovascular risk factor is still debated. Numerous studies support the deleterious vascular impact of IH in rodents but an overall interpretation is challenging owing to heterogeneity in the rodent species investigated and the severity and duration of IH exposure. It thus appeared as 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.

In this paper, we report a meta-analysis on rodent data to assess the impact of intermittent hypoxia (IH) on vascular structure and function. We included more than 100 studies in which rodents were exposed to IH and various vascular parameters were examined, the main ones being arterial pressure, arterial reactivity to vasodilators or vasoconstrictors, vascular remodeling, and atherosclerosis lesions in ApoE  $-/-$  mice.

In our study, we clearly demonstrate that IH, independently of confounders, has a major impact on systemic artery structure and function. Our findings support the interest of identifying and treating sleep apnea in routine cardiology practice.

Therefore, we think our results could raise much interest in the large community of both clinicians and researchers working in the fields of sleep apnea and of vascular physiopathology. We thus hope that you will consider our manuscript as well suited for publication in *ERJ*.

We suggest the following reviewers for this paper:

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We confirm that this article is not under consideration for publication in whole or in part elsewhere in any form. All authors have seen and approved submission of the manuscript to *ERJ*.

We look forward to your reply,

Respectfully yours,

**Anne Briançon-Marjollet (corresponding author) and co-authors**

# 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.

Word count : 4389

## 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 and included 104 articles in a meta-analysis, among them 92 using wild-type rodents and 12 using Apolipoprotein E knock-out mice. We used the standardized mean difference (SMD) to compare results between studies.

IH significantly increased mean arterial pressure (+ 13.77 mmHg (95% CI [11.73; 15.82]), systolic and diastolic blood pressure. Meta-regressions showed that hypoxia severity ( $FiO_2$ ) was associated with mean arterial pressure. 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 1.05, CI [0.52; 1.58], absolute values: + 3.23  $\mu$ m (1.61-5.00)). This increase was observed in mice but not in rats, and was negatively associated with age. Finally IH increased atherosclerotic plaque size in ApoE<sup>-/-</sup> mice (SMD 1.1, CI [0.82; 1.41]).

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,<sup>1</sup> and is characterized by the repetitive occurrence of apneas and hypopneas during sleep<sup>2</sup>. 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<sup>2-4</sup>. 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. 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<sup>5</sup>, 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<sup>5,6</sup>, structural vascular remodelling<sup>7-9</sup>, altered vascular reactivity<sup>10,11</sup>, and atherosclerosis progression<sup>12-14</sup>. However, some studies showed no effect of IH on vascular parameters (for example<sup>15-18</sup>) 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<sub>2</sub>, 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

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3 heterogeneity in results, with particular interest in assessing different rodent models and IH cycle  
4 characteristics.  
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## 6 7 **Methods** 8

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10 The protocol for the meta-analysis was recorded in the PROSPERO registry under the number  
11 CRD42020169940 ([https://www.crd.york.ac.uk/prospere/display\\_record.php?ID=CRD42020169940](https://www.crd.york.ac.uk/prospere/display_record.php?ID=CRD42020169940)) .  
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13 Owing to the very large amount of available data, this work focuses on structural and functional  
14 vascular outcomes.  
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### 16 **Search methods and study selection** 17

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19 We searched MEDLINE for articles published up to January 31st, 2020. The search terms were  
20 “Intermittent hypoxia” AND “Rodent” OR “mice” OR “rat” (see PROSPERO record for exact query). We  
21 also searched for keywords and MeSH related to each search term. After the initial electronic search,  
22 we screened the titles and the abstracts to retrieve relevant articles. Eligibility was considered if they  
23 were written in English and addressed vascular outcomes of intermittent hypoxia in rodents. Then, the  
24 full manuscripts were screened for inclusion and exclusion criteria. The final inclusion of a study was  
25 made by one author, and independently confirmed by a second author.  
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32 We included only controlled studies with a well-established control group i.e. normoxic animals, for  
33 adult rodents exposed to chronic intermittent hypoxia. An intermittent hypoxia cycle was defined as  
34 the repetitive occurrence of several hypoxia-reoxygenation sequences during the same day. Chronic  
35 IH was defined as a repetition of IH cycles over time, for a minimum of one day. All wild-type rodent  
36 models (mouse and rat, male and female, young or aged, lean or obese) with exposure to intermittent  
37 hypoxia and compared to a normoxic group were included.  
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43 The outcomes considered as mandatory for inclusion were variables allowing characterization of  
44 vascular structure or function. This included blood pressure (systolic (SBP)/diastolic  
45 (DBP)/mean/pulsed), arterial reactivity (vasodilatory response to 10<sup>-6</sup>M acetylcholine and  
46 vasoconstriction responses to 10<sup>-6</sup>M phenylephrine or 10<sup>-8</sup>M endothelin-1, both *ex vivo* in cannulated  
47 vessels or vessel rings); vascular remodeling (Intima-Media Thickness (IMT), internal vessel diameter),  
48 and atherosclerosis plaque size in apolipoprotein E knock-out (ApoE<sup>-/-</sup>) mice.  
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54 We excluded studies without any control group (normoxic and untreated mice), studies in which  
55 hypoxia was applied continuously (i.e. no hypoxia-normoxia cycles), or studies in which hypoxia was  
56 combined with hypercapnic or hypobaric conditions. We also excluded studies using IH exposure in  
57 prenatal or perinatal periods and studies using transgenic animals, except for ApoE<sup>-/-</sup> mice that are  
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3 the model of choice to study atherosclerosis plaques. Studies on pulmonary or cerebral vascular beds  
4 were also excluded.  
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### 6 7 8 **Assessment of methodological quality**

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10 The quality assessment of studies was performed using the SYRCLE tool described by Hooijmans et  
11 al.<sup>19</sup>. This contains several types of bias: selection bias (sequence generation, baseline characteristics,  
12 and allocation concealment), performance bias (randomized housing of animals, blinding of  
13 investigators), detection bias (random outcome assessment, blinding of outcome assessor), attrition  
14 bias (incomplete outcome data), and reporting bias (selective outcome reporting). Each risk of bias  
15 was scored as High, Low, or Unclear. Three authors were involved and every discrepancy was discussed  
16 to achieve a shared decision.  
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### 22 23 24 **Statistical analysis**

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26 We performed two separate meta-analyses for wild-type rodents and ApoE<sup>-/-</sup> mice. For each outcome,  
27 data were abstracted and analyzed using standardized mean difference,  $SMD = ((Mc - Me)) / SD$ , where  
28 Mc is the mean of the outcome measure in the control group, Me is the mean of the outcome measure  
29 in the experimental group, and SD is the pooled standard deviation of the two groups.<sup>20</sup> A  $SMD > 0.8$   
30 was considered as large, 0.5-0.8 as moderate, and 0.2-0.5 small<sup>21</sup>. In case of a missing SD we calculated  
31 or estimated it from confidence intervals, standard errors, t values, P values or F values.<sup>22</sup> The  
32 remaining SD were imputed using the mean outcome-specific SD from other included studies. All  
33 results are represented using orchard plots, an innovative data visualization tool well adapted for  
34 displaying the results of a large number of outcomes<sup>23</sup> (supplementary figure 1).  
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42 To facilitate interpretation of homogeneous and widely used outcomes we performed meta-analyses  
43 using natural mean differences for arterial pressure outcomes and we back-transformed some SMD to  
44 natural mean differences using the median SD from the control groups of included studies using the  
45 target unit<sup>24</sup>. SMD are expressed with 95% confidence intervals. For study descriptions, we used  
46 medians and interquartile ranges (IQR).  
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51 Given the high anticipated heterogeneity in included studies we performed random effect meta-  
52 analysis by the restricted maximum-likelihood estimator method.<sup>25</sup> Moreover, to account for  
53 correlation among multi-arm studies we constructed a hierarchical/mixed effect model with a random  
54 intercept for study. We explored sources of heterogeneity through pre-specified subgroup analyses  
55 and meta-regressions according to population (species, strain, sex, age, diet and body weight), year of  
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3 publication and details of intermittent hypoxia protocols (oxygen fraction ( $FiO_2$ ) during hypoxic phases,  
4 duration of hypoxic and normoxic phases, frequency, duration per day and total duration of exposure).  
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7 Given the large number of studied outcomes, we performed meta-regressions only on pre-specified  
8 primary outcomes: mean arterial blood pressure (MAP), intima-media thickness, response of vessel  
9 rings to acetylcholine, and atherosclerosis lesion size in ApoE<sup>-/-</sup> mice. We first performed univariate  
10 meta-regressions on study and animal characteristics (age and body weight were adjusted on species).  
11 Then, we added the significant predictors ( $p < 0.2$ ) in meta-regression models evaluating intermittent  
12 hypoxia (IH) protocol parameters. Given the exploratory nature of these analyses we considered all  $p$ -  
13 values  $< 0.05$  as significant. Lastly, to assess the robustness of the findings, we performed sensitivity  
14 analyses by excluding potential outliers for significant meta-regressions.  
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21 Funnel plot asymmetry was also explored for primary outcomes using Egger's regression test, as  
22 recommended by the Cochrane handbook for systemic reviews of interventions<sup>26</sup>, with  $p < 0.1$   
23 suggesting publication bias. We also performed a Trim and Fill analysis to assess the impact of small  
24 study effects on the meta-analyses results<sup>27</sup>.  
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28 All statistical analyses were performed using R statistical software (version 3.6.2).  
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## Results

Our literature review yielded 1886 references among which we ultimately selected 104 studies for inclusion in the meta-analysis, 92 concerning wild-type rodents and 12 using ApoE<sup>-/-</sup> 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 92 studies on wild-type rodents, 21 were performed in mice (20 in C57Bl/6 mice, 1 in 129S1 mice) and 71 in rats (50 in Sprague-Dawley, 18 in Wistar and 3 in other strains of rats). At study inclusion, median body weight and age were 26.5g (IQR 22.0-27.2) and 8.25 (7-10.5) weeks for mice and 275g (200-325) and 9 (8-13) weeks for rats respectively. Males were used in 83 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 76 studies and both standard and high-fat diet in 4 studies; diet was not specified in 12 studies.

For the studies on ApoE<sup>-/-</sup> mice, median weight was 27g (27.9-29.5) and median age was 13 (8- 14.25) weeks. Eight 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 5 studies and both standard and high fat in 2 studies.

Concerning IH protocols (Supplementary figure 2), median and IQ values of FiO<sub>2</sub> during hypoxic periods were of 5% (IQR 5-9), desaturation during 40 (30-90) seconds followed by 90 (30-232.5) seconds of reoxygenation and return to a FiO<sub>2</sub> of 21% (normoxia). Cycles were repeated on average for 8h/day for a median duration of 21 (10-35) days. Forty two percent of studies used a FiO<sub>2</sub> of 5%, 10% used a FiO<sub>2</sub> of 6%, and 20% used a FiO<sub>2</sub> of 10% during hypoxic phases. Fifteen percent of studies had a duration of 7 days, 18% a duration of 14 days, 12% a duration of 21 days, 10% a duration of 28 days and 12% a duration of 35 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<sup>-/-</sup> 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 1.33, CI [1.07-1.59],  $I^2 = 74.79\%$  corresponding to a mean increase of 13.77 mmHg (CI [11.73; 15.82]) after IH. Similarly, SBP increased by 13.73 [11.08; 16.37] mmHg, and DBP by 12.72 [9.03; 16.54] 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 (supplementary figure 4 and supplementary table 2). 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 was significantly associated with a higher MAP ( $p=0.02$ ) (Figure 2D and supplementary table 2). Total duration of exposure was significantly associated with MAP but this association disappeared after exclusion of outlier studies ( $p=0.13$ , supplementary table 2).

### 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=-2.37 [-3.72;-1.02],  $I^2 = 89.68\%$  (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 -2.22 [-3.53; 0.9] 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 supplementary table 2). However, after adjusting for the duration of hypoxia exposure, this association did not persist ( $p=0.27$ ).

### Impact of IH on vascular remodeling

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3 In wild-type rodents, intima-media thickness significantly increased after IH (SMD 1.05 [0.52; 1.58],  $I^2$   
4 = 76.4%) (Figure 4A) with an increase of IMT of 3.23 (1.61-5.00)  $\mu\text{m}$ . In contrast, inner vessel  
5 diameter did not significantly change after IH (Figure 4B). Subgroup analyses for IMT showed a much  
6 stronger effect of IH in mice (SMD 1.29 [0.44; 2.14]) than in rats (SMD 0.40 [-0.13; 0.93]) ( $p=0.05$  for  
7 subgroup difference) (supplementary figure 6). Univariate analysis also showed that age was  
8 negatively associated with IMT thickening ( $p=0.02$ , Figure 4C and supplementary table 2). Meta-  
9 regression analysis for IH parameters after adjustment for significant predictors in univariate meta-  
10 regression (strain, species, year of publication and age) were not significant for IMT at a  $p$ -value of  
11 0.05, but total duration of exposure tended to be associated with IMT thickening ( $p=0.07$ ) (Figure 4D  
12 and supplementary table 2).  
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### 21 **Impact of IH on atherosclerosis lesions in ApoE<sup>-/-</sup> mice**

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24 Since vascular remodeling is an early step in the process of atherogenesis and because wild-type  
25 C57Bl/6 mice are resistant to atherosclerosis, we included ApoE<sup>-/-</sup> mice in the meta-analysis, as a  
26 recognized model of susceptibility to atherosclerosis. The analysis showed that IH strongly increased  
27 atheromatous lesion size in ApoE<sup>-/-</sup> mice (SMD 1.1 [0.82; 1.41],  $I^2=27.3\%$ ) (Figure 5). Meta-regression  
28 analysis for IH parameters showed that IH parameters were not significantly associated with lesion size  
29 in ApoE<sup>-/-</sup> mice, except for a strong trend towards significance regarding the animal's body weight  
30 ( $p=0.06$ ) (supplementary table 2).  
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### 37 **Risk of bias of studies**

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40 The risk of bias of studies was assessed using the SYRCLE tool<sup>19</sup>. The results are presented in  
41 Supplementary Figure 7 and supplementary table 3. Items for which the risk of bias was low were  
42 selective outcome reporting (selection bias, 31% low risk), sequence generation (39% of studies are at  
43 low risk), baseline characteristics (43% low risk) and incomplete outcome data (attrition bias, 58% low  
44 risk). However, incomplete outcome data was also the criterion with the highest percentage of high-  
45 risk studies (20%). Finally, several outcomes, mainly categorized as performance and detection bias,  
46 were almost never mentioned and therefore scored as "unclear risk": allocation concealment,  
47 randomized housing, blinding of investigators, random outcome assessment, blinding of outcome  
48 assessor.  
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### 55 **Small study effect**

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3 Funnel plots are presented in supplementary figure 8 for MAP, ring dilation, IMT and plaque size. For  
4 these four items, the asymmetric distribution of studies and a significant Egger regression test  
5 indicating a clear small study effect were observed. However, the SMD remained significant for all  
6 outcomes after correcting for missing studies (Trim and Fill analysis), meaning a consistent effect of IH  
7 on the outcomes (Supplementary Table 4).  
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## 12 Discussion

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16 One of the main features of obstructive sleep apnea syndrome is intermittent hypoxia which  
17 represents the major trigger for cardiovascular complications<sup>28</sup>. A large corpus of studies in animals  
18 report diverse effect sizes for the impact of intermittent hypoxia on vascular parameters such as  
19 arterial pressure, altered vascular reactivity or remodeling. However, there is heterogeneity or even  
20 inconsistencies among the published results and, to date, no meta-analysis has been done to assess  
21 the impact of IH on these specific parameters. Our meta-analysis firmly establishes that intermittent  
22 hypoxia, in the absence of the confounders flawing human studies, triggers blood pressure elevation,  
23 alterations in vasodilation and atherosclerosis. Some of these responses were proportional to the  
24 hypoxic burden and duration of exposure. Another lesson was to delineate the different responses  
25 depending on the species, strains, sex and age of exposed animals.  
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### 34 *Impact of IH on vascular parameters*

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37 Our meta-analysis confirmed that IH has a clear and significant impact on the primary outcomes:  
38 arterial pressure, vessel reactivity, intima-media thickness and atherosclerotic lesions (standard mean  
39 differences always > 0.7). This is consistent with the known vascular effects of OSAS<sup>3,28</sup>, supporting the  
40 relevance of these IH models in rodents to the human pathology. In particular, while clinical studies  
41 are often difficult to interpret due to comorbidities, rodent studies suggest that IH per se may be the  
42 main cause of the vascular consequences of OSA.  
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48 Our meta-analysis showed that IH is associated with a significant increase in MAP, which is consistent  
49 with the elevation in sympathetic activity and blood pressure occurring in healthy volunteers  
50 submitted to 14 nights of intermittent hypoxia<sup>29</sup>. Interestingly, meta-regression analyses showed that  
51  $FiO_2$  (i.e. the hypoxic burden) was associated with MAP, suggesting that the severity of hypoxia could  
52 be the key element for increased risk of hypertension. This is of high clinical significance since  
53 epidemiological studies<sup>30,31</sup> already suggest a dose response relationship between OSA severity, as  
54 defined by the Apnea-Hypopnea Index, and hypertension. Also, responses to continuous positive  
55 airway pressure, the primary therapy for OSA, is related to the severity of hypoxia at the time of OSA  
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3 diagnosis (see meta-analyses<sup>32,33</sup>). This suggests that beyond confounders (such as obesity or  
4 metabolic syndrome) IH may be the main parameter accounting for increased blood pressure in OSA.  
5 It also suggests that parameters such as minimal oxygen saturation or time spent at <90% oxygen  
6 saturation should be used to describe more precisely OSA severity and hypoxic burden, rather than  
7 the AHI which does not necessarily reflect the patient's hypoxic burden.  
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12 In vascular reactivity studies, we observed that IH significantly altered endothelium-dependent  
13 vasodilation in response to acetylcholine. This is in line with studies in humans suggesting that OSA  
14 alters endothelial function<sup>34</sup>, and is associated with arterial stiffness<sup>35,36</sup>. Our group recently reported  
15 in an individual participant meta-analysis that among adults without overt CV disease, severe OSA (AHI  
16  $\geq 30$ ) was independently associated with an increased risk of endothelial dysfunction that may  
17 predispose to late CV events<sup>37</sup>. Moreover, vasoconstriction in response to endothelin-1 was enhanced,  
18 while vasoconstriction in response to phenylephrine was not altered by IH. Other vasoconstrictors,  
19 such as angiotensin II, have only been sparsely studied and the lack of data did not allow a meta-  
20 analysis. Interestingly, endothelin-1-induced vasoconstriction was largely studied with IH protocols  
21 that included 5% CO<sub>2</sub> in the air breathed by animals. IH combined with hypercapnia also increased the  
22 contractile response to ET-1<sup>38,39</sup>. Our meta-analysis thus suggests that IH, rather than hypercapnia, may  
23 thus be responsible for the ET-1 response.  
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28 In this meta-analysis, we did not have sufficient statistical power to allow comparison of the reactivity  
29 of different vascular beds after IH. However, some studies report some differences in the reactivity  
30 among vascular beds, in particular in small muscular vs large elastic arteries<sup>39</sup>. More studies are needed  
31 to allow a meta-analysis on the effects on various vascular beds.  
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36 IH-induced vascular remodeling in rodents, as characterized by an augmentation in the intima-media  
37 thickness, is consistent with what is observed in humans<sup>40,41</sup>. Our results are also consistent with a  
38 recent meta-analysis limited to aorta IMT in mice<sup>42</sup>. Although not reaching significance ( $p=0.07$ ), the  
39 IMT tended to be associated with the total duration of exposure. This suggests a progressive  
40 remodeling of arteries over time. Interestingly, internal vessel diameter was not modified in rodents  
41 while, in humans, it is postulated that obstructive sleep apnea could induce an increase in diameter,  
42 at least in some patients and vessels<sup>43,44</sup>. IH models in rodents might rapidly attain the late  
43 characteristics of the disease such as thickening the media following changes in the inner diameter of  
44 vessels. Other remodeling parameters, such as compliance or elasticity, could not be included in our  
45 meta-analysis due to insufficient studies. However, IH in rodents is known to induce disorganization of  
46 the elastin fiber network<sup>8,45,46</sup>, reduced vessel distension<sup>47,48</sup> and increased stiffness<sup>47,48</sup>. Taken together,  
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3 IH induces structural remodeling along with alterations in vasoreactivity (blunted vasodilation and  
4 increased vasoconstriction) that could act synergistically to increase blood pressure.  
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8 Since increased IMT suggests ongoing atherogenesis in wild-type rodents, we included ApoE<sup>-/-</sup> mice in  
9 the meta-analysis because they are susceptible to atherosclerosis and a model of choice to study the  
10 impact of IH on atherosclerotic lesions. As expected, we found that IH strongly increased  
11 atherosclerotic lesions, consistent with the remodeling observed in wild-type animals<sup>8</sup>, and with the  
12 known pro-atherogenic consequences of OSAS in humans<sup>49,50</sup>. Interestingly, diet (standard vs high fat)  
13 did not significantly modulate plaque size after IH, suggesting that IH is a robust inducer of plaques,  
14 independent of a high fat diet.  
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21 We performed meta-regression analyses to determine whether the variability of IH protocols could  
22 modulate the impact of IH. Apart from the associations mentioned above, other meta-regressions  
23 found no significant effect of IH parameters on the selected vascular outcomes. This would suggest  
24 that IH has a robust impact on these outcomes, whatever the duration or severity of IH (in the range  
25 of our inclusion criteria). Interestingly, FiO<sub>2</sub> was always ≤ 10% in the included studies, corresponding  
26 to the very severe hypoxia that occurs in the most severe OSAS patients. A less severe hypoxic burden  
27 has been little investigated in animal experiment designs. This needs investigation in future animal  
28 studies because the impact of OSA treatments in reducing cardiovascular consequences is mostly  
29 debated for the mild to moderate spectrum of the disease.  
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### 36 37 *Contribution of animal characteristics to IH impact*

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39 In univariate analyses, we investigated the contribution of strain, sex, age, diet, body weight and year  
40 of publication on IH effects. The species and/or the strain significantly impacted MAP and IMT,  
41 suggesting that the choice of species/strain of mice or rats is important when designing a study. MAP  
42 is consistently elevated in the most frequently used models such as C57Bl/6 mice, or Sprague-Dawley  
43 or Wistar rats. However, MAP was not found to be elevated in Fischer, Wistar kyoto or lean Zucker  
44 rats; although the very small number of studies using these strains probably accounts for this absence  
45 of statistical effect. Vascular remodeling as assessed by IMT is much more pronounced in mice than in  
46 rats; rats may thus not be a good model to study remodeling. Our meta-analysis may help researchers  
47 to choose the most appropriate models according to the objectives of their study.  
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56 Analysis by sex showed that the alteration of vessel dilation induced by IH is found in males, but not  
57 in females, despite the small number of studies using females (n=5), suggesting a robust difference in  
58 the impact of IH on vasodilation between males and females. This may reflect a sex-related sensibility  
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3 to the IH stimulus regarding this particular outcome, consistent with the known stimulation of  
4 endothelial-dependent vasodilation by oestrogens<sup>51</sup>. It may underlie the fact that, although most OSAS  
5 patients are men, specific studies of the vascular consequences of OSAS in women are necessary,  
6 although they are under-represented in the current literature<sup>35,52</sup>.  
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11 Animal age was inversely associated with IH-induced intima-media thickening, suggesting that young  
12 animals may be more sensitive to IH in terms of vascular remodeling. This is consistent with data in  
13 humans<sup>33</sup>. Age had no significant association with other parameters. However, the vast majority of  
14 studies were performed in young animals (8-9 weeks old) and the very few using animals older than  
15 50 weeks had to be considered as outliers. There is a lack of information about the effects of IH in older  
16 animals, indicating a need for further studies, particularly as obstructive sleep apnea is predominant  
17 in humans aged over 50 and not in young adults.  
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### 23 24 *Risk of bias and limitations of the analysis*

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27 Funnel plots and Egger regression tests evidenced a small study effect for all the outcomes studied.  
28 Such an effect could have multiple reasons: selective reporting of results or publication bias, poor  
29 methodological quality of small studies leading to overestimation of results, true heterogeneity in the  
30 results or chance<sup>53</sup>. However, interestingly the SMD remained stable for the four main outcomes after  
31 the Trim and Fill analyses correcting for missing values, suggesting a limited impact of the small study  
32 effect on the results. A publication bias is not unusual in animal studies and is probably mainly due to  
33 selective reporting such as non-publication of negative results and selection of publishable outcomes.  
34 This could be associated with the frequent reluctance of journals to publish negative results. To avoid  
35 this reporting bias, we suggest that journals accept to publish animal study protocols, as is done for  
36 clinical studies, as well as negative results. For many of the listed items the risk of bias assessed with  
37 the SYRACLE risk of bias tool was quite high. This is in line with the poor SYRACLE scores in many other  
38 animal study meta-analyses. We argue for improvement of research and publication practices with  
39 regard to laboratory animal studies and the widespread adoption and implementation of the SYRACLE  
40 guidelines.  
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51 Another limitation of this meta-analysis was the heterogeneity of the outcomes and units of  
52 measurement. The use of SMD was intended to deal with this, but our analyses still showed strong  
53 heterogeneity for most of the outcomes studied. Statistical analyses only partly succeeded in  
54 identifying factors that could explain this heterogeneity, although some characteristics such as species,  
55 sex or certain IH properties were suggested. There may be other underlying factors that could  
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3 potentially explain the heterogeneity of results that were not investigated in our study, such as  
4 laboratory-, experimentation- or investigator-dependent effects.  
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### 7 *Conclusion*

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10 To our knowledge, this is the first meta-analysis of animal studies on the vascular impact of  
11 intermittent hypoxia. The meta-analysis based on a large corpus of articles evidenced the clear impact  
12 of IH on arterial pressure, reactivity and vascular remodeling. We identified some features of IH, in  
13 particular  $FiO_2$  during hypoxia, which were sometimes associated with an amplified impact of IH.  
14 However, in most cases the impact of IH was independent of the precise pattern of IH exposure,  
15 suggesting that whatever its modality, aimed at mimicking obstructive sleep apnea in humans, IH had  
16 a robust effect on rodent vessel structure and function.  
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## 26 **Figure legends**

### 27 **Figure 1: Flow diagram of the study**

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32 **Figure 2: IH increases blood pressure in systemic vessels of wild-type animals.** Orchard plots showing  
33 SMD for: A) systolic blood pressure (SBP), B) diastolic blood pressure (DBP), C) mean arterial pressure  
34 (MAP). D) Significant association between MAP and  $FiO_2$  in meta-regression analysis ( $p=0.02$ , slope = -  
35 0.13).  
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40 **Figure 3: IH alters vasodilation with Acetylcholine and increases vasoconstriction with ET-1 but not**  
41 **vasoconstriction with Phenylephrine.** Orchard plots showing SMD for A) cannulated artery dilation, B)  
42 artery ring dilation with Acetylcholine ( $10^{-6}M$ ), C) artery ring constriction with Endothelin-1 ( $10^{-8}M$ ), D)  
43 artery ring constriction with Phenylephrine ( $10^{-6}M$ ). E) Association between ring vasodilation and  $FiO_2$   
44 ( $p<0.01$ , slope = -0.67).  
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50 **Figure 4: IH provokes vascular structural remodeling.** Orchard plots showing SMD for A) intima-media  
51 thickness (IMT) of systemic vessels, B) vessel luminal diameter. C) Univariate meta-regressions showing  
52 the negative correlation between IMT and rodent age ( $p=0.02$ , slope = -0.03). D) Univariate adjusted  
53 meta-regression showing a strong tendency toward positive correlation between IMT and total  
54 duration of exposure in days ( $p=0.07$ , slope = 0.03).  
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3 **Figure 5: IH increases atherosclerotic plaque size in ApoE<sup>-/-</sup> mice.** Orchard plot showing SMD for  
4 atherosclerosis lesion size.  
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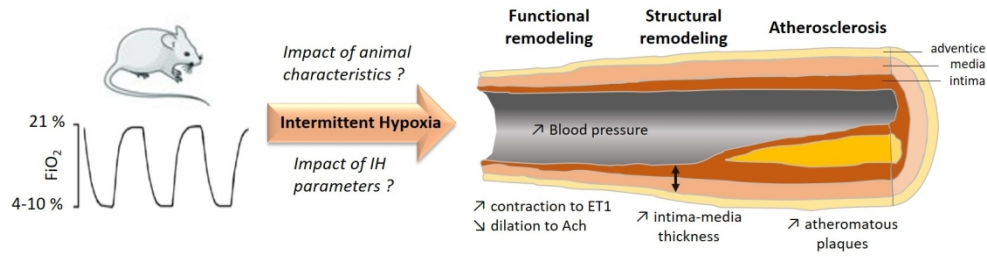
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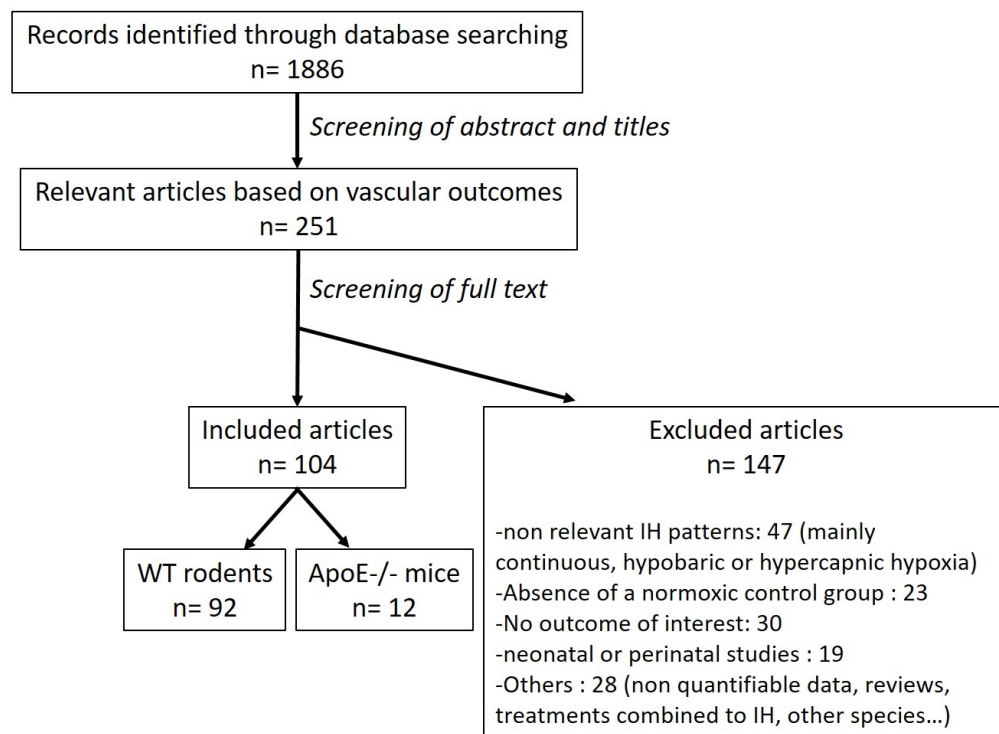




16 Our meta-analysis included rodents exposed to experimental intermittent hypoxia. We demonstrate that IH  
17 significantly increased blood pressure, altered vasodilation and increased vasoconstriction, increased intima-  
18 media thickness and atherosclerosis plaques. Altogether, IH is responsible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

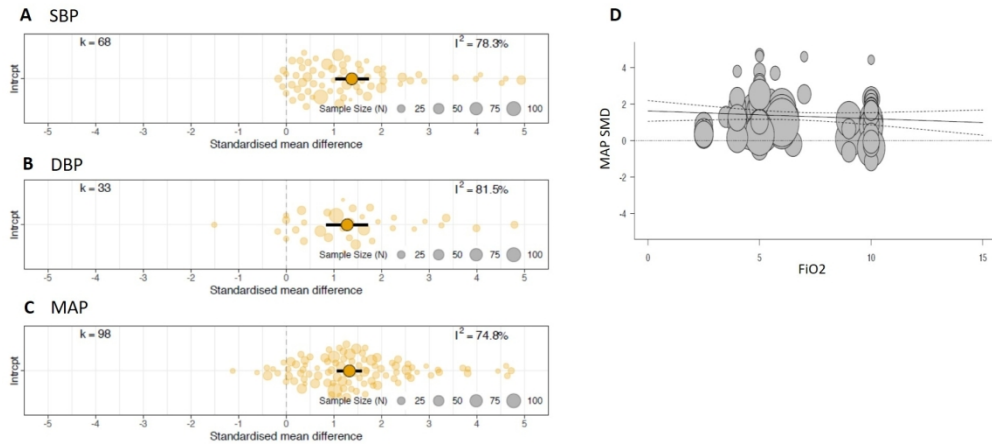
20 303x80mm (150 x 150 DPI)

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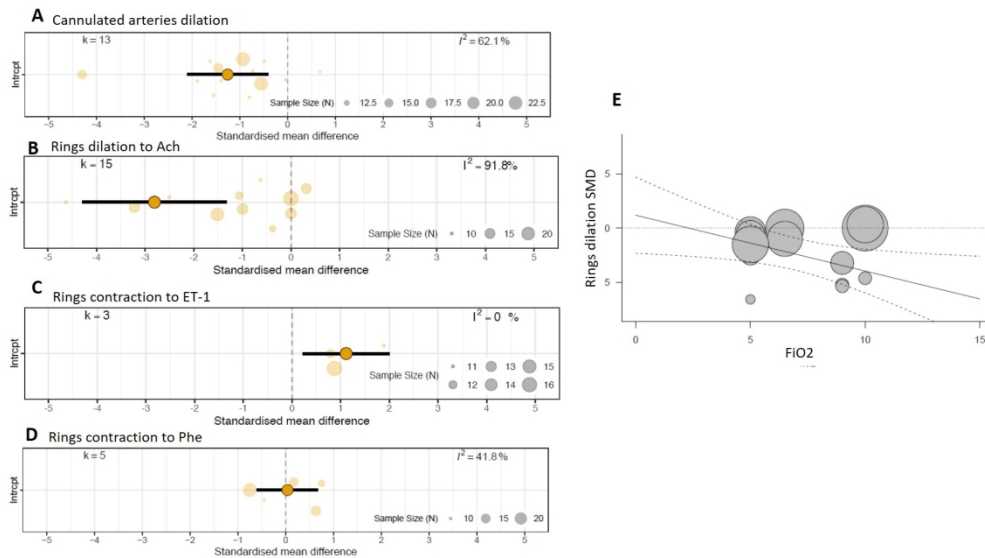
Flow diagram of the study

221x163mm (150 x 150 DPI)



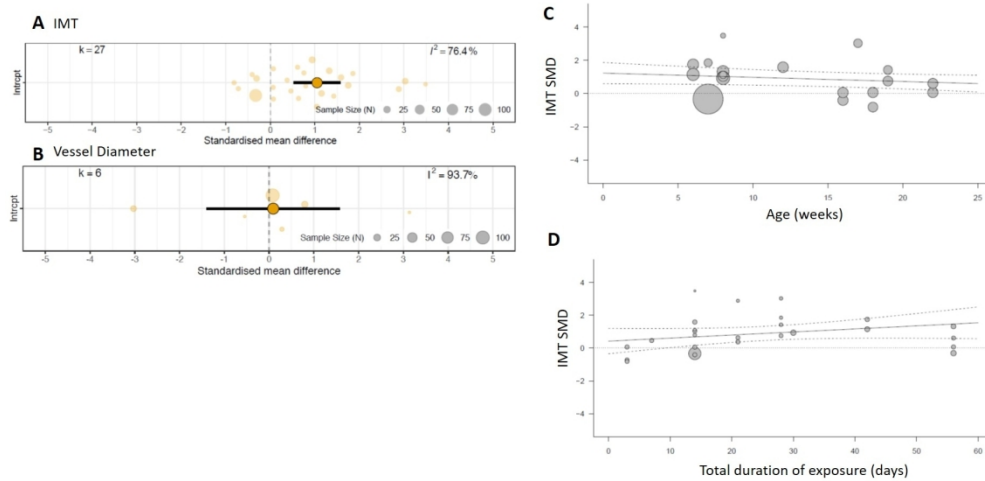
IH increases blood pressure in systemic vessels of wild-type animals. Orchard plots showing SMD for: A) systolic blood pressure (SBP), B) diastolic blood pressure (DBP), C) mean arterial pressure (MAP). D) Significant association between MAP and FiO<sub>2</sub> in meta-regression analysis ( $p=0.02$ , slope =  $-0.13$ ).

327x149mm (150 x 150 DPI)



IH alters vasodilation with Acetylcholine and increases vasoconstriction with ET-1 but not vasoconstriction with Phenylephrine. Orchard plots showing SMD for A) cannulated artery dilation, B) artery ring dilation with Acetylcholine (10-6M), C) artery ring constriction with Endothelin-1 (10-8M), D) artery ring constriction with Phenylephrine (10-6M). E) Association between ring vasodilation and  $FiO_2$  ( $p < 0.01$ , slope = -0.67).

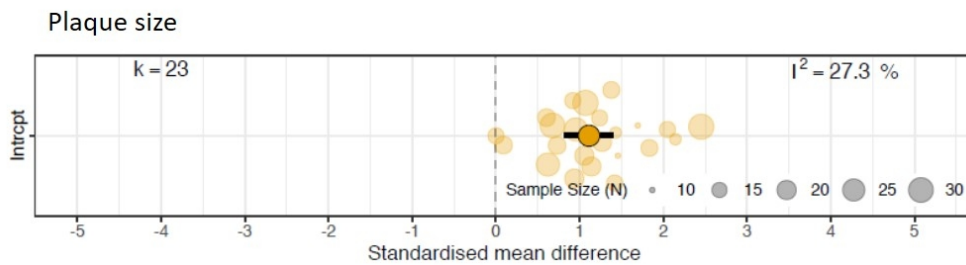
317x180mm (150 x 150 DPI)



IH provokes vascular structural remodeling. Orchard plots showing SMD for A) intima-media thickness (IMT) of systemic vessels, B) vessel luminal diameter. C) Univariate meta-regressions showing the negative correlation between IMT and rodent age ( $p=0.02$ , slope =  $-0.03$ ). D) Univariate adjusted meta-regression showing a strong tendency toward positive correlation between IMT and total duration of exposure in days ( $p=0.07$ , slope =  $0.03$ ).

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IH increases atherosclerotic plaque size in ApoE<sup>-/-</sup> mice. Orchard plot showing SMD for atherosclerosis lesion size.

171x47mm (150 x 150 DPI)

## Supplemental data

**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<sub>2</sub> 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).

**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<sup>-/-</sup> 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:** Meta-regression analyses for the main outcomes: MAP, dilation of artery rings, IMT, atherosclerosis lesions in ApoE<sup>-/-</sup> mice. 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. -> represents the evolution before -> after exclusion of outlier studies.

Moderator	MAP			Arteries rings dilation			IMT			Atherosclerosis lesions		
	n	slope	p-val	n	slope	p-val	n	slope	p-val	n	slope	p-val
Univariate metaregressions												
Strain	96		<b>0.00¥</b>	14		0.69	27		0.09¥	23		0.78
Diet	88		0.39	14		0.7	NA			23		0.24
Species	96		0.28	14		0.69	27		<b>0.05¥</b>	23		
Gender	94		0.86	14		<b>0.00¥</b>	22		0.89	22		0.35
Body weight	73	0.00	0.09	12	0.00	0.44	16	0.00	0.14	13	-0.15	0.06
Year of publication	96	0.04	<b>0.02¥</b>	14	-0.06	0.83	27	0.08	0.19¥	23	-0.02	0.64
Age	38->36	-0.01->0.02	0.01->0.65	10	-0.06	0.84	20	-0.03	<b>0.02</b>	18	0,00	0.96
Univariate adjusted metaregression on IH parameters												
FiO <sub>2</sub>	96	-0.13	<b>0.02</b>	14	-0.67	<b>0.00</b>	27	0.11	0.65	23	0.23	0.39
Duration of exposure	96->93	0.00->0.02	0.02->0.12	14	0.00	0.96	27	0.03	0.07	23	0.23	0.39
Duration of IH per day	96	-0.07	0.48	14	0.27	0.45	27	-0.1	0.76	23	0.11	0.31
Duration of reoxygenation phase	96	0,00	0.34	14	0,00	0.99	27	0.00	0.93	23	-0.01	0.5
Duration of hypoxic phase	96	0,00	0.53	14->13	-4.01->-0.01	0.04->0.80	27	0.00	0.69	23	0.00	0.7



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**Supplementary Table 3:** Risk of bias of studies, according to the 9 outcomes of the SYRCLE quality tool. L=Low, U= Unclear, H= High risk of bias.

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**Supplementary Table 4:** Trim and Fill analysis for correction of small study effect. The table indicates the corrected SMD, confidence interval and *I*<sup>2</sup> 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>I</i> <sup>2</sup>
MAP	21	117	0.96	0.7	1.22	85%
RVD	1	15	-1.77	-2.93	-0.61	91%
IMT	0	27	0.9	0.47	1.33	76%
LESION	6	29	0.89	0.61	1.16	50%

Author	Journal	pmid	Year	Species
Arnaud C	Am J Respir Crit Care Med.	21680945	2011	mouse
Arnaud C	J Am Heart Assoc	29371201	2018	mouse
Badran M	Oxid Med Cell Longev	31093313	2019	mouse
Badran M	Sleep Med.	24767726	2014	mouse
Campen MJ	J Appl Physiol	16002771	2005	mouse
Castro-Grattoni AL	Chest	26836908	2016	mouse
Castro-Grattoni-A	Respirology	31215129	2020	mouse
Chen L	Cardiovasc pathol	28985491	2017	rat
Chen YC	Am J Physiol Regul Integr Comp Physiol.	27252472	2016	rat
Coleman CG	J Neurosci.	20826673	2010	mouse
Cunningham JT	Hypertension.	22689746	2012	rat
Del Rio R	Eur Respir J.	22183481	2012	rat
Del Rio R	Eur Respir J.	19996187	2010	rat
Dematteis M	Am J Respir Crit Care Med.	17962641	2008	mouse
Diogo LN	Eur J Pharmacol.	26291659	2015	rat
do Carmo JM	Acta Physiol (Oxf).	30466186	2018	rat
Dopp JM	Respiration.	21846958	2011	rat
Fenik VB	Front Neurol.	22509173	2012	rat
Fletcher EC	J Appl Physiol	1601808	1992	rat
Fletcher EC	Hypertension.	1592450	1992	rat
González-Martín MC	Adv Exp Med Biol.	19536495	2009	rat
Gras E	J Appl Physiol	26679613	2016	mouse
Greenberg HE	J Appl Physiol	9887143	1999	rat
Gu H	Am J Physiol Heart Circ Physiol.	17693540	2007	rat
Guan P-A	J Cell Biochem.	30259991	2018	rat
Guo QH	Clin Exp Pharmacol Physiol.	23662699	2013	rat
Guo XL	Chin Med J	24033945	2013	rat
Hayashi T	Am J Physiol Heart Circ Physiol.	18326795	2008	mouse
Hernández-Guerra M	Hepatology.	23174804	2013	rat
Hinojosa-Laborde C	Hypertension.	16157795	2005	rat
Huang J	Respir Physiol Neurobiol.	20227529	2010	rat
Huang J	Respir Physiol Neurobiol.	19429526	2009	rat
Huang J	Respir Physiol Neurobiol.	17442632	2007	rat

1	Hui AS	Hypertension.	14597643	2003	rat
2	Hung MW	J Pineal Res.	23869411	2013	rat
3	Iturriaga R	Adv Exp Med Biol.	19536496	2009	rat
4	Iturriaga R	Adv Exp Med Biol.	20217364	2010	rat
5	Kc P	J Physiol.	20051497	2010	rat
6	Knight WD	Am J Physiol Regul Integr Comp Physiol.	21543638	2011	rat
7	Krause BJ	Front Physiol.	30087615	2018	rat
8	Krause BJ	J Hypertens	25629363	2015	rat
9	Kumar GK	J Physiol.	16777938	2006	rat
10	Kuo TB	Respir Physiol Neurobiol.	20863915	2011	rat
11	Lai CJ	J Appl Physiol	16484362	2006	rat
12	Lan XF	Sci Rep	28871193	2017	mouse
13	Lee MYK-A	J Appl Physiol	30091668	2018	mouse
14	Lefebvre B	Respir Physiol Neurobiol.	15979951	2006	rat
15	Lesske J	J Hypertens.	9488210	1997	rat
16	Li JR	plos one	29641598	2018	rat
17	Lin M	Am J Physiol Heart Circ Physiol.	17384123	2007	mouse
18	Liu P	J Cell Physiol.	30609027	2019	rat
19	Lu W	sleep breath	28078487	2017	rat
20	Lu W	Braz J Med Biol Res.	28076452	2017	rat
21	Marcus NJ	Respir Physiol Neurobiol.	22728949	2012	rat
22	Marcus NJ	Respir Physiol Neurobiol.	19013546	2009	rat
23	Mentek M	Invest Ophthalmol Vis Sci.	30383197	2018	rat
24	Moraes DJ	Hypertension	27480839	2016	rat
25	Moreau JM	Brain Res.	26183015	2015	rat
26	Moya EA	Oxid Med Cell Longev.	26798430	2016	rat
27	Nanduri J	J Physiol.	27506145	2016	rat
28	Olea E	J Appl Physiol	25103975	2014	rat
29	Peng YJ	J Appl Physiol	22016368	2012	rat
30	Perim RR	Exp Physiol.	26195236	2015	rat
31	Philippi NR	Respir Physiol Neurobiol.	19969108	2010	rat
32	Phillips SA	J Appl Physiol	16357071	2006	rat
33	Phillips SA	Am J Physiol Heart Circ Physiol.	14512283	2004	rat
34	Poulain L	Mediators Inflamm.	25873766	2015	mouse
35	Prabha K	Adv Exp Med Biol.	21445804	2011	rat
36	Quintero M	J Physiol.	26752660	2016	rat
37	Raghuraman G	Antioxid Redox Signal.	20836657	2011	rat

1	Ray AD	Am J Physiol Regul Integr Comp Physiol.	17459910	2007	rat
2	Ren H	Mol Med Rep	28983603	2017	rat
3	Ribon-Demars A	Acta Physiol (Oxf).	29947475	2018	rat
4	Sacramento JF	Respir Physiol Neurobiol.	26993367	2016	rat
5	Schulz R	J Hypertens.	24270180	2014	mouse
6	Shang J	Chin Med J	24033947	2013	rat
7	Sharpe AL	Am J Physiol Heart Circ Physiol.	24097432	2013	rat
8	Shirai M	Basic Res Cardiol.	25139633	2014	rat
9	Silva AQ	J Physiol.	21242253	2011	rat
10	Souza GM	Exp Physiol.	25631702	2015	rat
11	Suarez-Giron MC	Front Physiol.	29881356	2018	mouse
12	Tahawi Z	J Appl Physiol	11299297	2001	rat
13	Takahashi K	Sci Rep.	30560943	2018	mouse
14	Wu JG	J Cell Physiol.	29215742	2018	rat
15	Yamamoto K	Auton Neurosci.	23167993	2013	rat
16	Yang	J Am Heart Assoc	30757948	2019	rat
17	Yang R	Am J Physiol Heart Circ Physiol.	21278136	2011	mouse
18	Zhang Y	Biochem Biophys Res Commun.	28822761	2017	mouse
19	Zhou S	Oxid Med Cell Longev.	25177426	2014	mouse
20	Zoccal DB	Auton Neurosci.	17293169	2007	rat
21	Zoccal DB	Exp Physiol.	17085676	2007	rat

Number of studies per outcome

22	ApoE-/-				
23	Arnaud C	Atherosclerosis.	21917260	2011	souris
24	Drager LF	Am J Respir Crit Care Med.	23328524	2013	souris
25	Fang G	Am J Pathol.	22940439	2012	souris
26	Gautier-Veyret E	pharmacol res	29920371	2018	souris
27	Gautier-Veyret E	Eur Respir J.	23060635	2013	souris
28	Kato R	Eur J Pharmacol.	26276396	2015	souris
29	Li RC	Am J Respir Crit Care Med.	21493735	2011	souris
30	Poulain L	Eur Respir J.	24072212	2014	souris
31	Song D-A	Atherosclerosis	29407890	2018	souris
32	Tuleta I	Atherosclerosis.	25150938	2014	souris
33	Van Noolen L	Prostaglandins Leukot Essent Fatty Acids.	25139400	2014	souris
34	Zeng X	J Transl Med	29673358	2018	souris

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Number of studies per outcome

Strain	Body weight (grams)	Gender	Age	Diet	FiO <sub>2</sub> (%)	Duration of hypoxic phase (seconds)
C57BL/6	nm	m	8	standard	5	30
C57BL/6	nm	m	12	standard	5	30
C57BL/6	36	m	10	standard	5	30
C57BL/6	26.5	m	9	standard + HF	5	30
C57BL/6	nm	m	14	standard	5	30
C57BL/6	nm	m	6	standard	5	20
C57BL/6	21 and 24	f	8 and 72	standard	5	20
sprague dawley	195	m	adult	standard	9	90
wistar	nm	m	12	standard	5	90
C57BL/6	22	m	adult	standard	10	90
sprague dawley	300	m	nm	standard	10	180
sprague dawley	220	m	nm	standard	5	20
sprague dawley	200	m	nm	standard	5	20
C57BL/6	27.2	m	8	nm	4	30
wistar	308.4	m	9.5	standard	5	210
sprague dawley	nm	m	13	standard	7	120
sprague dawley	326	m	12.4	standard	10	120
sprague dawley	314.5	m	nm	standard	10	90
Wistar	312.5	m	nm	standard	4	15
wistar + sprague dawley	337.5	m	nm	standard	4	15
Wistar	290	m	adult	standard	10	40
C57BL/6	nm	nm	8	standard	5	30
sprague dawley	175	m	8	standard	7	30
Fischer 344	nm	nm	14	nm	10	90
sprague dawley	200	m	adult	standard	9	90
sprague dawley	195	M	nm	standard	9	60
wistar	225	M	8	standard	10	160
C57BL/6	21.6	m	9.5	nm	5	30
sprague dawley	237.5	m	matched	standard	9	120
sprague dawley	nm	m and f	adult	standard	10	180
sprague dawley	245	m	nm	standard	9	60
sprague dawley	245	m	nm	standard	9	60
sprague dawley	165	m	nm	standard	7.5	60

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2	sprague dawley	187.5	m	nm	standard	10	90
3	sprague dawley	nm	M	4	standard	5	30
4	sprague dawley	225	m	nm	nm	5	20
5	sprague dawley	200	m	nm	nm	5	20
6	sprague dawley	200	m	nm	nm	5	20
7	sprague dawley	200	m	nm	nm	5	20
8	sprague dawley	327.5	m	nm	standard	10	45
9	sprague dawley	300	m	adult	standard	10	180
10	sprague dawley	300	m	adult	standard	10	180
11	sprague dawley	200	m	nm	standard	5.5	20
12	sprague dawley	200	m	adult	standard	5	20
13	sprague dawley	200	m	adult	standard	5	20
14	sprague dawley	275	m	adult	nm	5	100
15	sprague dawley	275	m	adult	nm	5	100
16	sprague dawley	423	M	adult	standard	4	30
17	sprague dawley	423	M	adult	standard	4	30
18	sprague dawley	472	m	adult	standard	4	30
19	sprague dawley	472	m	adult	standard	4	30
20	C57BL/6	nm	f	7	nm	5	10
21	C57BL/6	27	m	4	standard + HF	5 / 10	240
22	C57BL/6	27	m	4	standard + HF	5 / 10	240
23	Wistar	2230	m	adult	standard	5	40
24	wistar + sprague dawley	312.5	m	nm	standard	3.5	15
25	wistar + sprague dawley	312.5	m	nm	standard	3.5	15
26	sprague dawley	205	m	adult	standard	9	90
27	sprague dawley	205	m	adult	standard	9	90
28	C57BL/6	nm	nm	14	nm	5.7	360
29	C57BL/6	nm	nm	14	nm	5.7	360
30	sprague dawley	225	m	nm	standard	5	30
31	sprague dawley	225	m	nm	standard	5	30
32	sprague dawley	200	m	nm	standard	5	30
33	sprague dawley	200	m	nm	standard	5	30
34	sprague dawley	190	m	nm	standard	5	30
35	sprague dawley	190	m	nm	standard	5	30
36	sprague dawley	311	m	13.5	standard	10	105
37	sprague dawley	311	m	13.5	standard	10	105
38	sprague dawley	nm	m	adult	standard	10	105
39	wistar	287	m	7	standard	5	30
40	wistar	287	m	7	standard	5	30
41	wistar	110	m	nm	nm	6	35
42	sprague dawley	110	m	nm	nm	6	35
43	sprague dawley	325	m	adult	standard	6.5	80
44	sprague dawley	325	m	adult	standard	6.5	80
45	sprague dawley	200	m	adult	standard	5	20
46	sprague dawley	200	m	adult	standard	5	20
47	sprague dawley	250	m	adult	standard	5	15
48	wistar	475	m	24	standard +HF	5	40
49	wistar	475	m	24	standard +HF	5	40
50	sprague dawley	250	m	nm	standard	5	100
51	sprague dawley	250	m	nm	standard	5	100
52	wistar	47.5	m	nm	standard	6	240
53	wistar	47.5	m	nm	standard	6	240
54	sprague dawley	435	m	from 14 to 24	standard	10	120
55	sprague dawley	435	m	from 14 to 24	standard	10	120
56	sprague dawley	325	m	matched	standard	10	60
57	sprague dawley	325	m	matched	standard	10	60
58	sprague dawley	325	m	matched	standard	10	60
59	C57BL/6	nm	m	17	standard	5	30
60	sprague dawley	327.5	m	adult	standard	5	15
	wistar	366.2	m	14 and 92	standard	5	40
	sprague dawley	225	m	adult	standard	5	15

Zucker (lean)	479	m	48	standard	4.5	90
wistar	191	m	9	standard	5	90
sprague dawley	275	f	9	standard	10	120
wistar	360	m	10.5	standard	5	210
C57BL/6	nm	m	8.5	standard	7	120
wistar	nm	M	8	standard	10	120
sprague dawley	312.5	M	nm	standard	10	180
sprague dawley	260	m	9	standard	4	90
sprague dawley	216	m	8	standard	6	240
wistar	242	f	8.5	standard	6	40
C57BL/6	nm	m	6	standard	5	20
sprague dawley	350	m	13	standard	2.5	15
C57BL/6	nm	m	8	standard	10	90
sprague dawley	275	m	nm	standard	7.5	60
sprague dawley	498	m	14	standard	9	360
sprague dawley	200	m	9	standard	6	80
C57BL/6	25	m	10	standard	5	30
C57BL/6	nm	m	7	standard + HF	6.5	30
129S1	nm	nm	nm	standard	8	60
wistar	295	m	nm	nm	6	240
wistar	293	m	nm	nm	6	240

ApoE -/-	26.3	m	15	standard + HF	5	30
ApoE -/-	25.5	m	12	HF	6.5	30
ApoE-/-	nm	m+f	nm	standard	6	30
ApoE -/-	30	m	14	standard	5	30
ApoE-/-	nm	m	14	standard	5	30
ApoE -/-	nm	m	8	HF	5	30
ApoE -/-	nm	nm	nm	HF	5.7	90
ApoE -/-	29	m	20	standard	5	30
ApoE -/-	27	m+f	7	standard + HF	6	30
ApoE -/-	nm	m+f	11	HF	5	60
ApoE -/-	nm	m	14	standard	5	30
ApoE -/-	nm	m	8	HF	6	10



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Duration of reoxygenation phase (seconds)	Duration of cycles per day (hours)	Total duration of exposure (days)	SBP	DBP	MAP	IMT	inner vessel diameter
30	8	14				•	•
30	8	7 / 14	•			•	
30	12	56					
30	12	42					
30	12	35			•		
40	6	42				•	
40	6	56				•	
90	8	7 / 14 / 21	•			•	
90	8	28			•		
90	8	14 / 35	•		•		
180	8	7			•		
280	8	21	•	•	•		
280	8	21			•		
30	8	14			•	•	
420	10.5	35	•	•	•		
180	8	7			•		
120	12	14					
90	10	7 / 21 / 35	•				
15	7	35	•	•	•		
15	7	20 / 30 / 35	•	•	•		
80	8	15			•		
30	8	14				•	
30	8	30			•		
270	12	35			•		
90	8	7 / 21 / 35	•	•			
60	8	/ 9 / 12 / 15 / 21	•				
140	8	30			•	•	•
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180	8	21			•		
60	8	35	•	•			

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30	8	7 / 14 / 21	•				
280	8	21			•		
280	8	7 / 14 / 21			•		
300	8	10			•		
180	8	7			•		
280	8	35			•		•
280	8	21	•	•	•	•	•
300	8	10			•		
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45	6	7 / 14 / 30			•		
80	8	28				•	
120	8	28			•		
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15	7	20 / 30 / 35			•		
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60	8	7 / 14 / 28	•	•	•		
60	8	7 / 14 / 21	•				
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30	8	14	•	•	•	•	•
540	8	10			•		
120	8	7 / 95	•	•	•		
280	8	7	•	•			
300	8	Oct-30			•		
80	8	14			•		
300	8	14			•		
300	8	10	•	•	•		
120	12	3 / 14 / 28 / 56				•	
240	12	14			•	•	
240	12	14			•		•
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300	8	10	•	•	•		

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210	8	7 / 14 / 21	•	•	•		
190	8	7 / 35	•	•	•		
420	10.5	28 / 35			•		
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120	8	28			•		
180	8	7			•		
90	8	42			•		
300	8	14			•		
500	8	35	•	•	•		
40	6	42				•	
15	7	8 / 14 / 20 / 26 / 32 / 35			•		
210	8	14 / 28	•				
30	8	7 / 14 / 21 / 28	•				
240	8	7			•		
40	8	42	•	•			
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30	12	42					
60	12	3 / 7 / 21 / 56				•	
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300	8	35	•	•	•		

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90	12	70					
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Author	Journal	PMID	year	Sequence generation
Arnaud C	J Am Heart Assoc	29371201	2018	L
Arnaud C	Am J Respir Crit Care Med.	21680945	2011	L
Badran M	Oxid Med Cell Longev	31093313	2019	U
Badran M	Sleep Med.	24767726	2014	U
Campen MJ	J Appl Physiol	16002771	2005	U
Castro-Grattoni	Respirology	31215129	2020	L
Castro-Grattoni AL	Chest	26836908	2016	L
Chen L	Cardiovasc pathol	28985491	2017	L
Chen YC	Am J Physiol Regul Integr Comp Physiol.	27252472	2016	U
Coleman CG	J Neurosci.	20826673	2010	L
Cunningham JT	Hypertension.	22689746	2012	U
Del Rio R	Eur Respir J.	22183481	2012	L
Del Rio R	Eur Respir J.	19996187	2010	L
Dematteis M	Am J Respir Crit Care Med.	17962641	2008	L
Diogo LN	Eur J Pharmacol.	26291659	2015	L
do Carmo JM	Acta Physiol (Oxf).	30466186	2018	U
Dopp JM	Respiration.	21846958	2011	U
Fenik VB	Front Neurol.	22509173	2012	U
Fletcher EC	Hypertension.	1592450	1992	U
Fletcher EC	J Appl Physiol	1601808	1992	U
González-Martín MC	Adv Exp Med Biol.	19536495	2009	U
Gras E	J Appl Physiol	26679613	2016	L
Greenberg HE	J Appl Physiol	9887143	1999	U
Gu H	Am J Physiol Heart Circ Physiol.	17693540	2007	U
Guan P	J Cell Biochem.	30259991	2018	L
Guo QH	Clin Exp Pharmacol Physiol.	23662699	2013	L
Guo XL	Chin Med J	24033945	2013	L
Hayashi T	Am J Physiol Heart Circ Physiol.	18326795	2008	L
Hernández-Guerra M	Hepatology.	23174804	2013	L
Hinojosa-Laborde C	Hypertension.	16157795	2005	U
Huang J	Respir Physiol Neurobiol.	20227529	2010	L
Huang J	Respir Physiol Neurobiol.	19429526	2009	L
Huang J	Respir Physiol Neurobiol.	17442632	2007	L
Hui AS	Hypertension.	14597643	2003	U
Hung MW	J Pineal Res.	23869411	2013	L
Iturriaga R	Adv Exp Med Biol.	20217364	2010	H
Iturriaga R	Adv Exp Med Biol.	19536496	2009	H
Kc P	J Physiol.	20051497	2010	U
Knight WD	Am J Physiol Regul Integr Comp Physiol.	21543638	2011	U
Krause BJ	Front Physiol.	30087615	2018	U
Krause BJ	J Hypertens	25629363	2015	L
Kumar GK	J Physiol.	16777938	2006	U

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2	Kuo TB	Respir Physiol Neurobiol.	20863915	2011	L
3	Lai CJ	J Appl Physiol	16484362	2006	U
4	Lan XF	Sci Rep	28871193	2017	L
5	Lee MYK	J Appl Physiol	30091668	2018	L
6	Lefebvre B	Respir Physiol Neurobiol.	15979951	2006	U
7	Lesske J	J Hypertens.	9488210	1997	U
8	Li JR	Plos one	29641598	2018	L
9	Lin M	Am J Physiol Heart Circ Physiol.	17384123	2007	U
10	Liu P	J Cell Physiol.	30609027	2019	L
11	Lu W	sleep breath	28078487	2017	L
12	Lu W	Braz J Med Biol Res.	28076452	2017	L
13	Marcus NJ	Respir Physiol Neurobiol.	22728949	2012	U
14	Marcus NJ	Respir Physiol Neurobiol.	19013546	2009	U
15	Mentek M	Invest Ophthalmol Vis Sci.	30383197	2018	U
16	Moraes DJ	Hypertension	27480839	2016	U
17	Moreau JM	Brain Res.	26183015	2015	U
18	Moya EA	Oxid Med Cell Longev.	26798430	2016	U
19	Nanduri J	J Physiol.	27506145	2016	U
20	Olea E	J Appl Physiol	25103975	2014	U
21	Peng YJ	J Appl Physiol	22016368	2012	U
22	Perim RR	Exp Physiol.	26195236	2015	U
23	Philippi NR	Respir Physiol Neurobiol.	19969108	2010	U
24	Phillips SA	J Appl Physiol	16357071	2006	L
25	Phillips SA	Am J Physiol Heart Circ Physiol.	14512283	2004	U
26	Poulain L	Mediators Inflamm.	25873766	2015	U
27	Prabha K	Adv Exp Med Biol.	21445804	2011	U
28	Quintero M	J Physiol.	26752660	2016	U
29	Raghuraman G	Antioxid Redox Signal.	20836657	2011	U
30	Ray AD	Am J Physiol Regul Integr Comp Physiol.	17459910	2007	U
31	Ren H	Mol Med Rep	28983603	2017	L
32	Ribon-Demars A	Acta Physiol (Oxf).	29947475	2018	U
33	Sacramento JF	Respir Physiol Neurobiol.	26993367	2016	U
34	Schulz R	J Hypertens.	24270180	2014	L
35	Shang J	Chin Med J	24033947	2013	L
36	Sharpe AL	Am J Physiol Heart Circ Physiol.	24097432	2013	U
37	Shirai M	Basic Res Cardiol.	25139633	2014	U
38	Silva AQ	J Physiol.	21242253	2011	U
39	Souza GM	Exp Physiol.	25631702	2015	U
40	Suarez-Giron MC	Front Physiol.	29881356	2018	L
41	Tahawi Z	J Appl Physiol	11299297	2001	U
42	Takahashi K	Sci Rep.	30560943	2018	U
43	Wu JG	J Cell Physiol.	29215742	2018	L
44	Yamamoto K	Auton Neurosci.	23167993	2013	L
45	Yang	J Am Heart Assoc	30757948	2019	L
46	Yang R	Am J Physiol Heart Circ Physiol.	21278136	2011	U
47	Zhang Y	Biochem Biophys Res Commun.	28822761	2017	U
48	Zhou S	Oxid Med Cell Longev.	25177426	2014	U
49	Zoccal DB	Auton Neurosci.	17293169	2007	U
50	Zoccal DB	Exp Physiol.	17085676	2007	U
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<b>ApoE -/- studies</b>				
Arnaud C	Atherosclerosis.	21917260	2011	<b>U</b>
Drager LF	Am J Respir Crit Care Med.	23328524	2013	<b>U</b>
Fang G	Am J Pathol.	22940439	2012	<b>U</b>
Gautier-Veyret E	pharmacol res	29920371	2018	<b>L</b>
Gautier-Veyret E	Eur Respir J.	23060635	2013	<b>L</b>
Kato R	Eur J Pharmacol.	26276396	2015	<b>U</b>
Li RC	Am J Respir Crit Care Med.	21493735	2011	<b>U</b>
Poulain L	Eur Respir J.	24072212	2014	<b>U</b>
Song D	Atherosclerosis	29407890	2018	<b>L</b>
Tuleta I	Atherosclerosis.	25150938	2014	<b>L</b>
Van Noolen L	Prostaglandins Leukot Essent Fatty Acids.	25139400	2014	<b>U</b>
Zeng X	J Transl Med	29673358	2018	<b>L</b>

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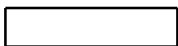
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U	U	U	U	L	U	H	U
H	U	U	U	U	U	L	U
L	U	U	U	U	U	H	U
L	U	U	U	U	U	U	U
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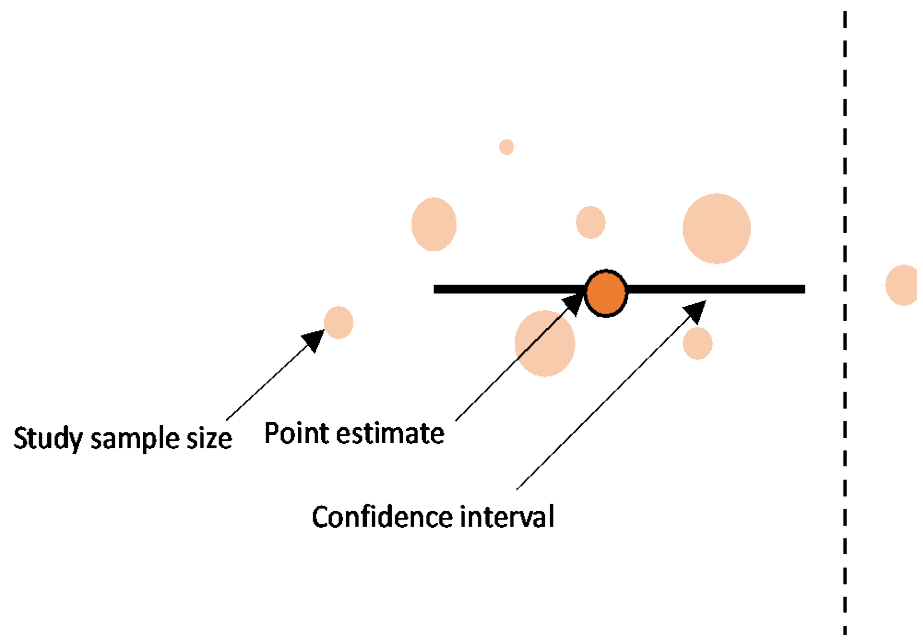
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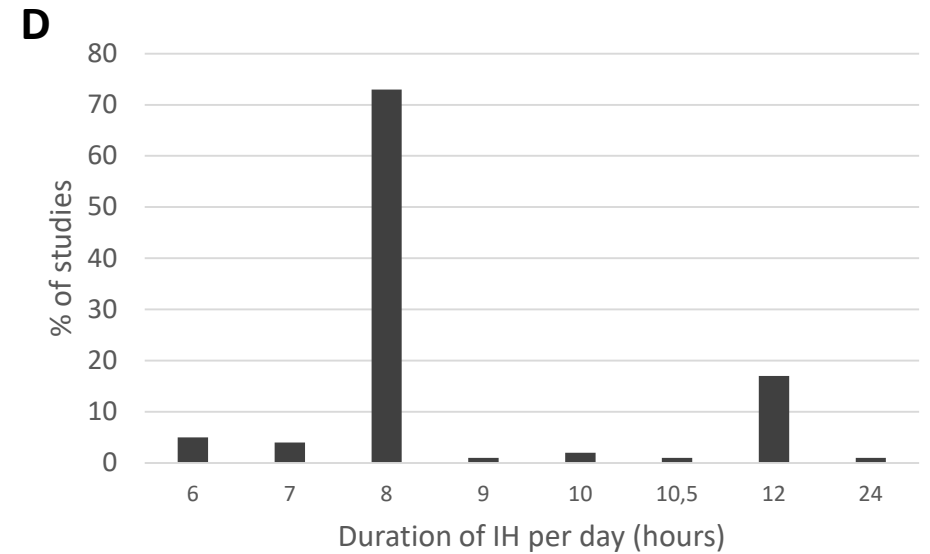
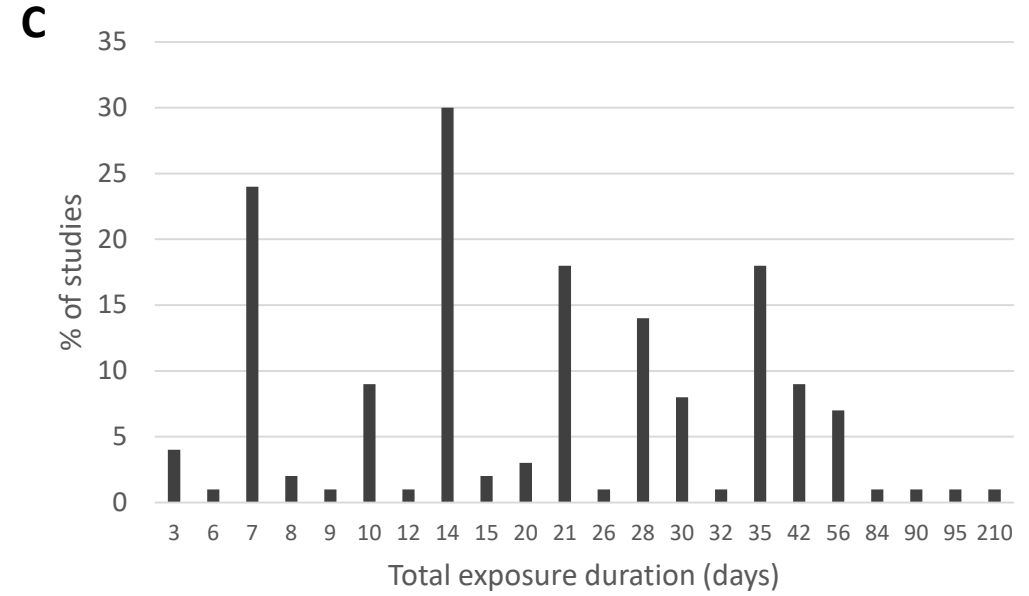
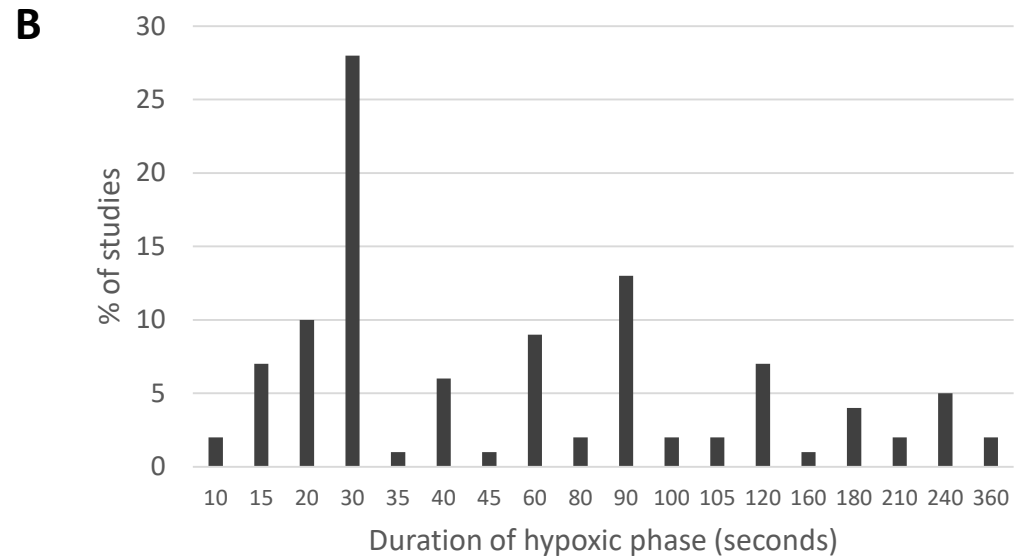
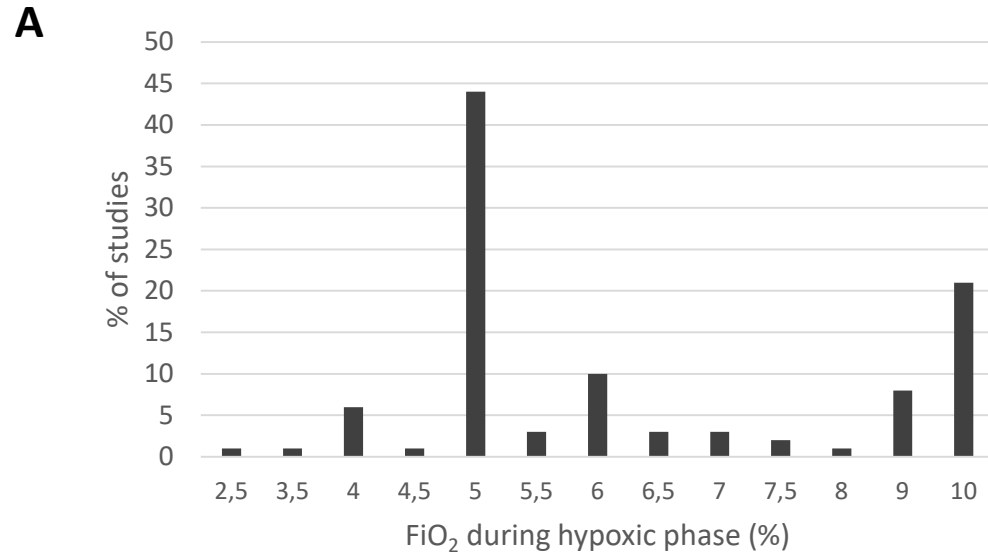


# Supplementary Figure 1

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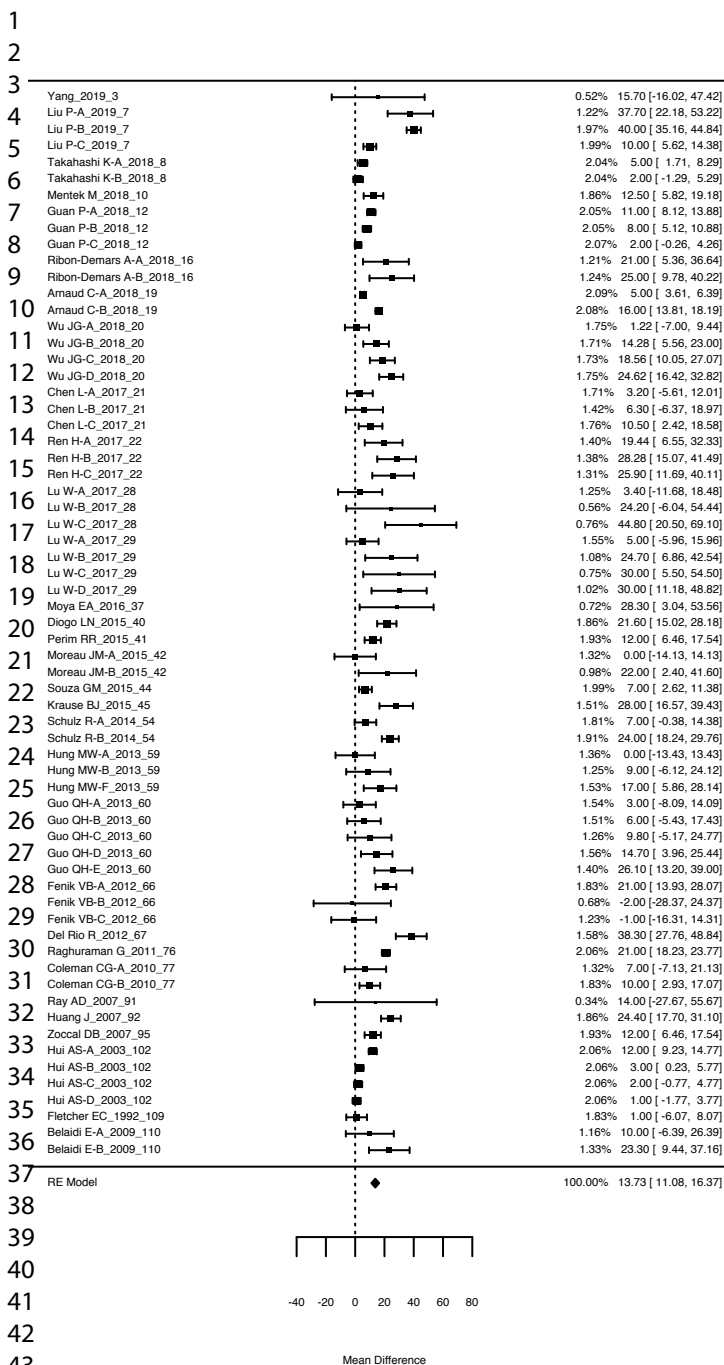


## Supplementary Figure 2

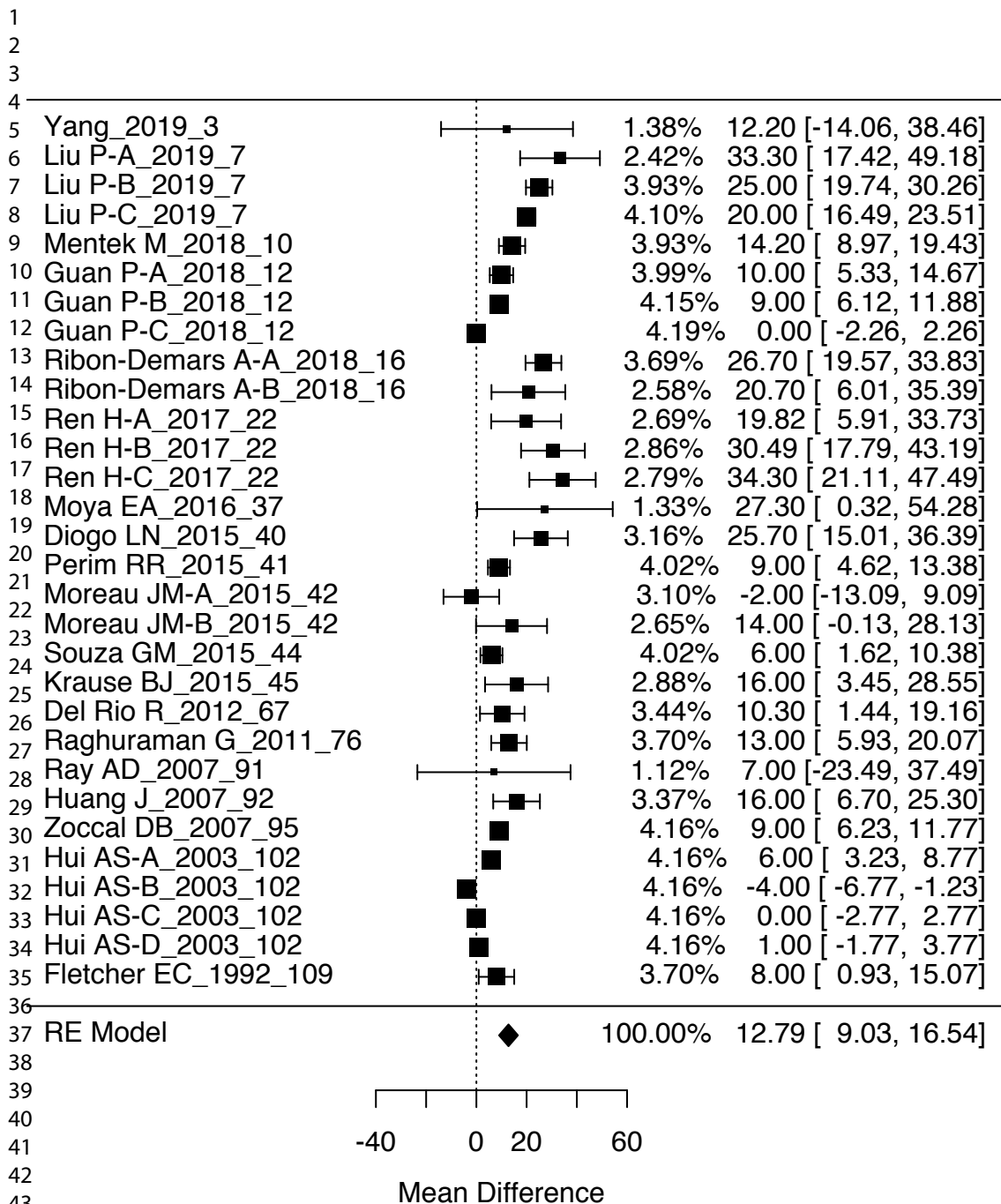


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## Supplementary Figure 3A

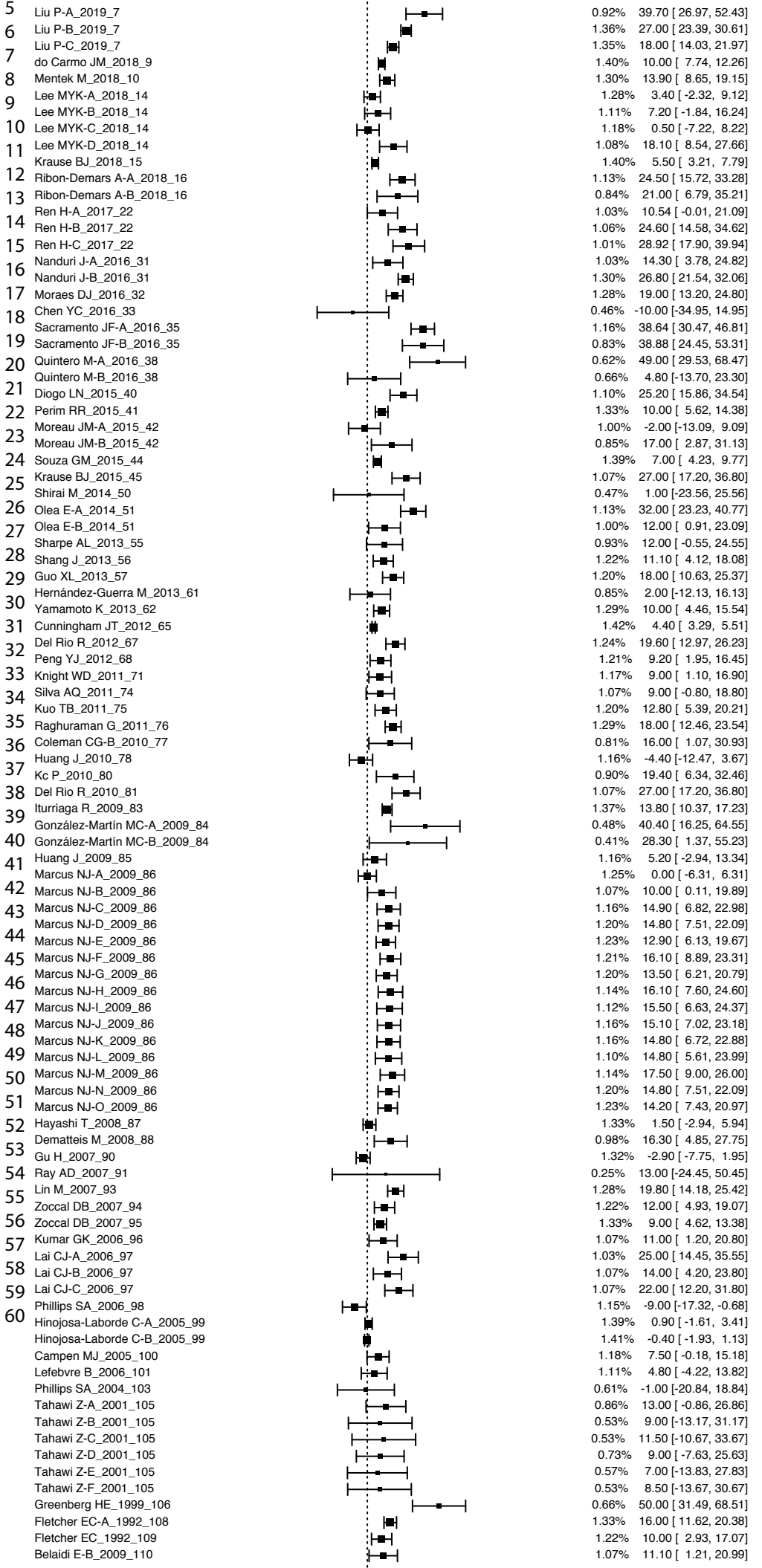


## Supplementary Figure 3B

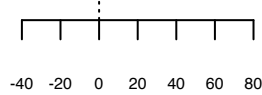


Supplementary Figure 3C

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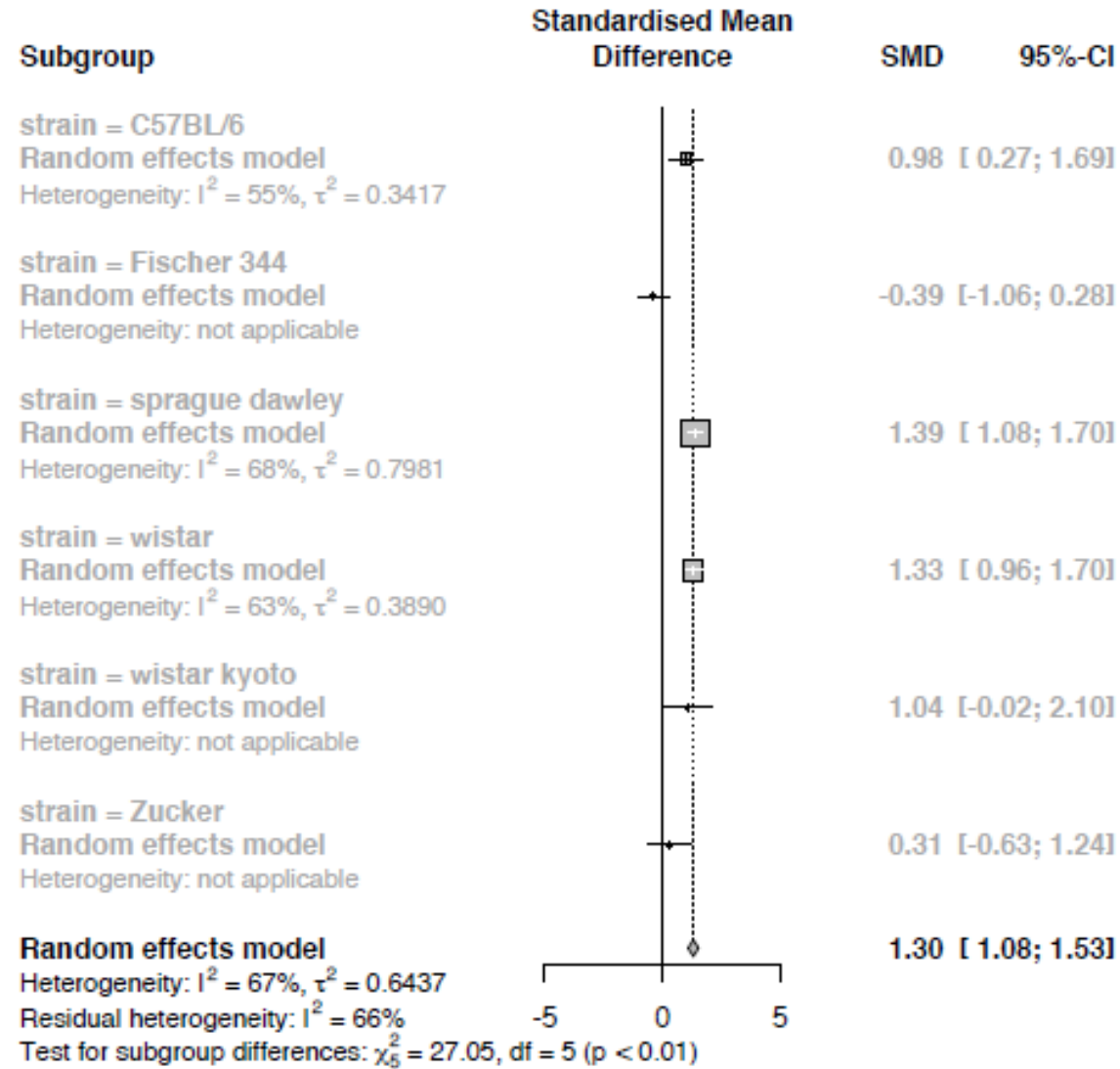


RE Model 100.00% 13.77 [ 11.73, 15.82]



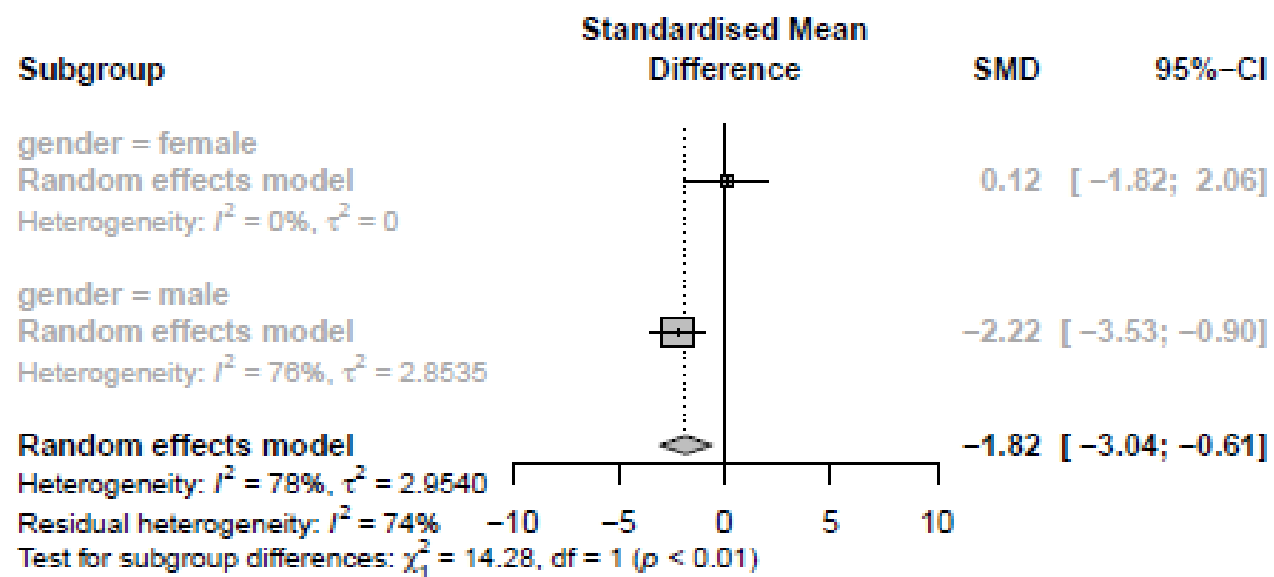
Mean Difference

## Supplementary Figure 4

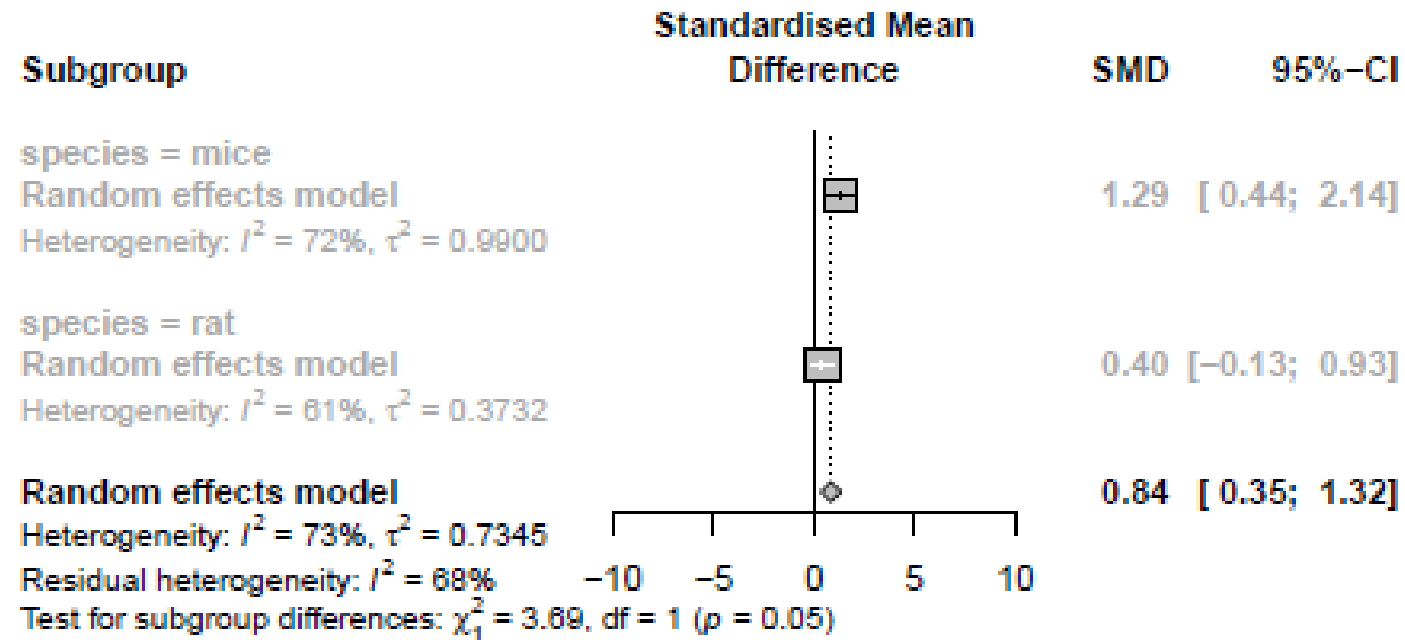




## Supplementary Figure 5

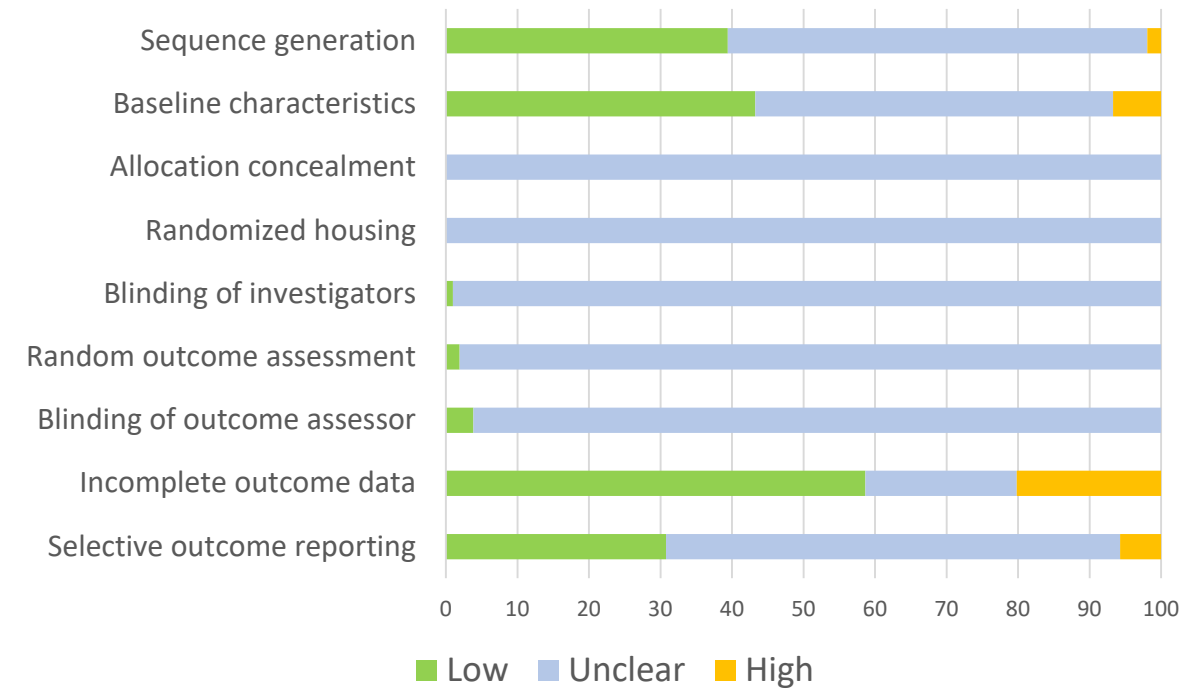


## Supplementary Figure 6

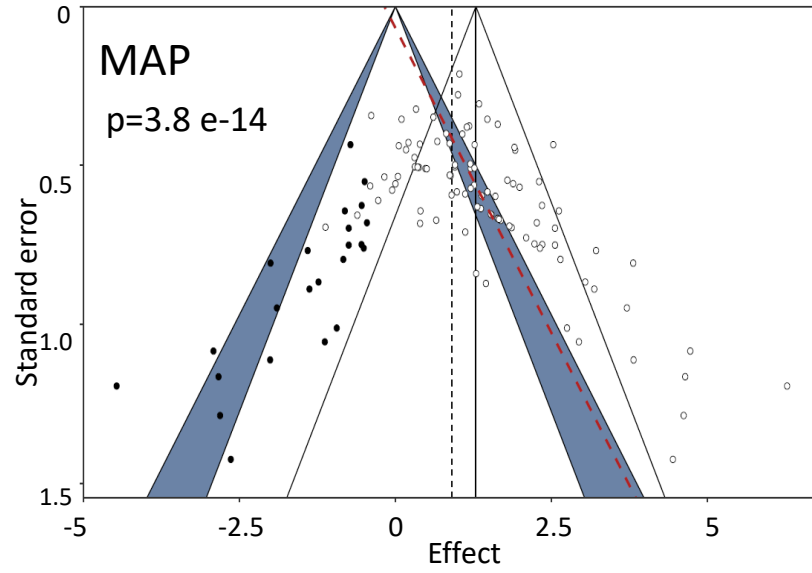


# Supplementary Figure 7

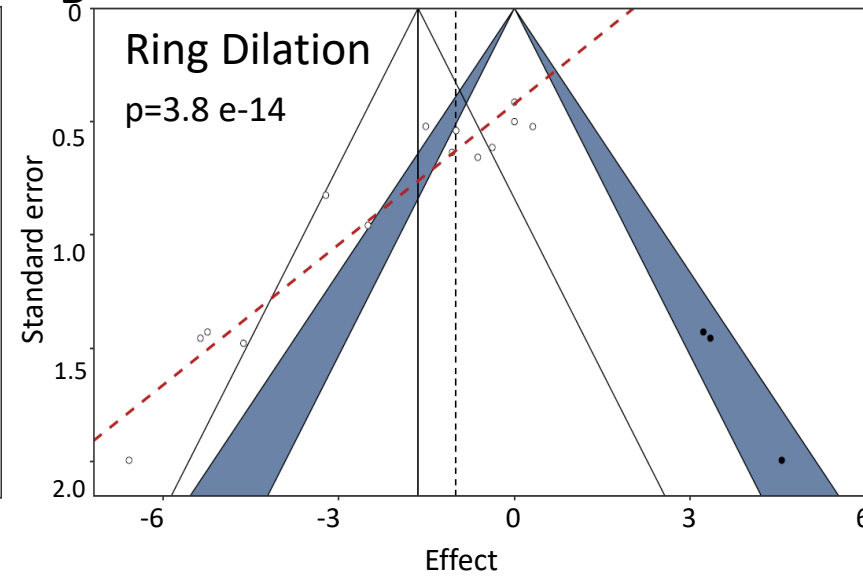
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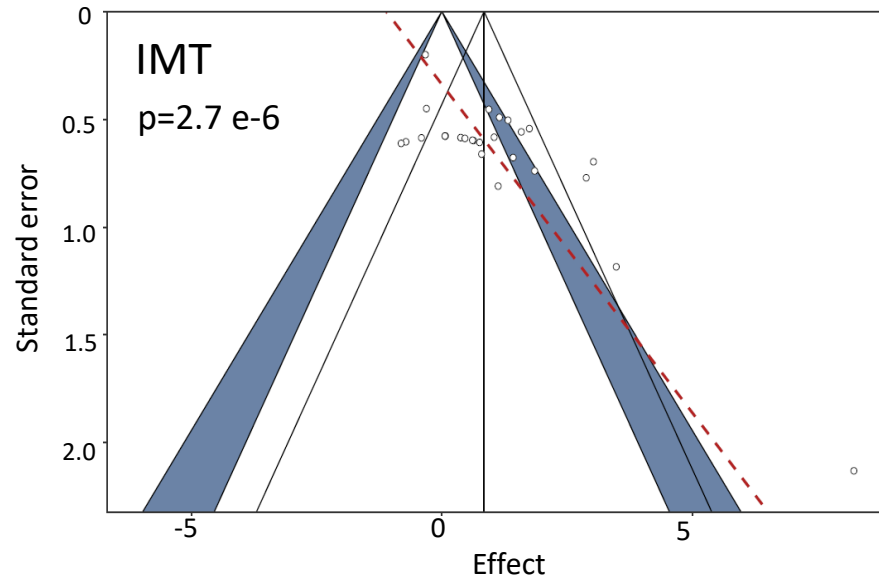
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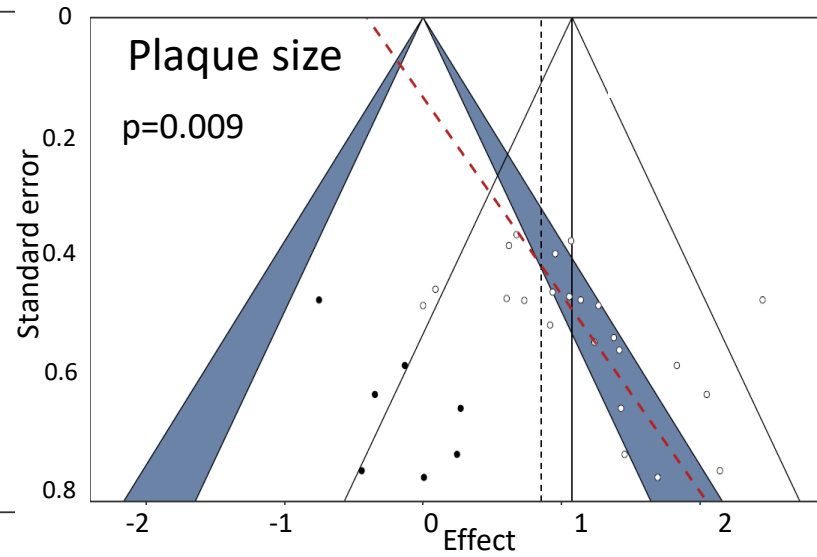
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**C**



**D**



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# PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
<b>TITLE</b>			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
<b>ABSTRACT</b>			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
<b>INTRODUCTION</b>			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3-4
<b>METHODS</b>			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	4
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4-5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	PROSPERO
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	4
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	4+ PROSPERO
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	5
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., $I^2$ ) for each meta-analysis.	5



# PRISMA 2009 Checklist

Page 1 of 2

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6
<b>RESULTS</b>			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	7+Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Supplementary Tables 1 and 2
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	9+ Supplementary Figure 7 and supplementary table 3
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	8-9+Figures 2,3,4,5
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	8-9+Figures 2,3,4,5
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	9+ Supplementary Figure 7 and supplementary table 3
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	10+ supplementary figure 8+ Supplementary Table 4
<b>DISCUSSION</b>			



# PRISMA 2009 Checklist

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Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10-13
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	13
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	10-13
<b>FUNDING</b>			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	15

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: [www.prisma-statement.org](http://www.prisma-statement.org).