

Curcumin prevents chronic intermittent hypoxia-induced myocardial injury

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Abstract

Background: Chronic intermittent hypoxia (IH), the hallmark feature of obstructive sleep apnoea syndrome, contributes to infarct size enhancement after myocardial ischemia–reperfusion (I/R). Curcumin (Curc), the natural pigment of *Curcuma longa*, has been demonstrated to be beneficial in the context of myocardial injury. In this study, we assessed the effects of Curc on the maladaptive cardiac response to IH, and particularly on IH-induced hypoxia inducible factor-1 (HIF-1) expression, oxidative stress, inflammation, endoplasmic reticulum (ER) stress and apoptosis.

Methods: Swiss/SV129 mice were exposed to normoxia or IH (21–5% FiO₂, 60s cycles, 8h per day, for 21 days) and treated orally with Curc (100 mg kg⁻¹day⁻¹, oral gavage) or its vehicle. Mice were then either euthanised for heart sampling in order to perform biochemical and histological analysis, or subjected to an *in vivo* ischemia–reperfusion protocol in order to measure infarct size.

Results: IH increased nuclear HIF-1 α expression and superoxide anion (O₂⁻) production as well as nuclear factor kappa B (NF- κ B) p65, glucose-regulated protein (Grp78) and C/EBP homologous protein (CHOP) expression. IH also induced apoptosis and increased infarct size after I/R. The IH-induced HIF-1 activation, oxidative stress, inflammation, ER stress and apoptosis were abolished by chronic Curc treatment. Curc also significantly decreased infarct size only in mice exposed to IH.

Conclusion: Curc prevents IH-induced myocardial cell death signalling. Curc might be used as a combined therapy with continuous positive airway pressure in sleep apnoea patients with high cardiovascular risk.

Keywords: curcumin, intermittent hypoxia, myocardial injury

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Introduction

Myocardial infarction (MI), associated with acute mortality and long-term consequences, represents a major public health concern. Post-MI functional and structural recovery is a key contributor for long-term quality of life and risk of cardiovascular event recurrence. Indeed, maladaptive response to ischemia–reperfusion (I/R) injury increases incidence of heart failure. This stimulated research to better understand the I/R injury process, to identify key contributors of I/R maladaptive response, in order to develop pharmacological strategies for cardiomyocyte protection.¹ Co-morbidities, including obstructive sleep apnoea syndrome

(OSA), represent a major contributing factor for myocardial injury initiation and progression. OSA is one of the most frequent chronic diseases that contributes to atherosclerosis progression, arrhythmia occurrence and recurrence, coronary artery disease and myocardial infarction.^{2–5} Moreover, at 3 months following MI, patients with sleep apnoea display deleterious cardiac remodelling with larger infarct size than non-OSA patients.⁶

OSA corresponds to the repetitive occurrence of partial or complete pharyngeal collapse during sleep responsible for oxygen (O₂) desaturation–resaturation sequences leading

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to chronic intermittent hypoxia (IH).⁷ It is now well accepted that IH is the major trigger of detrimental OSA cardiovascular consequences, promoting oxidative stress, low grade inflammation and endoplasmic reticulum (ER) stress as well as the hypoxia inducible factor-1 (HIF-1) activation.⁸ All of these intermediary mechanisms cooperate and participate in a cascade of molecular mechanisms inducing OSA cardiovascular and metabolic consequences.^{9,10} In this context of chronic IH, HIF-1 seems to be the key player in this vicious circle, and the following has been established: (1) oxidative stress is a potent inducer of the expression and stabilisation of HIF-1 α and in HIF-1 transactivation¹¹; (2) vascular inflammation, characterised by an increase in nuclear factor-*kappa*B (NF- κ B) activation is abolished in HIF-1 α ^{+/-} mice¹²; and (3) myocardial ER stress inhibition is responsible for a decrease in HIF-1 activity.¹³ Most importantly, HIF-1 is responsible for the IH-induced increase in infarct size following I/R.¹³⁻¹⁴ Thus, targeting these interconnected mechanisms as a therapeutic approach for the treatment of OSA-related cardiovascular complications remains a major challenge in the field.⁸ Such a strategy might increase myocardial cell viability and might prevent heart failure progression in OSA patients.¹⁵

Curcumin (Curc; diferuloylmethane) is the natural yellow pigment extracted from roots of *Curcuma longa*.¹⁶ Curc has already demonstrated beneficial effects in acute coronary syndrome, with a decrease in total cholesterol, low density lipoprotein and C-reactive protein in post-coronary artery bypass grafting.¹⁷ A reduction in malonaldehyde and N-terminal Pro-B-type natriuretic peptide levels has also been reported.¹⁸ In rodent models, acute administration of Curc before or after myocardial I/R reduced infarct size in rats, specifically through its anti-oxidative action and through the stimulation of the SAVE and the RISK pathways.¹⁹⁻²² In the context of MI, Curc also demonstrated protective effect through its anti-inflammatory action.²³

Thus, we hypothesised that Curc has the ability to abolish IH-induced deleterious effects. Specifically, we aimed to investigate the potential beneficial effects of Curc in: (a) reducing IH-induced increase

in infarct size; (b) reducing IH-induced increase in HIF-1 α expression, oxidative stress, inflammation and ER stress, which are all intermediary mechanisms recognised to participate to OSA-cardiovascular consequences.

Methods

Animals

A total of 60 male Swiss/SV129 mice (8–12 weeks old, 25–30 g) were used. They were bred at the ‘plateforme de haute technologie animale (number: C38 516 10006) of Grenoble Alpes University. Swiss/SV129 mice were previously used to reproduce deleterious consequences of our IH stimulus.¹³ All animals received humane care in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (directive 2010/63/UE, decree 2013–118 and orders of 1 February 2013). The study protocol was approved by the French ministry (number: #963- 2015061813053181) according to 3R rules. Animals were housed in the conventional animal care facility of the HP2 laboratory (number: C38 516 10006) in a controlled-temperature and hygrometry environment and 12-h light/dark cycle. Mice were housed (six per cage) in Plexiglas[®] cages (1285/5L; 530 cm²) with environmental enrichment. Mice had unlimited access to water and standard chow (Rod-18R, Genobios, Laval, France). During the entire experiment, mice were visited every day and body weight was measured. No mice were excluded for reasons of abnormal behaviour or weight loss.

Experimental design

The 60 mice were divided randomly into two groups (procedure 1 or procedure 2, $n=28$ and 32 , respectively). In each group, they were then randomised into four sub-groups, exposed to normoxia (N) or intermittent hypoxia (IH) and treated with Curc or vehicle ($n=6-8$ mice per group; Figure 1A). Two sets of experiments were randomly performed. The first procedure (procedure 1) aimed at performing immunohistological and biochemical analysis on myocardial tissue to determine HIF-1 α expression, oxidative stress, inflammation, ER stress and apoptosis. Procedure 2 aimed at investigating myocardial infarct size

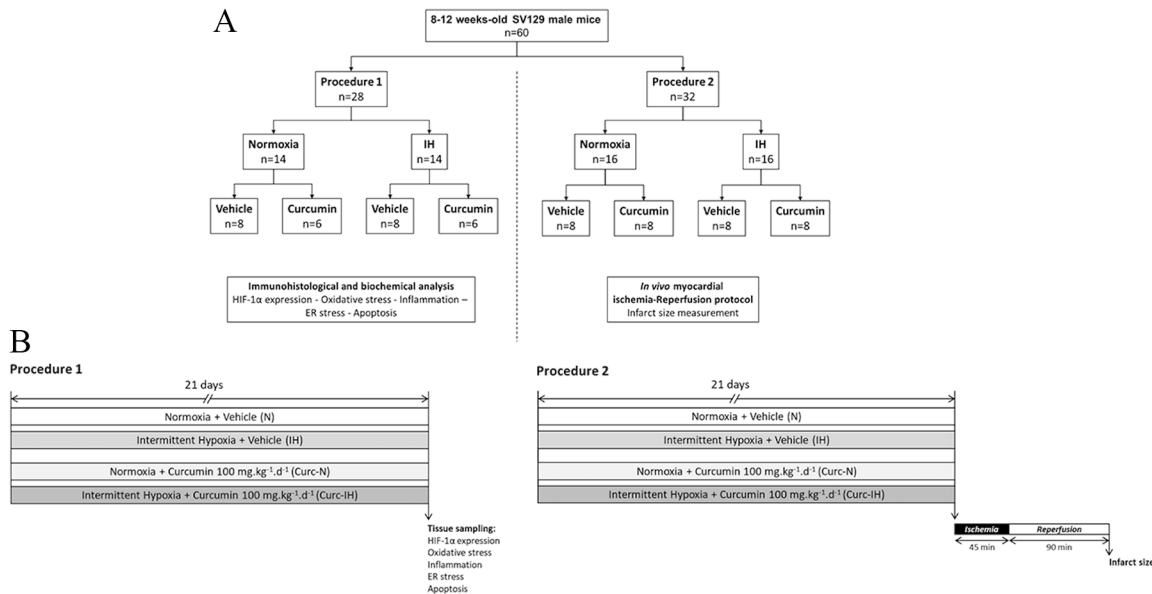


Figure 1. Experimental study groups and procedures. A total of 60 mice were randomised in two procedures. In each procedure, mice were exposed to 21 days of N or IH, and treated by oral gavage with Curc ($100 \text{ mg kg}^{-1} \text{ day}^{-1}$) or vehicle ($10 \text{ ml kg}^{-1} \text{ day}^{-1}$) (A). After N or IH exposure, two procedures were performed: hearts were removed for western-blot, immunohistochemistry and histology [$n = 6 - 8$ per group, procedure (a)]; or mice were submitted to *in vivo* ischemia-reperfusion to measure infarct size [$n = 8$ per group, procedure (b)] (B). N, normoxia; IH, intermittent hypoxia; Curc, curcumin; I/R, ischemia-reperfusion.

following *in vivo* I/R protocol (Figure 1B). This repartition was planned taking into account exposure, treatment and subsequent experiments in order to avoid mice mixing accordingly to breeding. For the first set, each experiment was performed with a number of animals depending on the experimental conditions and techniques used.

Intermittent hypoxia

Mice were exposed in their housing cages to 21 days of IH or N, 8 h per day during their sleeping period. The IH stimulus consisted in 60 s cycles alternating 30 s of hypoxia (hypoxic plateau at 5% FiO_2) and 30 s of N (normoxic plateau at 21% FiO_2). Normoxic control mice were exposed to similar air-air cycles in order to control for noise and air turbulences related to gas flow.

Pharmacological treatment

Curc treatment (Sigma, Saint Quentin-Fallavier, France) was administered by oral gavage at a dose of $100 \text{ mg kg}^{-1} \text{ day}^{-1}$ (in vehicle carboxymethylcellulose sodium 0.1%)^{24,25} during N or IH exposure (Curc-N and Curc-IH groups). Control groups were daily fed with vehicle (carboxymethylcellulose

sodium 0.1%, $10 \text{ ml kg}^{-1} \text{ day}^{-1}$) during N or IH exposure (N and IH groups).

Procedure 1

Myocardial tissue preparation. After cervical dislocation, nearly 40 mg of heart was rapidly frozen in optimum cutting temperature compound to perform immuno-histochemistry and histology; and nearly 80 mg of heart was immediately frozen in liquid nitrogen to perform western blot.

Expression of HIF-1 α , NF- κ B, Grp78 and CHOP by immunohistochemistry and western blot

Immunohistochemistry. Frozen cryopreserved hearts were cryosectioned ($8 \mu\text{m}$ thickness). Slices were fixed in 100% ice-cold acetone and blocked successively with hydrogen peroxide (3%), avidin-biotin kit (Vectorlab, Abcys, Paris, France) and serum (10% in PBS). Primary antibody of HIF-1 α (1:1000, Abcam, Cambridge, UK), NF- κ B p65 (1:1500, Abcam), 78 kDa glucose-regulated protein (Grp78, 1:50, Covalab, Villeurbanne, France) and C/EBP homologous protein (CHOP, 1:50, Santa Cruz Biotechnology, Dallas, TX, USA) were incubated for 30 min, and secondary antibody-coupled to biotin (1/500 or 1/200, Vectorlab), was

incubated for 30 min. Slices were then treated with amplifying complex AB (Vectorlab) and colorimetric-substrate was added for 5 min (Histo Green, Abcys, Paris, France). Slices were then stained with haematoxylin before dehydration. Vecta-Mount Permanent medium (Vectorlab) was used to mount the slides. Images were then acquired using the axioscan microscope (Zeiss, Göttingen, Germany, x20, x40) and Zen® software. Two slides per heart and two areas per slide were analysed by Image J® software (National Institutes of Health, Bethesda, MD, USA). In accordance with the experiment design, seven of the eight N and IH hearts (random choice) and all six hearts from Curc-N and Curc-IH were analysed. According to experimental conditions, the numbers of images analysed are specified in the figure legends.

Western blot. Heart tissue samples were homogenised (Precellys® 24, 6000 rpm, 3×10s-5s, Bertin Technologies, Montigny le Bretonneux, France). Cytoplasmic and nuclear fractions were obtained using a nuclear extract kit (Active Motif, La Hulpe, Belgium) according to the manufacturer's recommendations, and protein concentration was measured with Bradford reagent (Sigma-Aldrich, St. Louis, MO, USA) and expressed relative to bovine serum albumin (BSA) standard. Proteins (30 µg) were separated by SDS-PAGE (8–12% acrylamide) and transferred to nitrocellulose membranes. Membranes were blocked with 5% non-fat milk or BSA in Tris-buffered saline (TBS) with Tween 20 (0.05%). Then, membranes were placed in TBS-Tween20 (0.05%)–1% BSA or non-fat milk and incubated at 4°C overnight with the following primary antibodies: HIF-1α (1:500, Abcam), NF-κB p65 (1:1000, Abcam), Grp78 (1:500, Covalab), CHOP (1:500, Santa Cruz Biotechnology). Then, appropriate horseradish peroxidase-conjugated anti-IgG (1:5000, Jackson ImmunoResearch, West Grove, PA, USA) were incubated for 1 h at room temperature. Enhanced chemiluminescence was performed with the ECL western-blot detection kit (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions and video acquisition (ChemiDoc™ XRS+ System, Bio-Rad). Equal loading was evaluated by tubulin for cytoplasmic protein and p84 for nuclear protein (Sigma-Aldrich). Density of protein bands was analysed (image J software, National Institutes of Health) and expressed relative to the appropriate control group. Four frozen hearts from the N and IH groups and three frozen hearts of Curc-N and Curc-IH groups were

randomly included in order to process to Western blot in optimised conditions. Each sample intensity was expressed as fold increase of the mean of the N group.

Determination of superoxide anion production by dihydroethidium staining. Superoxide anion (O_2^-) production was evaluated using dihydroethidium (DHE) staining (Sigma-Aldrich). Cells are permeable to DHE, which, upon reaction with O_2^- , forms a red fluorescent product (ethidium bromide) that intercalates with DNA. Frozen cryopreserved tissues samples were cryosectioned (14 µm thickness) and allowed to air dry for 10 min at room temperature. Sections were stained with DHE 10 µM in PBS Tween 0.1% for 5 min at room temperature in a dark moist chamber. After washing, the reaction was stopped with acetone (–20°C), the fluorescent ethidine signal was recorded using confocal microscopy (LSM5 510 Meta confocal microscope; x40; Zeiss, Oberkochen, Germany) and two slides per heart were analysed using Image J® software (National Institutes of Health). All animals included in Procedure 1 ($n=8$ in N and IH and $n=6$ in Curc groups) were used. Staining were performed in two time sequences and images were acquired and analysed by a blinded experimenter.

Apoptosis evaluation through terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay. The analysis of DNA fragmentation in apoptosis was evaluated by applying TUNEL assay staining (Abcam) following the kit protocol.

Frozen heart tissues were cryosectioned at 8 µm. An Axioscan fluorescent microscope (Zeiss, Göttingen, Germany, x20) was used to visualise TUNEL-positive red cells, and sections were counter-stained with 4',6-diamidino-2-phenylindole (DAPI). Two slides per heart and two areas per slide were analysed using Image J® software (National Institutes of Health). Red dots were expressed as the percentage of myocardium area. Six hearts per group from procedure 1 were freshly cut in order to perform the staining with the same kit (six of eight hearts from N and IH groups were randomly assigned to the experiment). TUNEL assay was performed in two time sequences, and images were acquired and analysed by a blinded experimenter. According to experimental conditions, the numbers of analysed images are specified in figure legends.

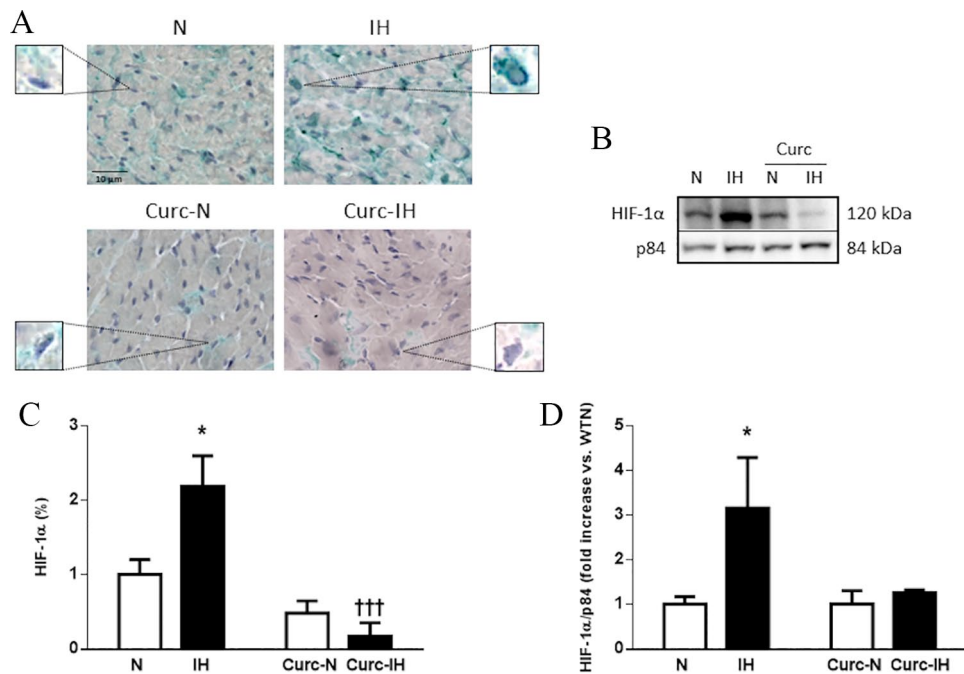


Figure 2. Curcumin significantly reduced the IH-induced increase in HIF-1 α expression. Myocardial expression of HIF-1 α assessed by immunohistochemistry (A) and western blot (B). HIF-1 α content was expressed as the percentage of myocardium area, ($n=6-7$) (A) or expressed relative to p84 in nuclear proteins extract, ($n=3-4$) (B). Nuclear localisation was illustrated using a $\times 40$ acquisition and $\times 2.5$ numerical zoom.

Values are mean \pm SEM. * $p < 0.05$ IH versus N; *** $p < 0.001$ Curc versus vehicle.

Curc, curcumin; IH, intermittent hypoxia; HIF-1 α , hypoxia inducible factor-1 alpha; N, normoxia; SEM, standard error of the mean.

Procedure 2

In vivo I/R and infarct size assessment. Mice were anaesthetised with pentobarbital (70 mg kg^{-1} , i.p.), ventilation was maintained by intubation (tidal volume $170 \mu\text{l}$; ambient air), body temperature was maintained at 37°C using a rectal probe connected to a heating plate. After a left thoracotomy, a 45-min myocardial ischemia was induced by ligation of the left coronary artery followed by 90 min of reperfusion, as previously described.¹³ At the end of the reperfusion period, the coronary artery was re-occluded and staining of area at risk (AAR) and area of necrosis (AN) was performed using Uniprse blue dye and 1% triphenyltetrazolium chloride, respectively, as previously described. AAR and AN were measured by planimetric analysis (Image J® software, National Institutes of Health) and expressed relative to heart weight. Eight animals per group were submitted to the *in vivo* ischemia-reperfusion protocol, realised by the same experimenter. In the N and IH groups, one animal died during the first minutes of reperfusion. Images were acquired and analysed by a blinded experimenter.

Statistical analysis

All analysis was performed using GraphPad Prism 6 Software (San Diego, CA, USA). All data are presented as the mean \pm standard error of the mean (SEM). D'Agostino-Pearson test was performed to test normality. A two-way ANOVA followed by Tukey's *post hoc* test was used to analyse the effect of IH and Curc treatment. If normal distribution was assumed, two-way ANOVA was performed and a *post hoc* Tukey test was conducted only if 'F' was significant. If normal distribution was not assumed, a non-parametric Kruskal-Wallis test was performed. Statistical significance was set at a value of $p < 0.05$.

Results

Curcumin treatment during IH decreased HIF-1 α expression

As a major actor of the response to IH, expression of HIF-1 α was quantified after immunohistochemistry (Figure 2A and C) and western blot (Figure 2B and D). As expected, chronic IH increased HIF-1 α expression. In addition to

expression, Figure 2A (insets) illustrates HIF-1 α localisation and reveals that, under hypoxic conditions, HIF-1 α was located in the nucleus, suggesting its activation. Curc treatment significantly decreased the amount of HIF-1 α , especially in hearts from mice exposed to IH.

Curcumin treatment during IH decreased oxidative stress

Detection of O₂⁻ has been used as an indicator of oxidative stress. The quantification of O₂⁻, measured by DHE staining, is presented in Figure 3. Chronic IH exposure resulted in a significant increase in O₂⁻ staining (red staining, Figure 3A) and quantified relative to the normoxic control group (Figure 3B). Curc treatment, administered all along the IH exposure, blunted O₂⁻ over-expression in IH mice only (Figure 3B).

Curcumin treatment during IH decreased inflammation

As it was previously demonstrated to be increased by IH in several tissues, we chose to assess NF- κ B p65 expression as a marker of myocardial inflammation. As shown in Figure 4, chronic IH exposure induced a significant increase in NF- κ B p65 expression, which was strongly decreased by Curc (Figure 4A–D).

Curcumin treatment during IH decreased ER stress

Detection of the ER stress sensor Grp78 and the terminal pro-apoptotic unfolded protein response effector CHOP were also performed by immunohistochemistry (Figure 5A and B, respectively) and Western-blot (Figure 5E and F, respectively). Immunohistochemical quantifications show that chronic IH exposure significantly increased Grp78 and CHOP expression (Figure 5C and D, respectively). This was confirmed by western blot quantification analysis (Figure 5G and H, respectively). Curc treatment administered during IH significantly reduced Grp78 content (Figure 5C and G), and also limited the IH-induced increase in CHOP expression (Figure 5D and H).

Curcumin treatment during IH decreased apoptosis and infarct size

TUNEL assay was performed in order to assess the impact of IH *per se* on basal apoptosis. Chronic

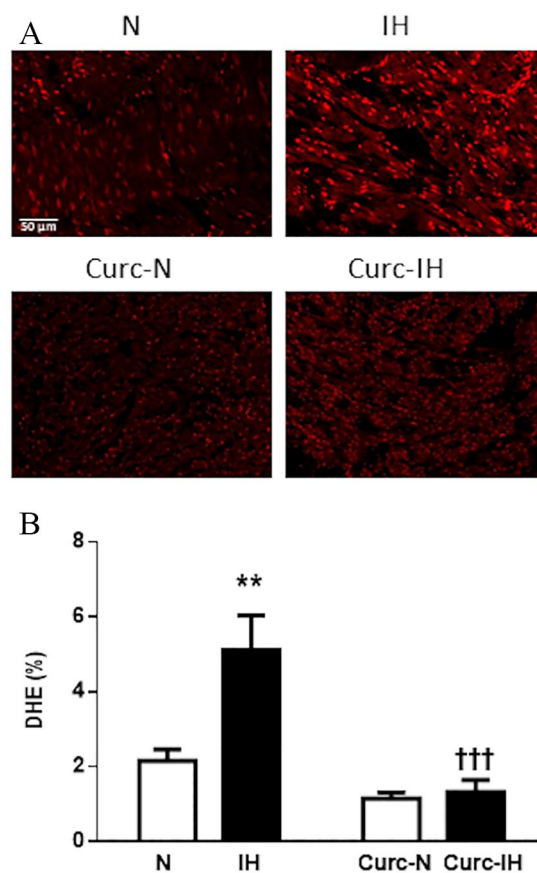


Figure 3. Curcumin significantly reduced IH-induced oxidative stress. Myocardial level of O₂⁻ assessed by DHE staining [A]. O₂⁻ quantity was expressed as the percentage of myocardium area, (n = 6–8) [B]. Values are mean ± SEM. **p < 0.01 IH versus N; †††p < 0.001 Curc versus vehicle. Curc, curcumin; N, normoxia; IH, intermittent hypoxia; DHE, dihydroethidium; O₂⁻, superoxide anion; SEM, standard error of the mean.

exposure to IH increased apoptotic cells, which was prevented by Curc treatment (Figure 6A and B). Interestingly, whereas areas at risk of all groups were similar, IH dramatically increased infarct size following I/R. Curc treatment also significantly reduced the IH-induced increase in infarct size following I/R (Figure 6C).

Discussion

The present study demonstrates that chronic oral administration of Curc during IH exposure prevents IH-induced HIF-1 activation, oxidative stress (O₂⁻), inflammation (NF- κ B p65) as well as ER stress (Grp78 and CHOP) and apoptosis (Figure 7). Consistent with these beneficial

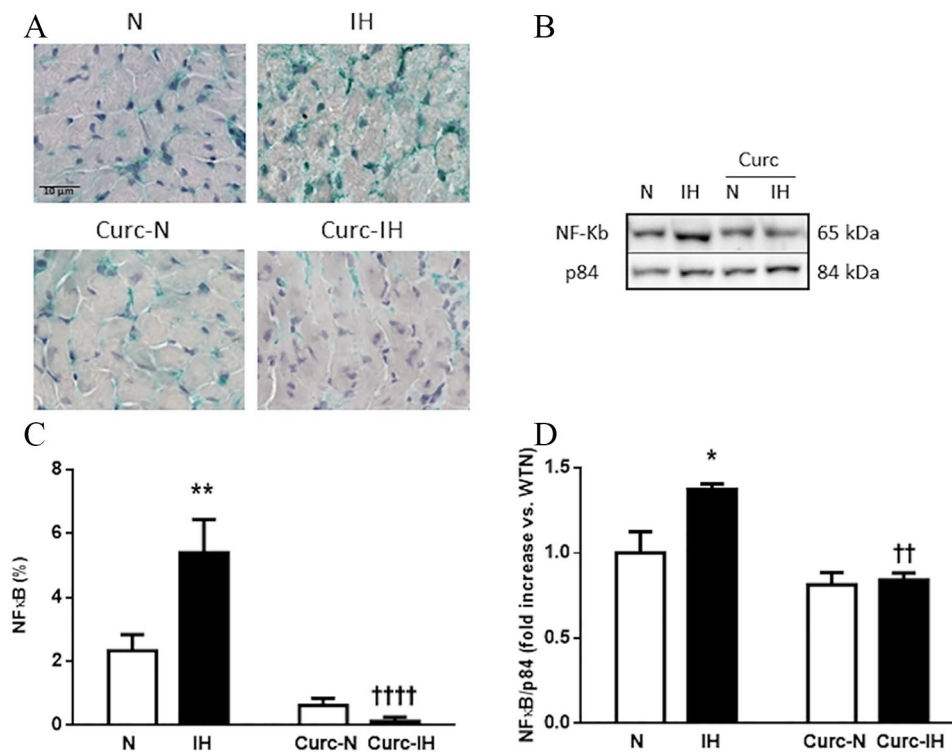


Figure 4. Curcumin significantly reduced the IH-increase in NF-κB p65 expression. Myocardial level of NF-κB p65 assessed by immunohistochemistry (A) and Western blot (B). NF-κB p65 subunit content was calculated as the percentage of myocardium area, ($n=5-6$) (C) or expressed relative to p84 in nuclear proteins extract ($n=3-4$) (D). Values are mean \pm SEM. * $p < 0.05$, ** $p < 0.01$ IH versus N; †† $p < 0.01$, †††† $p < 0.0001$ Curc versus vehicle. Curc, curcumin; N, normoxia; IH, intermittent hypoxia; NF-κB, nuclear factor kappa B; SEM, standard error of the mean.

effects, Curc also decreases IH-induced infarct size enhancement, demonstrating an overall clinically relevant impact. These results support the concept that Curc could be an effective therapeutic modality to limit cardiovascular consequences in severe OSA and, in particular, improving post-MI recovery.^{16,26}

Improving cardiomyocyte viability after MI is one of the most important factors contributing to myocardial structural and functional recovery, and strategies aiming at limiting post-MI myocardial alterations exert beneficial effects on long-term morbi-mortality.²⁷ Another valuable strategy would be to identify underlying conditions or diseases, such as OSA, that first increase infarct size and contribute to deleterious post-MI remodelling. Our data are deciphering and targeting specific mechanisms involved in 'OSA-IH' myocardial injury. As mentioned previously, sleep apnoea syndrome is well recognised to represent an independent risk factor for coronary artery disease and OSA is known to be

associated with an increase in infarct size and a poor functional recovery post-MI.^{6,7} Our group has contributed significantly to identifying several deleterious mechanisms induced in response to chronic IH exposure in rodent models, such as oxidative stress, inflammation and ER stress.⁸ Although the cellular and molecular mechanisms of IH-induced increase in myocardial infarct size are not yet completely elucidated, HIF-1 activation seems to play a major role.⁸ In the present study, HIF-1 α was overexpressed in hearts from mice exposed to IH. This is in accordance with previous studies demonstrating an increase in HIF-1 activation in both rats and mice exposed to chronic IH.^{13,28} Daily Curc treatment during IH significantly reduced HIF-1 α content. This effect of Curc on the HIF pathway has already been demonstrated in other chronic diseases such as diabetes, liver fibrosis and cancer,²⁹ and our study demonstrates for the first time that Curc also modulates HIF-1 activation in the specific context of IH, the hallmark of OSA.

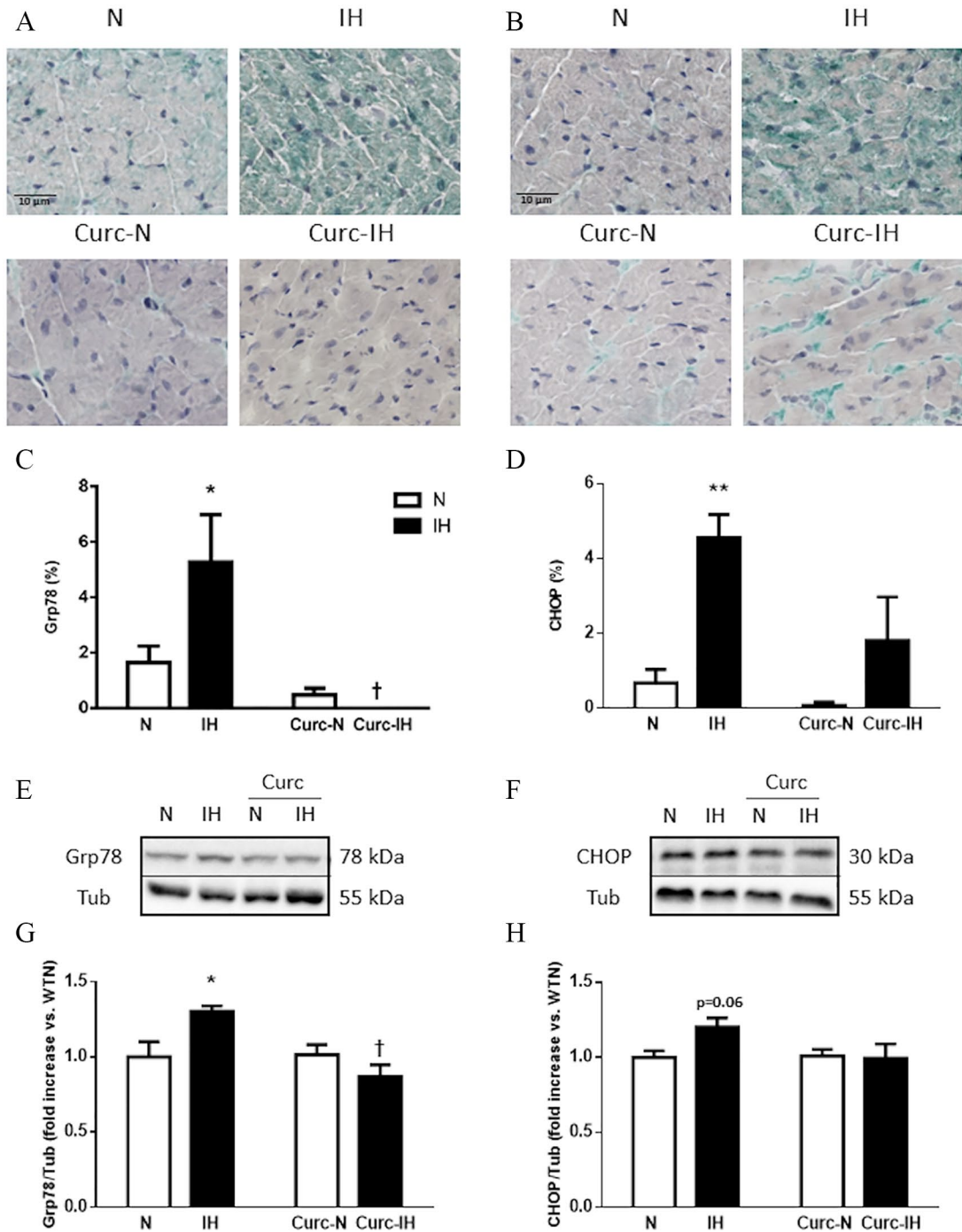


Figure 5. Curcumin significantly reduced the IH-induced ER stress. Expression of ER stress markers was assessed by immunohistochemistry (A, Grp78 and B, CHOP) and Western blot (E, Grp78 and F, CHOP). Grp78 and CHOP contents were calculated as the percentage of myocardium area, ($n=5-7$) (C, Grp78 and D, CHOP) or expressed relative to tubulin in cytosolic proteins extract, ($n=3-4$) (G, Grp78 and H, CHOP). Values are mean \pm SEM. * $p < 0.05$, ** $p < 0.01$ IH versus N; † $p < 0.05$ Curc versus vehicle. Curc, curcumin; N, normoxia; IH, intermittent hypoxia; CHOP, C/EBP homologous protein; ER, endoplasmic reticulum; Grp78, glucose-regulated protein (Grp78); SEM, standard error of the mean.

Sleep apnoea syndrome is characterised by an imbalance of pro/anti-oxidant status,³⁰ and oxidative stress is known to be a powerful inducer of HIF-1 activity.¹¹

Consistent with previous preclinical studies,^{31,32} we observed that IH exposure induced an increase in myocardial O_2^- expression and that Curc abolished

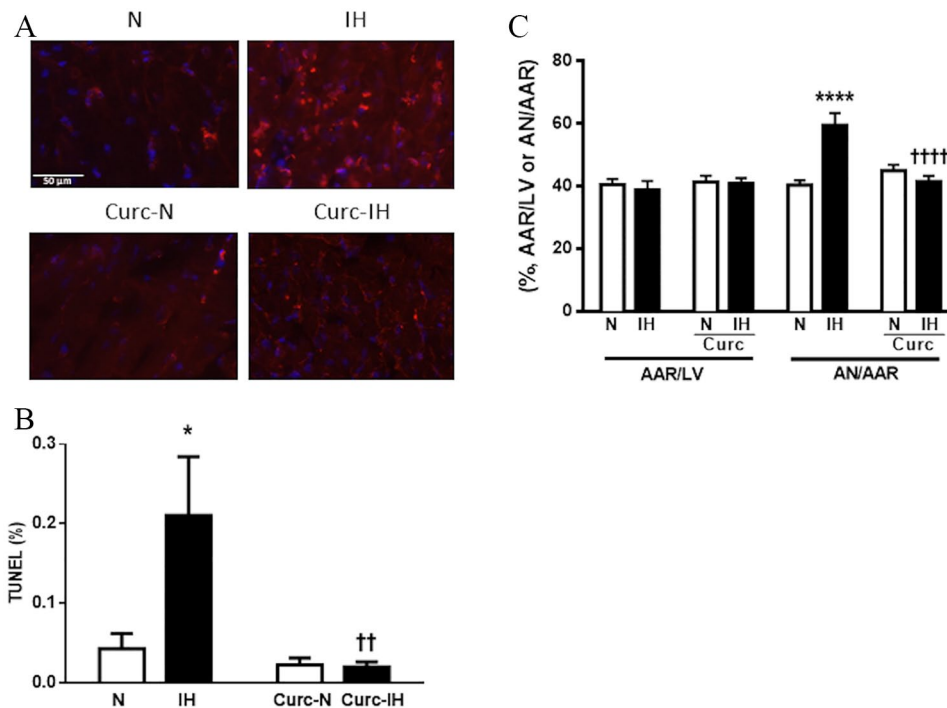


Figure 6. Curcumin significantly reduced the IH-induced myocardial cell death and an increase in infarct size. Myocardial cell death was assessed by TUNEL staining (A). Red dots were quantified and expressed as the percentage of myocardium area ($n=4-6$) (B). Infarct size was determined by colorimetry and planimetry following *in vivo* I/R. AAR relative to LV and AN relative to AAR, ($n=7-8$) (C). Curc significantly attenuated IH-induced myocardial cell death and infarction.

Values are mean \pm SEM. * $p < 0.05$, **** $p < 0.0001$ IH versus N; †† $p < 0.01$, †††† $p < 0.0001$ Curc versus vehicle.

Curc, curcumin; N, normoxia; IH, intermittent hypoxia; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling; I/R, ischemia-reperfusion; AAR, area at risk; LV, left ventricle; AN, area of necrosis; SEM, standard error of the mean.

this effect. This is in accordance with the well-known anti-oxidant properties of Curc (i.e. increase in anti-oxidant enzyme content, reactive oxygen species scavenging).³³

Tissue inflammation has also been described to be induced by chronic IH in OSA patients and rodent models.^{12,34,35} Particularly, Gras *et al.* demonstrated that IH induced an increase in vascular NF- κ B activity that was abolished by partial HIF-1 α deletion in mice.¹² In the current study, chronic Curc administration blunted the IH-induced increase in NF- κ B p65 sub-unit expression in the myocardium. This is also in accordance with previous studies demonstrating the anti-inflammatory effects of Curc in both pre-clinical and clinical studies,^{36,37} and on several markers such as interleukin-6, monocyte chemoattractant protein 1 or early growth factor-1.^{36,38,39}

More recently, ER stress has been shown to be a key player in the deleterious consequences of

IH on the brain and heart,⁴⁰⁻⁴² and we have demonstrated that it is a potent inducer of HIF-1 activation, leading to an increase in infarct size following I/R.¹³ Due to the recent nature of these experimental results, only one clinical study has investigated the link between OSA and ER stress markers. In apnoeic and obese patients, continuous positive airway pressure (CPAP, the first line therapy of OSA) decreased adipose tissue mRNA content of several ER stress markers.⁴³ In the present study, we confirmed that IH increased the myocardial expression of Grp78 and CHOP, a pro-apoptotic effector of the unfolded protein response, and showed that Curc prevents these effects. This is in accordance with the beneficial myocardial effects of Curc in reducing ER stress markers in the context of acute myocarditis,⁴⁴ as well as in reducing lactate dehydrogenase or creatine kinase in the context of myocardial dysfunction induced by isoproterenol treatment.^{45,46}

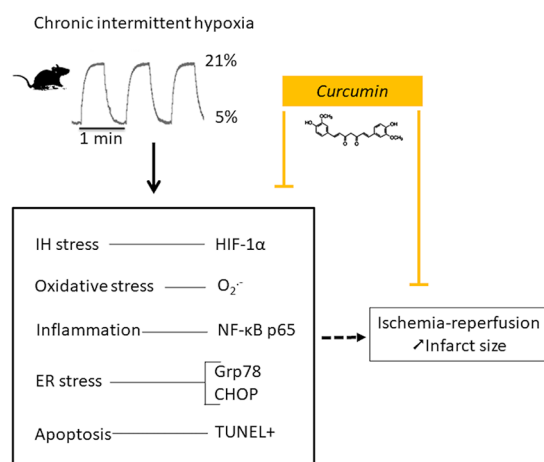


Figure 7. Beneficial effects of Curcumin against IH-associated myocardial injuries. Curc reduced the IH-induced increase in HIF-1 α subunit expression, $O_2^{\cdot-}$ production, expression of NF- κ B p65 subunit and ER stress markers (i.e. Grp78 and CHOP). Curc also limited the IH-induced apoptosis [number of apoptotic nuclei (TUNEL)]. All these beneficial effects on myocardial cellular death signalling are associated with a beneficial effect of Curc against the IH-induced increase in infarct size following myocardial I/R. IH, intermittent hypoxia; HIF-1, hypoxia inducible factor-1; $O_2^{\cdot-}$ superoxide anion; NF- κ B, nuclear factor-kappa B; ER, endoplasmic reticulum; Grp78, glucose regulated protein 78 kDa; CHOP, C/EBP homologous protein; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling.

Finally, in accordance with the literature,⁴⁷ we confirmed that chronic exposure to IH induced myocardial apoptosis, which was avoided by chronic Curc treatment. This is in agreement with a recent study demonstrating that Curc also attenuates chronic IH-induced apoptosis in other cell types such as neurons.²⁴

Based on the fact that (a) all IH-induced mechanisms, alone, or through interactions with each other, are potentially responsible for an increase in myocardial vulnerability to I/R; (b) IH is a potent inducer of HIF-1 activation; and (c) Curc reduces HIF-1 activation and associated oxidative stress, inflammation, ER stress and apoptosis; we speculate that chronic oral Curc administration might represent an interesting new avenue to limit the IH-induced increase in infarct size following myocardial I/R.

Interestingly, whereas several studies demonstrated that acute bolus administration of Curc at the onset of I/R stress decreases myocardial

injury^{19,20,22}; our results showed that chronic administration of Curc had no beneficial in limiting infarct size in normoxic mice. This discrepancy could be in part explained by the differences in Curc administration pattern (acute *versus* chronic, moment of administration). Our experiment, with daily Curc administration throughout IH exposure, was designed to demonstrate that limiting the IH-induced intermediate mechanisms could prevent the increase in infarct size.

Study limitations

Study limitations are the following: (i) we demonstrated that chronic administration of Curc is specifically protective against IH-induced increase in infarct size. This is associated with an inhibition of IH-induced HIF-1 nuclear localisation, inflammation, oxidative stress and ER stress. All these mechanisms could be interrelated, and the present study cannot explain whether Curc exerts pleiotropic beneficial effects on all these mechanisms or whether these effects depend on Curc-induced HIF-1 inhibition and subsequent beneficial effects on inflammation, oxidative stress and ER stress.⁸ (ii) OSA is a complex pathology characterised by IH, sleep fragmentation and intra-thoracic pressure swing. Although IH is described by the international scientific community as the major deleterious consequence of OSA in terms of cardiovascular repercussions, we cannot exclude a significant contribution of the other mechanisms on deleterious cardiovascular complications in OSA patients. This should be considered before bench-to-bedside transfer. (iii) Finally, the present study assessed only the short-term post-MI consequences through infarct size determination. Although infarct size is a well-known predictive parameter of post-MI structural and functional recoveries; in a further study, it would be interesting to also evaluate the effect of Curc on long-term post-MI cardiac remodelling and contractile dysfunction.

Conclusion

In conclusion, the current study demonstrates that daily treatment with Curc during IH exposure protects against the maladaptive response to IH and improves the myocardial response to I/R injury, resulting in a reduction in infarct size. Cardioprotection was associated with attenuation of $O_2^{\cdot-}$ production and of nuclear HIF-1 α , NF- κ B p65, Grp78 and CHOP expression. Through all

these beneficial effects, Curc could prevent apoptosis in myocardium subjected to IH (Figure 7). Even if CPAP therapy is recognised to be beneficial regarding quality of life and neurocognitive functions, CPAP therapy alone did not reduce cardiovascular events.⁴⁸ Thus, adjunctive therapies have to be considered in order to adverse pathophysiological-related mechanistic consequences shown to be involved in cardiovascular consequences. Since Curc is a safe food additive, it might be used as a combined therapy with CPAP when a high cardiovascular risk has to be addressed in OSA patients.

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Author contributions

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Data analysis: SM, SB, EB

Manuscript preparation: SM, JLP, DG, CA, EB

Study design: SM, CA, EB


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Conflict of interest statement

The authors declare that there is no conflict of interest.

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References

1. Bulluck H, Yellon DM and Hausenloy DJ. Reducing myocardial infarct size: challenges and future opportunities. *Heart* 2016; 102: 341–348.
2. Benjafield AV, Ayas NT, Eastwood P, *et al.* An estimate of the global prevalence and burden of obstructive sleep apnoea. *Lancet Respir Med* 2019; 7: 687–698.
3. Baguet JP, Hammer L, Levy P, *et al.* The severity of oxygen desaturation is predictive of carotid wall thickening and plaque occurrence. *Chest* 2005; 128: 3407–3412.
4. May AM, Van Wagoner DR and Mehra R. OSA and cardiac arrhythmogenesis: mechanistic insights. *Chest* 2017; 151: 225–241.
5. Drager LF, McEvoy RD, Barbe F, *et al.* Sleep apnea and cardiovascular disease: lessons from recent trials and need for team science. *Circulation* 2017; 136: 1840–1850.
6. Buchner S, Satz A, Debl K, *et al.* Impact of sleep-disordered breathing on myocardial salvage and infarct size in patients with acute myocardial infarction. *Eur Heart J* 2014; 35: 192–199.
7. Levy P, Kohler M, McNicholas WT, *et al.* Obstructive sleep apnoea syndrome. *Nat Rev Dis Primers* 2015; 1: 15015.
8. Belaidi E, Morand J, Gras E, *et al.* Targeting the ROS-HIF-1-endothelin axis as a therapeutic approach for the treatment of obstructive sleep apnea-related cardiovascular complications. *Pharmacol Ther* 2016; 168: 1–11.
9. Ryan S, Arnaud C, Fitzpatrick SF, *et al.* Adipose tissue as a key player in obstructive sleep apnoea. *Eur Respir Rev* 2019; 28: 190006.
10. Aron-Wisnewsky J, Clement K and Pepin JL. Nonalcoholic fatty liver disease and obstructive sleep apnea. *Metabolism* 2016; 65: 1124–1135.
11. Semenza GL. Regulation of oxygen homeostasis by hypoxia-inducible factor 1. *Physiology (Bethesda)* 2009; 24: 97–106.
12. Gras E, Belaidi E, Briancon-Marjollet A, *et al.* Endothelin-1 mediates intermittent hypoxia-induced inflammatory vascular remodeling through HIF-1 activation. *J Appl Physiol (1985)* 2016; 120: 437–443.
13. Belaidi E, Thomas A, Bourdier G, *et al.* Endoplasmic reticulum stress as a novel inducer of hypoxia inducible factor-1 activity: its role in the susceptibility to myocardial ischemia-reperfusion induced by chronic intermittent hypoxia. *Int J Cardiol* 2016; 210: 45–53.
14. Moulin S, Thomas A, Arnaud C, *et al.* Cooperation between HIF-1 and ATF4 in sleep-apnea mediated myocardial injury, in press *Can J Cardiol*, 13 April 2020. DOI: <https://doi.org/10.1016/j.cjca.2020.04.002>

15. Khattak HK, Hayat F, Pamboukian SV, *et al.* Obstructive sleep apnea in heart failure: review of prevalence, treatment with continuous positive airway pressure, and prognosis. *Tex Heart Inst J* 2018; 45: 151–161.
16. Kunnumakkara AB, Bordoloi D, Padmavathi G, *et al.* Curcumin, the golden nutraceutical: multitargeting for multiple chronic diseases. *Br J Pharmacol* 2017; 174: 1325–1348.
17. Alwi I, Santoso T, Suyono S, *et al.* The effect of Curcumin on lipid level in patients with acute coronary syndrome. *Acta Med Indones* 2008; 40: 201–210.
18. Wongcharoen W, Jai-Aue S, Phrommintikul A, *et al.* Effects of Curcuminoids on frequency of acute myocardial infarction after coronary artery bypass grafting. *Am J Cardiol* 2012; 110: 40–44.
19. Duan W, Yang Y, Yan J, *et al.* The effects of Curcumin post-treatment against myocardial ischemia and reperfusion by activation of the JAK2/STAT3 signaling pathway. *Basic Res Cardiol* 2012; 107: 263.
20. Liu H, Wang C, Qiao Z, *et al.* Protective effect of Curcumin against myocardium injury in ischemia reperfusion rats. *Pharm Biol* 2017; 55: 1144–1148.
21. Wang NP, Wang ZF, Tootle S, *et al.* Curcumin promotes cardiac repair and ameliorates cardiac dysfunction following myocardial infarction. *Br J Pharmacol* 2012; 167: 1550–1562.
22. Yang Y, Duan W, Lin Y, *et al.* SIRT1 activation by Curcumin pretreatment attenuates mitochondrial oxidative damage induced by myocardial ischemia reperfusion injury. *Free Radic Biol Med* 2013; 65: 667–679.
23. Ahmed S, Khan H and Mirzaei H. Mechanics insights of Curcumin in myocardial ischemia: where are we standing? *Eur J Med Chem* 2019; 183: 111658.
24. Wang B, Li W, Jin H, *et al.* Curcumin attenuates chronic intermittent hypoxia-induced brain injuries by inhibiting AQP4 and p38 MAPK pathway. *Respir Physiol Neurobiol* 2018; 255: 50–57.
25. Sun X, Liu Y, Li C, *et al.* Recent advances of Curcumin in the prevention and treatment of renal fibrosis. *Biomed Res Int* 2017; 2017: 2418671.
26. Arzt M, Hetzenecker A, Steiner S, *et al.* Sleep-disordered breathing and coronary artery disease. *Can J Cardiol* 2015; 31: 909–917.
27. Vogel B, Claessen BE, Arnold SV, *et al.* ST-segment elevation myocardial infarction. *Nat Rev Dis Primers* 2019; 5: 39.
28. Belaidi E, Joyeux-Faure M, Ribuot C, *et al.* Major role for hypoxia inducible factor-1 and the endothelin system in promoting myocardial infarction and hypertension in an animal model of obstructive sleep apnea. *J Am Coll Cardiol* 2009; 53: 1309–1317.
29. Bahrami A, Atkin SL, Majeed M, *et al.* Effects of Curcumin on hypoxia-inducible factor as a new therapeutic target. *Pharmacol Res* 2018; 137: 159–169.
30. Lavie L. Obstructive sleep apnoea syndrome—an oxidative stress disorder. *Sleep Med Rev* 2003; 7: 35–51.
31. Ramond A, Godin-Ribuot D, Ribuot C, *et al.* Oxidative stress mediates cardiac infarction aggravation induced by intermittent hypoxia. *Fundam Clin Pharmacol* 2013; 27: 252–261.
32. Totoson P, Fhayli W, Faury G, *et al.* Atorvastatin protects against deleterious cardiovascular consequences induced by chronic intermittent hypoxia. *Exp Biol Med (Maywood)* 2013; 238: 223–232.
33. Pulido-Moran M, Moreno-Fernandez J, Ramirez-Tortosa C, *et al.* Curcumin and health. *Molecules* 2016; 21: 264.
34. Guven SF, Turkkani MH, Ciftci B, *et al.* The relationship between high-sensitivity C-reactive protein levels and the severity of obstructive sleep apnea. *Sleep Breath* 2012; 16: 217–221.
35. Arnaud C, Beguin PC, Lantuejoul S, *et al.* The inflammatory preatherosclerotic remodeling induced by intermittent hypoxia is attenuated by RANTES/CCL5 inhibition. *Am J Respir Crit Care Med* 2011; 184: 724–731.
36. Bulboaca AE, Boarescu PM, Bolboaca SD, *et al.* Comparative effect of Curcumin versus liposomal Curcumin on systemic pro-inflammatory cytokines profile, MCP-1 And RANTES in experimental diabetes mellitus. *Int J Nanomedicine* 2019; 14: 8961–8972.
37. Mirzaei H, Shakeri A, Rashidi B, *et al.* Phytosomal Curcumin: a review of pharmacokinetic, experimental and clinical studies. *Biomed Pharmacother* 2017; 85: 102–112.
38. Li C, Miao X, Li F, *et al.* Curcuminoids: implication for inflammation and oxidative stress in cardiovascular diseases. *Phytother Res* 2019; 33: 1302–1317.

39. Wang NP, Pang XF, Zhang LH, *et al.* Attenuation of inflammatory response and reduction in infarct size by postconditioning are associated with downregulation of early growth response 1 during reperfusion in rat heart. *Shock* 2014; 41: 346–354.
40. Chou YT, Zhan G, Zhu Y, *et al.* C/EBP homologous binding protein (CHOP) underlies neural injury in sleep apnea model. *Sleep* 2013; 36: 481–492.
41. Ding W, Cai Y, Wang W, *et al.* Adiponectin protects the kidney against chronic intermittent hypoxia-induced injury through inhibiting endoplasmic reticulum stress. *Sleep Breath* 2016; 20: 1069–1074.
42. Bourdier G, Flore P, Sanchez H, *et al.* High-intensity training reduces intermittent hypoxia-induced ER stress and myocardial infarct size. *Am J Physiol Heart Circ Physiol* 2016; 310: H279–H289.
43. Perrini S, Cignarelli A, Quaranta VN, *et al.* Correction of intermittent hypoxia reduces inflammation in obese subjects with obstructive sleep apnea. *JCI Insight* 2017; 2.
44. Mito S, Thandavarayan RA, Ma M, *et al.* Inhibition of cardiac oxidative and endoplasmic reticulum stress-mediated apoptosis by Curcumin treatment contributes to protection against acute myocarditis. *Free Radic Res* 2011; 45: 1223–1231.
45. Boarescu PM, Boarescu I, Bocsan IC, *et al.* Antioxidant and anti-inflammatory effects of Curcumin nanoparticles on drug-induced acute myocardial infarction in diabetic rats. *Antioxidants (Basel)* 2019; 8: 504.
46. Boarescu PM, Boarescu I, Bocsan IC, *et al.* Curcumin nanoparticles protect against isoproterenol induced myocardial infarction by alleviating myocardial tissue oxidative stress, electrocardiogram, and biological changes. *Molecules* 2019; 24: 2802.
47. Ding W, Zhang X, Huang H, *et al.* Adiponectin protects rat myocardium against chronic intermittent hypoxia-induced injury via inhibition of endoplasmic reticulum stress. *PLoS One* 2014; 9: e94545.
48. Ayas NT, Foster GE, Shah N, *et al.* Could adjunctive pharmacology mitigate cardiovascular consequences of obstructive sleep apnea? *Am J Respir Crit Care Med* 2019; 200: 551–555.

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