High-intensity training reduces intermittent hypoxia-induced ER stress and myocardial infarct size

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1Grenoble Alpes University, HP2 Laboratory, Grenoble, France; 2Institut National de la Santé et de la Recherche Médicale, U1042, Grenoble, France; and 3Institut de Recherche Biomédicale des Armées, Operational Environments, Brétigny/Orge, France

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Bourdier G, Flore P, Sanchez H, Pepin JL, Belaidi E, Arnaud C. High-intensity training reduces intermittent hypoxia-induced ER stress and myocardial infarct size. Am J Physiol Heart Circ Physiol 310: H279–H289, 2016. First published November 13, 2015; doi:10.1152/ajpheart.00448.2015.—Chronic intermittent hypoxia (IH) is described as the major detrimental factor leading to cardiovascular morbimortality in obstructive sleep apnea (OSA) patients. OSA patients exhibit increased infarct size after a myocardial event, and previous animal studies have shown that chronic IH could be the main mechanism. Endoplasmic reticulum (ER) stress plays a major role in the pathophysiology of cardiovascular disease. High-intensity training (HIT) exerts beneficial effects on the cardiovascular system. Thus, we hypothesized that HIT could prevent IH-induced ER stress and the increase in infarct size. Male Wistar rats were exposed to 21 days of IH (21-5% fraction of inspired O2, 60-s cycle, 8 h/day) or normoxia. After 1 wk of IH alone, rats were submitted daily to both IH and HIT (2 × 24 min, 15-30 min/min). Rat hearts were either rapidly frozen to evaluate ER stress by Western blot analysis or submitted to an ischemia-reperfusion protocol ex vivo (30 min of global ischemia/120 min of reperfusion). IH induced cardiac proapoptotic ER stress, characterized by increased expression of glucose-regulated protein kinase 78, phosphorylated protein kinase-like ER kinase, activating transcription factor 4, and C/EBP homologous protein. IH-induced myocardial apoptosis was confirmed by increased expression of cleaved caspase-3. These IH-associated proapoptotic alterations were associated with a significant increase in infarct size (35.4 ± 3.2% vs. 22.7 ± 1.7% of ventricles in IH + sedenary and normoxia + sedenary groups, respectively, P < 0.05). HIT prevented both the IH-induced proapoptotic ER stress and increased myocardial infarct size (28.8 ± 3.9% and 21.0 ± 5.1% in IH + HIT and normoxia + HIT groups, respectively, P = 0.28). In conclusion, these findings suggest that HIT could represent a preventive strategy to limit IH-induced myocardial ischemia-reperfusion damages in OSA patients.

obstructive sleep apnea; intermittent hypoxia; ischemia-reperfusion; high-intensity aerobic training; endoplasmic reticulum stress

NEW & NOTEWORTHY

We demonstrated that intermittent hypoxia induced cardiac proapoptotic ER stress and increased infarct size, which were prevented by high-intensity aerobic training. These results strengthen the need for early identification of patients with sleep apnea at risk for cardiovascular complications and suggest that exercise can be used as a new preventive strategy for these patients.

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tion factor (ATF)6 also serves as a preferential transcription activator (47). The three arms of the UPR coordinately regulate the transcription of UPR-related genes encoding ER chaperones and protein folding enzymes to reduce the accumulation of unfolded proteins (31). However, during long-term activation of the ER stress response, the UPR fails to control the level of unfolded and misfolded proteins, and ER-initiated proapoptotic signaling is induced (47). Indeed, PERK, through ATF4, contributes to apoptotic cell death via activation of C/EBP homologous protein (CHOP) (25) and caspase pathway (i.e., caspase-3) activation (51), and the ATF6 branch is also involved in apoptotic cell death (50). Few studies have investigated the role of ER stress in the context of IH. ER stress has been shown to underlie neural injuries in an animal model of IH (11), and two recent studies have described a beneficial effect of both adiponectin (18) and metallothionein (70) on IH (11), and two recent studies have described a beneficial effect of both adiponectin (18) and metallothionein (70) on IH-induced myocardial ER stress and cell death. Therefore, we first hypothesized that IH induces proapoptotic ER stress that could underlie cardiac damage after myocardial infarction.

Beneficial effects of aerobic exercise on cardiovascular health are well established (32). In animals, the most commonly used exercise model is running at a moderate intensity on a treadmill (i.e., 30–60 min of running at treadmill speeds of 15–20 m/min for at least 5–12 wk), and this kind of exercise has been shown to reduce myocardial infarct size after ischemia-reperfusion (19, 20, 29). Shorter low-intensity exercise protocols also attenuate postischemic myocardial injuries, as 1–3 consecutive days of exercise result in both enhanced postischemic recovery and reduced infarct size (17, 23, 24, 68). In addition to protocol duration, it seems that training intensity may also represent a crucial factor in exercise-induced cardioprotection (1, 44, 60), and a growing body of evidence from both human and animal studies suggests a greater effect of high-intensity aerobic training (HIT) compared with moderate-intensity training (MIT) on cardiovascular, muscle, and metabolic adaptations (38, 49, 61, 64, 66). Among the putative mechanisms that have been proposed to explain exercise-induced cardioprotection, several studies have focused on the exercise-associated increase in myocardial antioxidant capacity (29, 56) and improvement of Ca2+ homeostasis (20, 23, 24, 34, 65). More recently, it has been shown that exercise could also exert beneficial effects through a reduction of ER stress in several contexts such as Alzheimer’s disease (36), insulin resistance (14), and muscle apoptosis (39). To our knowledge, there is only one study that has reported a protective effect of exercise in the context of IH. The authors reported a beneficial effect of exercise on IH-induced myocardial oxidative stress and apoptosis (10). In the present study, we first tested whether HIT would exert greater effects on performance and mitochondrial adaptations than MIT. We then hypothesized that HIT would reduce IH-induced ER stress and the associated postischemic myocardial damages.

As mentioned above, the firstline treatment of OSA is CPAP, which appears to be insufficient to reduce cardiovascular risk in many patients. Therefore, a combination of CPAP with other therapeutic strategies is a growing field of research. If our hypotheses are verified, targeting IH-induced ER stress by HIT could represent a new promising preventive strategy to limit IH-induced postischemic myocardial damage in OSA patients.

METHODS

Animals

This investigation conformed with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1996).

Adult male Wistar rats (2 mo old, 300–350g) were used in this study. Rats were obtained from Janvier Labs (Le Genest-Saint-Ismé, France). The study was approved by the University Grenoble Alpes Animal Research Ethic Committee (authorization no. 184_UHTA_U1042_CA_03). Rats were housed (n = 4 rats/cage) at the animal care facility of the HP2 Laboratory (approval no. A38 516 10006) under a 12:12-h light-dark cycle at 20–22°C and allowed free access to standard food and water.

Experimental Design

The first set of experiments was designed to validate the type of aerobic training. Rats were randomized to sedentary (Sed), MIT, or HIT conditions. Exercise effects on muscle citrate synthase (CS) activity, maximal aerobic speed (MAS), and endurance were then evaluated after 2 wk of training (n = 4–8 rats/condition).

Another group of rats was randomly assigned to normoxia or IH for a 21-day exposure. In both conditions, rats were assigned to Sed or HIT conditions to constitute the following four groups: normoxia (N)/Sed, IH/Sed, N/HIT, and IH/HIT. Training began after the first week of normoxia or IH and lasted until the end of exposure. In each group, two sets of experiments were performed: the first set of rats was used to investigate the effects of IH and HIT on ER stress (n = 6 rats/condition) and the second set was used to evaluate the effects of IH and HIT on myocardial infarct size (n = 11–15 rats/condition). Arterial pressure was recorded in all animals (Fig. 1).

Aerobic Trainings

Training was carried out on a motorized treadmill (0% grade, Bioserb, Vitrolles, France). Rats were first accustomed to the treadmill

![Fig. 1. Experimental design. Wistar rats were randomly submitted to normoxia (N) or intermittent hypoxia (IH) during 21 days and subjected to two different conditions, sedentary (Sed) or high-intensity interval training (HIT), during the last 10 days of exposure. Thus, the following four subgroups of animals were used in the present study: N/Sed, N/HIT, IH/Sed, and IH/HIT. Two sets of measurements were realized to evaluate infarct size and investigate the involvement of endoplasmic reticulum (ER) stress for each condition.](http://ajpheart.physiology.org/DownloadedFrom)}
Running for 4 days during the first week of exposure (no more than 15 min/session). MIT or HIT was then performed for 10 days during the last 2 wk of normoxia or IH exposure (5 days/wk).

**MIT protocol.** MIT was performed at a constant speed of 15 m/min corresponding to 60% MAS during 60 min. Twenty-four hours after the last training session, rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg), and muscle samples were taken.

**HIT protocol.** HIT consisted in 2 bouts of 24-min exercise interspaced by 30 min of recovery. The treadmill speed was set at 15 m/min for the first step and corresponded to a 6-min warmup (50% MAS) followed by six steps of 3 min with increased intensity to reach 30 m/min (corresponding to 65%, 70%, 75%, 80%, 85%, and 90% MAS). The highest intensity corresponded to ~90% MAS. Twenty-four hours after the last training session, rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg), and muscle samples were taken.

**CS Assay**

Muscles devoid of connective tissue (5 mg) were homogenized in extraction buffer [containing 5 mM HEPES, 1 mM EGTA, and 0.10% (vol/vol) Triton X-100] and incubated for 60 min on ice. DTT (1 mM) was externopimately added. The assay mixture contained measurement buffer [containing 1 M Tris-HCl, 10 mM 5,5′-dithiobis(2-nitrobenzoic acid), 10 mM acetyl-Co, and milli-Q water] and diluted sample (100 μg soluble proteins/ml total assay). Sample absorbance at 412 nm was monitored in 1.5-ml cuvettes using a spectrophotometer with the thermostat at 30°C (3 min). CS activity was measured with the addition of 10 mM oxaloacetate during 3 min (with data expressed in IU/g fresh weight).

**MAS and Endurance**

MAS can be used as an indicator of performance, owing to the linear relationship between O2 consumption and exercise intensity (8, 49, 59). MAS was individually determined using an incremental exercise protocol at 0° inclination consisting of a 6-min warmup at 13 m/min; speed was then increased by 3 m/min every 2 min until the rat was unable to run. MAS is the speed corresponding to the last entire stage completed by the animal.

Endurance was defined in this experiment as the running time to exhaustion at 75% MAS. After a 6-min warmup at 13–18 m/min, speed was increased to reach an intensity of 75% MAS. Exercise was stopped when the rat was no longer able to run.

**IH Protocol**

Animals were exposed daily to 8 h of IH or normoxia during their daytime sleep period for 21 days. The IH stimulus was performed using a specifically designed device, as previously described (2). It consisted of 1-min cycles with alternating 30 s of hypoxia (5% fraction of inspired O2 [FiO2]) and 30 s of normoxia (21% FiO2). FiO2 was monitored throughout the experiment with a gas analyzer (ML206, AD Instruments, Oxford, UK). Normoxic rats were exposed to air streams to reproduce equivalent levels of noise and turbulence related to gas circulation as IH without hypoxia. At the end of the exposure, rats were anesthetized with intraperitoneal injection of pentobarbital sodium (60 mg/kg). Arterial BP was recorded, blood was collected, and hearts were rapidly excised and either rapidly frozen (for Western blot and quantitative PCR analyses) or used for the Langendorff technique.

**Arterial BP Measurement**

The temperature of anesthetized rats was maintained at 37°C and adjusted using a rectal probe connected to a thermal pad (Harvard Apparatus, Les Ulis, France). Arterial BP was measured using an arterial carotid catheter linked to a mecanotransducer. Systolic BP, diastolic BP, mean BP, and heart rate were recorded using a PowerLab data-acquisition system (Powerlab, AD Instruments).

**Blood Samples**

After arterial BP measurement, animals were heparinized (500 U/kg). Blood was collected in capillary tubes and centrifuged (13,000 rpm for 7 min at 21°C) to measure hematocrit.

Venous blood was also collected from the inferior cava vein with EDTA and antioxidant solution [950 mg EGTA, 600 mg glutathione (pH 6–7), 10 ml H2O2, 10 μl/ml] and rapidly centrifuged (13,000 rpm for 10 min at 4°C) to collect plasma and for the catecholamine assay.

**Catecholamine Assay**

Epinephrine and norepinephrine were measured in venous plasma samples using the CatCombi ELISA kit (IBL, Hamburg, Germany). Data are expressed in nanograms per milliliter.

**Western Blot Analysis**

Frozen hearts were homogenized (Precells 24, 6,500 rpm, 3X20s-5s, Bertin Technology, Montigny le Bretonneux, France) to extract total proteins (sample lysis buffer: 5 mM EDTA, 1 mM Na2VO4, 20 mM NaF, 1 mM DTT, and protease inhibitor cocktail). The protein concentration was calculated using a Bradford assay (Bradford reagent, Sigma-Aldrich, Saint-Quentin Fallavier, France). Depending on the proteins analyzed, 30–100 μg of protein were separated by SDS polyacrylamide gels (8–12%) and transferred to polyvinylidene difluoride membranes. Next, membranes were blocked with 5% nonfat milk in Tris-buffered saline (TBS) with Tween 20 (0.1%). Membranes were then incubated overnight at 4°C with the following primary antibodies in TBS-Tween 20-5% BSA or nonfat milk: phospho-eIF2α, eIF2α, ATF4, and cleaved caspase-3 (1:500, Cell Signaling Technology, Hitchin, UK); ATF6 (1:200), phospho-PERK, PERK, CHOP, and Grp78 (1:500, Santa Cruz Biotechnology, Heidelberg, Germany), and actin (1:2,000, Sigma-Aldrich). The following day, membranes were incubated for 1 h at room temperature with the appropriate horseradish peroxidase-conjugated anti-IgG (1:5,000, Santa Cruz Biotechnology). Enhanced chemiluminescence was performed with the Western Blot ECL substrate (Clarity, Bio-Rad, Marnes-la Coquette, France) according to the manufacturer’s instructions and video acquisition (chemidoc-xrs-system, Bio-Rad). The relative amount of protein was quantified by densitometry (Image Lab, Bio-Rad) and expressed as a ratio of the loading control. Phosphorylated proteins were expressed relative to total protein, and nonphosphorylated proteins were expressed relative to actin. Finally, protein expressions of IH/Sed, N/HIT, and IH/HIT groups were expressed relative to the N/Sed group, which was normalized to 1.

**Quantitative Real-Time RT-PCR Analysis**

Total RNA was isolated from the whole heart using TRI reagent (Sigma-Aldrich) according to the manufacturer’s specifications. Total RNA (0.5 μg) was reversely transcribed to cDNA using iScript Reverse Transcription Supermix (C-1000 Thermal Cycler, Bio-Rad). Quantitative real-time PCR was performed using SsoAdvanced Universal SYBR Green Supermix (Bio-Rad) and PCR primers (Sigma-Aldrich) for the following rat ER stress-related genes: spliced XBP1, forward 5′-GAATCATTGGGA-3′ and reverse 5′-GTGCAGCTGGGAG-3′; and total XBP1, forward 5′-GTGCAGGCCCAGTGTGCCAC-3′ and reverse 5′-TCTGGGTAGACTCTGTTCCGA-3′. Cycling parameters were as follows: 95°C for 30 s, 95°C for 5 s, and 58°C for 10 s and increments of 0.5°C for 5 s from 65 to 95°C. All PCR assays were performed in triplicate (CFX96 Touch RT PCR, Bio-Rad). The PCR fluorescence signals for spliced XBP1 and total XBP1 were standardized to PCR fluorescent signals obtained from endogenous reference genes (actin and cyclin A; CFX Manager 3.1, Bio-Rad). Comparative and relative quantification of the
spliced XBPI product were normalized to total XBPI and calculated by the 2^ΔΔCt method (where Ct is threshold cycle).

Ex Vivo Ischemia-Reperfusion Protocol

Langendorff perfusion. Hearts of anaesthetized rats were rapidly excised and immediately immersed in 4°C Krebs-Henseleit buffer solution [containing (in mM) 118 NaCl, 4.7 KCl, 1.8 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 25.2 NaHCO₃, and 11 glucose]. The aortic stump was cannulated, and the heart was perfused using the Langendorff technique at constant pressure (75 mmHg) with oxygenated Krebs-Henseleit buffer. The myocardial temperature was maintained at 37°C. A water-filled latex balloon, coupled with a pressure transducer, was inserted into the left ventricular (LV) cavity via the left atrium for pressure recording. LV end-diastolic pressure was adjusted to ~7 mmHg. After 20 min of stabilization, no-flow global and total ischemia was induced by stopping the perfusion for 30 min. Thereafter, the heart was reperfused for 120 min. Hemodynamic variables were continuously recorded [LV end-diastolic pressure, LV developed pressure, cardiac contractility (dP/dt), and heart rate, Labchart, AD Instruments] (35).

Infarct size determination. At the end of ischemia-reperfusion protocol, atria were removed, and the heart was frozen at ~20°C for 10 min. The heart was then cut into 2-mm transverse sections from the apex to base (6–7 slices/heart). Once thawed, the slices were incubated at 37°C with 1% triphenyltetrazolium chloride in phosphate buffer (pH 7.4) for 20 min and fixed for 1 day in 10% formaldehyde to clearly distinguish stained viable tissue from unstained unviable tissue. Infarct size was determined by a computerized planimetric technique (ImageJ software, NIH) and expressed as a percentage of the ventricular size (35).

Statistical Analysis

Statistics were performed using GraphPad Prism 6 software (San Diego, CA). Data are expressed as means ± SE. Differences between groups over time were determined by two-way ANOVA with a subsequent Tukey’s post hoc test for multiple group comparisons. P values of <0.05 were considered statistically significant.

RESULTS

HIT Versus MIT Effects on Performance and Mitochondrial Adaptations

We assessed metabolic and functional variables to determine which protocol (MIT or HIT) of equal volume (i.e., total energy expenditure, although different intensity) promoted more beneficial outcomes after short-term training. CS is a pacemaking enzyme of the tricarboxylic acid cycle used as an indicator of cellular aerobic metabolism (43). HIT significantly increased maximal CS activity in soleus muscle (56.7 ± 4.1 vs. 37.9 ± 4.8 U/I in HIT and Sed groups, respectively, P < 0.05; Fig. 2A), whereas MIT had no significant effect. Functional capacity was assessed as MAS and endurance at 75% MAS. HIT induced a greater increase in MAS than MIT (50.8 ± 1.5 vs. 35.7 ± 3.2 m/min in HIT and before training/HIT groups, respectively, n = 4, P < 0.05; and 44.3 ± 4.3 vs. 34.4 ± 2.6 m/min in MIT and before training/MIT, respectively, n = 4, P < 0.15; Fig. 2B). Finally, endurance at 75% MAS tended to be higher in the HIT group compared with the MIT group (69.3 ± 8.6 vs. 48.2 ± 10.1 min in HIT and MIT groups, respectively, P = 0.08; Fig. 2C). Based on the greater short-term effect of HIT on performance (MAS and endurance) and mitochondrial adaptations (CS activity), we retained this protocol for the trained groups of the present study.

HIT Prevents IH-Induced Cardiac Proapoptotic ER Stress

Chronic IH triggered cardiac ER stress, as characterized by a significant enhancement of Grp78 (ER chaperone) expression (2.6 ± 0.6-fold increase, P < 0.01; Fig. 3A) and increased phosphorylation of PERK (1.7 ± 0.2-fold increase, P < 0.05; Fig. 3B) and eIF2α (1.5 ± 0.1-fold increase, P = 0.058; Fig. 3C). Whereas ATF6 expression and XBPI mRNA splicing were not increased by IH (Fig. 3, E and F), IH also induced ER-initiated proapoptotic signaling with a significant raise in ATF4 expression (1.6 ± 0.2-fold increase, P < 0.05; Fig. 3D) and a trend of increased CHOP expression (1.5 ± 0.4-fold increase; Fig. 3G), which were associated with increased expression of cleaved caspase-3 (3.4 ± 1.3-fold increase, P < 0.05; Fig. 3H).

HIT prevented this IH-induced proapoptotic ER stress, as shown by the absence of a significant increase in GRP78 expression, PERK and eIF2α phosphorylation, and proapoptotic ATF4 and CHOP expression in the IH/HIT group. ATF6 expression was significantly reduced, and splicing of XBPI mRNA was not modified. Finally, HIT also prevented the IH-induced increase in cleaved caspase-3 (Fig. 3).

In the normoxic group, HIT alone induced slight ER stress, as shown by the significant increase in GRP78 expression, PERK and eIF2α phosphorylation, and proapoptotic ATF4 expression in the IH/HIT group. However, this HIT-induced ER stress was different from that induced by IH, as HIT did not activate the UPR (i.e., no significant effect of HIT on PERK and eIF2α phosphorylation or on ATF4,

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**Fig. 2.** HIT effects. A–C: maximal activity of the mitochondrial enzyme citrate synthase (CS; A), maximal aerobic speed (MAS; B), and postraining endurance at 75% MAS (C) in rats under Sed, traditional moderate-intensity training (MIT), or HIT conditions before (PRE) and after (POST) endurance trainings (n = 4–8 rats/conditions). Data are means ± SE. *P < 0.05 vs. the Sed group; #P < 0.05 vs. the PRE/HIT group.
CHOP, and ATF6 expression and XBP1 mRNA splicing) or cleaved caspase-3 (Fig. 3).

**HIT Prevents the IH-Induced Increase in BP and Infarct Size**

IH was associated with a significant increase in heart rate (Fig. 4A) and mean BP (134 ± 3.8 vs. 119 ± 3.6 mmHg, *P* < 0.05; Fig. 4B). This IH-induced elevation of mean BP was demonstrated by significant increases in both systolic and diastolic BP (systolic BP: 155 ± 5.1 vs. 138 ± 3.6 mmHg and diastolic BP: 123 ± 3.3 vs. 109 ± 3.7 mmHg in IH/Sed and N/Sed groups, respectively, *P* < 0.05) and was associated with a trend of an increase in sympathetic activity that was assessed indirectly through measurement of plasma catecholamine levels. In Sed animals, norepinephrine concentrations tended to be greater in hypoxic rats (27.7 ± 5.4 vs. 13.3 ± 1.8 ng/ml in IH/Sed and N/Sed; groups respectively, *P* = 0.08; Fig. 4C). HIT prevented both IH-induced increases in heart rate and BPs (mean BP: 122 ± 5.0 and 113 ± 4.3 mmHg, systolic BP:
145 ± 6.0 and 135 ± 5.1 mmHg, and diastolic BP: 111 ± 5.2 and 103 ± 4.1 mmHg in IH/HIT and N/HIT groups, respectively; Fig. 4) and the IH-associated increased plasma norepinephrine levels (17.5 ± 5.9 and 11.96 ± 2.7 ng/ml in IH/HIT and N/HIT groups, respectively; Fig. 4C). Finally, under Sed conditions, infarct size was significantly higher in hypoxic than normoxic groups (34.5 ± 2.9% vs. 22.7 ± 1.7% in IH/Sed and N/Sed groups, respectively, P < 0.05). This IH-induced increase in infarct size was prevented by HIT (28.8 ± 3.9% and 21.0 ± 5.1% in IH/HIT and N/HIT groups, respectively), whereas HIT alone had no effect in normoxic animals (Fig. 5). Hemodynamic Langendorff variables were not different between the four groups at any time of the experiment (Table 1).

**DISCUSSION**

In the present study, we demonstrated that chronic exposure to IH induces sustained cardiac proapoptotic ER stress, an elevation of arterial BP, and increased myocardial infarct size after ischemia-reperfusion, which were all prevented by short-term HIT. Therefore, to limit myocardial damage after an ischemic event, early diagnosis and treatment of sleep apnea are particularly relevant. Furthermore, specific aerobic training might represent a potent preventive strategy to improve the prognosis in OSA patients presenting with myocardial infarction.

**Duality of the IH Stimulus**

It is well known that hypoxia is an ambivalent stimulus that can exert both beneficial and detrimental effects, in particular in the myocardium. We have previously demonstrated that the duration, depth, and pattern of the hypoxic stimulus are crucial to determine whether the effect is protective or detrimental. On one hand, we showed that 4 h of IH (1-min cycle, 10% FIO₂) reduced myocardial infarct size in rats, whereas 30 min of IH or 4 h of continuous hypoxia at 10% FIO₂ did not (5), suggesting that myocardial preconditioning can be achieved with a very specific protocol of acute IH. On the other hand, a more severe IH stimulus (5% FIO₂) is detrimental for the myocardium (i.e., increases myocardial infarct size) when applied either in an acute (4 h) or a chronic (several weeks) protocol (5, 35, 54). The specific IH stimulus used in the present study (1-min cycle, 5% FIO₂, for several weeks) is now commonly used to emulate OSA-like IH (16).

**Chronic Exposure to IH Promotes Cardiac Proapoptotic ER Stress**

In the present study, we have shown that chronic exposure to IH induces sustained cardiac ER stress. This ER stress was characterized by significant upregulation of the chaperone Grp78, increased phosphorylation of both PERK and eIF2α, and a significant increase in ATF4 expression and a trend of...
increases in ATF6 and CHOP expression. Finally, this IH-induced ER stress was associated with myocardial apoptosis, as we further demonstrated increased expression of cleaved caspase-3 in rats exposed to IH. These results are consistent with recent studies in rats and mice that have demonstrated that chronic IH induces proapoptotic ER stress in both the myocardium (18, 70) and neurons (11, 71). Furthermore, the link between ER stress and apoptosis is now well described. In particular, CHOP seems to be the main ER actor involved in apoptosis regulation, as it reduces the expression of the anti-apoptotic factor Bcl-2 (46) and upregulates the proapoptotic factor BIM (69), subsequently activating caspase signaling. Supporting this, CHOP deficiency is protective against ER stress-induced apoptosis and myocardial dysfunction (52). In our model, we cannot exclude that IH could directly induce apoptosis independently of ER stress, through the classical extrinsic or intrinsic mitochondrial apoptotic pathways. In fact, sympathetic activation and oxidative stress, both activated by IH, can also induce myocardial apoptosis, subsequent to ER stress activation or in an ER stress-independent manner (13, 15, 33, 58, 63).

**IH-Induced Increase in BP and Infarct Size: Potential Role of ER Stress**

We confirmed results of previous studies indicating that IH was associated with chronic sympathetic activation (for a review, see Ref. 21) and an increase in arterial BP (6, 35, 57, 62). As ER stress inhibitors have been shown to prevent both ANG II-induced systemic hypertension (37) and hypoxia-induced pulmonary arterial hypertension (42) in mice, our data suggest that prolonged ER stress induced by IH could contribute to the IH-induced elevation of arterial BP. We further confirmed that chronic exposure to IH was associated with an increased response to myocardial ischemia-reperfusion (i.e., increased infarct size), which is consistent with previous studies in both rodents (6, 35, 54, 57, 62) and humans (9). This result is particularly relevant as it suggests that a subject previously exposed to chronic IH will have a poor prognosis after an acute myocardial event. This raises the need to better understand mechanisms that could be activated by chronic IH and involved in the IH-increased infarct size after myocardial ischemia-reperfusion to identify and treat at-risk patients. Again, ER stress could represent a good intermediate mechanism as its role in the pathophysiology of cardiovascular diseases is well documented (26, 47). CHOP-deficient mice failed to increase infarct size after myocardial ischemia-reperfusion (48), and, in the context of chronic IH, previous studies have demonstrated the role of IH-induced ER stress on myocardial apoptosis and the subsequent contractile dysfunction (18, 70). The results of the present study support this and suggest that IH-induced proapoptotic ER stress could explain the IH-associated increased in myocardial infarction.

**HIT Prevents IH-Associated Myocardial ER Stress and Increased Infarct Size**

Interestingly, we further demonstrated that HIT inhibits IH-induced ER stress and also prevents the IH-induced increase in arterial BP and infarct size. The beneficial effect of HIT in decreasing proapoptotic ER stress has been previously described in skeletal muscles of rats and was associated with a decrease in fasting plasmatic glucose and insulin (39). Exercise training has also been shown to reduce ER stress-induced apoptosis in Alzheimer’s disease in mice (36). As previously mentioned, proapoptotic ER stress has been correlated with myocardial damage in several pathophysiological conditions, such as ischemia-reperfusion, myocardial infarction, and heart failure (47). Therefore, in our study, the normalization of myocardial ER stress by HIT could explain the limitation of...
IH-induced increase in infarct size. Accordingly, two studies have recently demonstrated that other cardioprotective interventions (i.e., methallothionein and adiponectin) were also efficient against IH-induced ER stress and associated cardiomyocyte death (18, 70), independently of any ischemia-reperfusion protocol.

**HIT Activates Adaptive ER Stress**

We chose HIT as a preventive strategy to limit both IH-induced myocardial ER stress and increased infarct size, as only 2 wk has the potential to exert beneficial effects on performance and mitochondrial adaptations. Interestingly, in the normoxic group, HIT induced slight ER stress, different from the stress induced by IH. These data suggest that HIT may activate the adaptive pathway of the UPR, which maintains ER homeostasis upon luminal stress and allows myocardium adaptations to exercise. In accordance, exercise-induced ER stress has been well documented in different organs, such as the skeletal muscle (67), liver (27), and brain (40), and is considered as a prosurvival, and not a proapoptotic, response of the UPR. However, in our study, HIT-induced ER stress was not associated with any beneficial or deleterious effect on cardiovascular parameters (i.e., arterial BP and infarct size).

**HIT as a Preventive Strategy Against OSA-Related Cardiovascular Consequences**

Exercise is described as an efficient strategy for the primary and secondary prevention of cardiovascular disease. It is associated with a decrease in cardiovascular mortality, through a lowering of resting heart rate, improved vascular endothelial function, increased vasculogenesis, and several metabolic adaptations of the myocardium, which result in improved tolerance to ischemia-reperfusion injuries in both human and animal models is well documented (22). Although it is generally admitted that MIT is sufficient to reduce the risk and recurrence of cardiovascular diseases, the beneficial effects of exercise may vary according to its intensity and duration (22). Moreover, high-intensity exercise brings greater short-term cardiovascular effects than MIT (28). In the context of a 21-day exposure to IH, current animal models of long-term moderate aerobic training were not suitable. Thus, we opted for an efficient short-term intense protocol aimed at improving cardiovascular variables and that could be applied together with IH exposure. We demonstrated that only 10 sessions of HIT elicited greater effects than traditional MIT on performance (i.e., MAS and endurance) and muscle adaptation (i.e., CS activity) and was also associated with an improvement of cardiovascular parameters (i.e., arterial BP and infarct size). These results are consistent with previous studies that have demonstrated that HIT can decrease sympathetic activation and arterial BP in humans (12, 53). Concerning animal studies, HIT has been shown to decrease arterial BP, preserve endothelial function (30), and improve myocardial contractile function both under basal conditions (38) and in a model of postinfarction heart failure (34, 49) in rats.

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**Table 1. Hemodynamic variables in Langendorff-perfused rat hearts**

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<td><strong>Heart rate</strong></td>
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<td>N/Sed</td>
<td>273 ± 9</td>
<td>175 ± 22</td>
<td>173 ± 48</td>
<td>181 ± 26</td>
</tr>
<tr>
<td>IH/Sed</td>
<td>273 ± 10</td>
<td>150 ± 28</td>
<td>198 ± 17</td>
<td>204 ± 20</td>
</tr>
<tr>
<td>N/HIT</td>
<td>272 ± 8</td>
<td>162 ± 17</td>
<td>221 ± 34</td>
<td>192 ± 12</td>
</tr>
<tr>
<td>IH/HIT</td>
<td>256 ± 8</td>
<td>201 ± 21</td>
<td>199 ± 14</td>
<td>193 ± 15</td>
</tr>
<tr>
<td><strong>Coronary flow</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N/Sed</td>
<td>14.7 ± 1.0</td>
<td>5.2 ± 0.3</td>
<td>4.8 ± 0.3</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td>IH/Sed</td>
<td>16.3 ± 1.2</td>
<td>5.0 ± 0.6</td>
<td>4.3 ± 0.5</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td>N/HIT</td>
<td>14.4 ± 0.6</td>
<td>5.3 ± 0.9</td>
<td>4.7 ± 0.6</td>
<td>4.0 ± 0.4</td>
</tr>
<tr>
<td>IH/HIT</td>
<td>14.3 ± 1.3</td>
<td>6.6 ± 0.9</td>
<td>6.2 ± 0.8</td>
<td>5.4 ± 0.8</td>
</tr>
<tr>
<td><strong>Left ventricular end-diastolic pressure, mmHg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N/Sed</td>
<td>6.0 ± 1.0</td>
<td>71.5 ± 6.6</td>
<td>58.2 ± 7.2</td>
<td>45.6 ± 6.7</td>
</tr>
<tr>
<td>IH/Sed</td>
<td>7.8 ± 1.1</td>
<td>69.8 ± 6.7</td>
<td>55.3 ± 6.7</td>
<td>53.7 ± 6.7</td>
</tr>
<tr>
<td>N/HIT</td>
<td>7.0 ± 0.9</td>
<td>66.3 ± 2.7</td>
<td>58.7 ± 2.9</td>
<td>49.5 ± 3.2</td>
</tr>
<tr>
<td>IH/HIT</td>
<td>6.9 ± 1.1</td>
<td>69.1 ± 4.9</td>
<td>56.6 ± 4.3</td>
<td>43.9 ± 3.3</td>
</tr>
<tr>
<td><strong>Left ventricular developed pressure, mmHg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N/Sed</td>
<td>90.9 ± 5.5</td>
<td>18.1 ± 4.1</td>
<td>32.0 ± 4.1</td>
<td>25.4 ± 3.9</td>
</tr>
<tr>
<td>IH/Sed</td>
<td>96.3 ± 10.0</td>
<td>13.2 ± 2.6</td>
<td>33.6 ± 7.4</td>
<td>25.1 ± 5.1</td>
</tr>
<tr>
<td>N/HIT</td>
<td>92.2 ± 7.0</td>
<td>28.4 ± 6.2</td>
<td>34.3 ± 5.1</td>
<td>26.5 ± 2.9</td>
</tr>
<tr>
<td>IH/HIT</td>
<td>108.6 ± 8.1</td>
<td>19.1 ± 4.9</td>
<td>29.6 ± 4.7</td>
<td>25.8 ± 3.2</td>
</tr>
<tr>
<td><strong>dP/dt</strong>&lt;sub&gt;max&lt;/sub&gt;, mmHg/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N/Sed</td>
<td>1685 ± 98</td>
<td>431 ± 60</td>
<td>602 ± 78</td>
<td>528 ± 57</td>
</tr>
<tr>
<td>IH/Sed</td>
<td>1792 ± 187</td>
<td>478 ± 160</td>
<td>626 ± 133</td>
<td>431 ± 66</td>
</tr>
<tr>
<td>N/HIT</td>
<td>1771 ± 147</td>
<td>583 ± 92</td>
<td>709 ± 74</td>
<td>571 ± 56</td>
</tr>
<tr>
<td>IH/HIT</td>
<td>1960 ± 117</td>
<td>474 ± 103</td>
<td>618 ± 92</td>
<td>545 ± 63</td>
</tr>
<tr>
<td><strong>dP/dt</strong>&lt;sub&gt;max&lt;/sub&gt;, mmHg/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N/Sed</td>
<td>2217 ± 179</td>
<td>506 ± 97</td>
<td>785 ± 115</td>
<td>660 ± 80</td>
</tr>
<tr>
<td>IH/Sed</td>
<td>2308 ± 345</td>
<td>584 ± 219</td>
<td>613 ± 88</td>
<td>524 ± 76</td>
</tr>
<tr>
<td>N/HIT</td>
<td>2282 ± 230</td>
<td>749 ± 166</td>
<td>939 ± 141</td>
<td>772 ± 81</td>
</tr>
<tr>
<td>IH/HIT</td>
<td>2939 ± 303*</td>
<td>567 ± 110</td>
<td>784 ± 130</td>
<td>736 ± 91</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 11–15 rats/group. Heart rate, coronary flow, left ventricular end-diastolic pressure, left ventricular developed pressure, and cardiac contractility (dP/dt) were determined after 20 min of stabulation (Stab), 30 min of reperfusion (R30), 60 min of reperfusion (R60), and 120 min of reperfusion (R120) in animals exposed for 21 days to either intermittent hypoxia (IH) or normoxia (N) and submitted to sedentary (Sed) or high-intensity training (HIT) conditions. *P < 0.05 vs. N/Sed and N/HIT groups.
Conclusions

HIT prevents the IH-dependent increase in myocardial infarction after ischemia-reperfusion, possibly through a down-regulation of the proapoptotic ER stress pathway (Fig. 6). Thus, our results suggest that HIT could represent a very promising preventive strategy to limit IH-induced myocardial ischemia reperfusion-related damages in OSA patients and improve their prognosis. The severity of sleep apnea can also be reduced by exercise only (41); therefore, rehabilitation programs should be implemented and evaluated in OSA patients that present with high cardiovascular risk.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: G.B., E.B., and C.A. performed experiments; G.B., E.B., and C.A. analyzed data; G.B., E.B., and C.A. interpreted results of experiments; G.B. and C.A. prepared figures; G.B. and C.A. drafted manu-
REFERENCES


